An investigation into the acute effects of treatments created and developed from either date fruit or date seeds on the mood and cognitive performance of healthy young volunteers

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A thesis submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy to Newcastle University, Newcastle Upon-Tyne

The research described within this thesis was undertaken in the Department of Population Health Science Institute, Faculty of Medical Sciences, Newcastle University

Submitted 2022

Abstract

The first aim of this thesis was to assess and compare the effects of an acute dose of two varieties of mature unripe freeze-dried date fruit (Barhi and Khassab) on cognitive performance, mood, and blood glucose concentration. An acute randomised, double-blind, placebo-controlled, crossover study with a week washout period between visits was conducted on thirty-five healthy young participants (18–35 years). Cognitive function was assessed using computerised tests for attention, working and episodic memory before and 45, 90 and 135 minutes after treatment. Participants consumed the equivalent of 115 g of fresh weight fruit, which differed in the total phenolics content. The vehicle was yoghurt (150 g per portion), and the placebo was yoghurt with added sugars matching the treatments for sugars. There were no significant differences in cognitive responses for the individual task outcomes (p = 00625), nor for the cognitive indices outcomes (p = 0.0017 after Bonferroni correction).

Roasted date seed drink is a popular beverage in Arab countries, so the second aim of this thesis was to assess the acute effect of a “coffee-like beverage” made from commercial date seeds on mood and cognitive function. It was postulated that some potential benefits may be related to the content of phenolic compounds in the date seeds which were characterised using HPLC. A randomised, double-blind, placebo-controlled, crossover study was conducted on fifty-two healthy young participants. Cognitive function was assessed as in the first trial, that is, before and 45 and 90 minutes after treatment. The experimental date beverage was tested against a positive control “regular coffee” and a placebo. The trial was designed to have 85% power to detect an effect size of half the published effect of regular coffee.

The vehicle was hot water (280 ml per cup), with the date beverage obtained from 45 g of roasted ground date seeds, the regular coffee from 6 g of roasted ground coffee, and the placebo was hot water with brown food colour matched to the treatments. There were no significant differences in cognitive effects among the three treatments, indicating not only that the date beverage did not affect cognitive function, but that the published effect of coffee also may not be consistently reproducible

Acknowledgements

First and foremost, thanks to my supervisors

I would like to express my sincerest gratitude to Dr Kirsten Brandt and Dr Anthony Watson for their invaluable advice, continuous support and guidance along my PhD journey. In particular, for their input in both the trial design and assistance in finalising the thesis. I cannot thank you enough for all your patience and for being both understanding and compassionate. Additionally, I would like to extend this to Dr Chris Seal who was my supervisor for the second year of my PhD before taking retirement.

Thank you to Prof Keith Wesnes and Helen Brooker

I would like to extend my thanks to Prof Keith Wesnes for his generous cooperation in providing me with access to the online assessment tool (CogTrack) free of charge, which was utilised in both my experiments to assess the cognitive function. His tragic passing was a great loss to both science and the cognitive psychology field, and I wish to express my deepest sympathy to Prof Keith Wesnes' family. A huge thank you to Helen Brooker for all of her support, guidance and cooperation during the planning, setting and conduction of the first experiment.

Thank you to all of those involved in the date and yoghurt study

Thank you to Rebecca Clayton for assisting me in the recipe development stage, and with the daily running of the trial and gathering of the data. Thank you to both Wendy Ball and Waseem Iqbal for all your assistance in making the study treatments at 8:00 am for various months. Also, thank you to Steve Hall for using your knowledge to convert the paper questionnaires into electronic versions.

Thank you to all of those involved in the GI study

I am deeply grateful to Ines Mendes for your assistance in making the study treatments at 8:00 am for many months.

Thank you to all of those involved in the date seeds drink study

I would like to offer my special thanks to Sarah Hakeem for assisting me in the smooth running of the second human intervention. Additionally, for all your hard work in preparing the treatments at 8:00 am for various months.

Thank you to all of those involved in the NU-Food Research Facility

I would like to offer my thanks to all of the staff at NU-Food for their friendship, but particularly to those who assisted in the booking of the facilities. Thank you to Wendy Bal, Nicky Parker, Alison Young and the rest of the NU-Food team for your help.

Thank you to all the participants in the two trials

This research would not be possible without you all. I thoroughly enjoyed working with each person whom I met, and I am enormously grateful for both your commitment and patience throughout each stage of the study.

Thank you to all of my friends and colleagues

Thank you to all of those who I shared an office, memories and love with, Sarah Hakeem, Abrar Babateen, Mohammed Almqbel, Albatul Almushayti, Katie Harrison, Ruyuf Alfuraihi, Sara Corsi, Mohammed Salman and Charles Ojobor. We laughed together, we cried together, and we shared both food and coffees together. Most prominently, we made it through both the good and the bad together.

Thank you to all of my beloveds

Thank you to my four incredibly amazing kids, Juri, Bader, Maha and Seba. You have been so patient and understanding. I thank you for accepting and loving me unconditionally, through all the hard days which resulted in me being irritated and losing my temper. You remained so thankful, even when I had to serve you ready meals instead of freshly cooked ones. You were extremely understanding when I had to go to university during the weekend instead of taking you out or spending more time

with you. Thank you for making it as easy as it could be for me when we had to be away from each other during my writing up year. Overall, I want to thank all of you for always being yourselves.

Last but not least

Thank you to me for being able to make it through, the journey was not easy at all. I definitely need to thank myself for never losing faith, for always holding my passion tightly and for showing a great deal of resilience. Finally, I am so thankful to be able to say I kept my promise to my parents who sadly passed away.

Dedication

"*To my wonderful parents who passed away I say: sleep in rest, I kept my promise*"

Declaration

This work has not been submitted for any other award. Except for where due acknowledgement has been given, in all experimental chapters of this thesis, the author had sole responsibility for the data collection, analysis, and interpretation.

Name: Duaa Altuwairki

Signed

Date

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List of Abbreviations

Abbreviation	Explanation
BG	Blood glucose
CAE	Chlorogenic acid equivalent
CAE	Chlorogenic acid equivalent
CAS No	Chemical abstract service
CBF	Cerebral blood flow
CC	Caffeine content
CCP	Critical Control Points
CGA	Chlorogenic acid
Cmax	Maximum concentration
DC	Decaf coffee
DCP	Decaffeinated coffee powder
DSD	Date seeds drink

DSDP	Date seeds drink power
DSE	Date seed extract
DW	Dry weight
EEG	Electroencephalogram
EGCG	Epigallocatechin gallate
EPE	Epicatechin equivalent
FW	Fresh Weight
GAE	Gallic acid equivalent
GI	Glycaemic index
GL	Glycaemic load
GTC	Green tea catechins
HACCP	Hazard analysis and critical control points
HPLC	High performance liquid chromatography
ILSI	International Life Sciences Institute

KSA	Kingdom of Saudi Arabia
MCAO	Middle cerebral artery occlusion
MML	Linear mixed model analyses
MMRM	Mixed model repeated measure
NIRS	Near infrared spectroscopy
P	Placebo
RC	Regular coffee (positive control)
Rpm	Reps per minute
SD or StDev	Standard deviation
SOD	Antioxidant enzyme activity
TPC	Total phenolic content
VASs	Visual Analogue Scales

Chapter 1. Introduction

1.1 General introduction

Homeostasis is sustained by essential nutrients, which are defined as substances that must be obtained from the diet as the body is unable to make them in sufficient quantity to meet its needs, giving rise to the significance of food in everyday life. Bioactive compounds, which can promote both health and disease prevention, can occur in particular foods (Liu, 2013). These are often referred to as “functional foods” or “super foods”. Functional foods which originate from plant sources, in addition to their nutritional and energy capacity, can provide modulate one or more targeted functions in the body by contributing to reducing the risk of disease and/or enhancing a physiological response (Nicoletti, 2012). Various effects have been proposed, including but not limited to, stimulant attributes (Haskell et al., 2007), mitigation of stress (Weeks, 2009), protection from cardiovascular disease (Francis et al., 2006), cognitive enhancement (Kennedy et al., 2007), neuro-protection (Kim et al., 2010), and defence against carcinogenesis (Bishayee et al., 2011). The particular compound and chemical structure influence the effects observed. A variety of compounds have displayed favourable outcomes *in vitro,* however, there is a deficiency of conclusive outcomes *in vivo* in humans. The potential *in vivo* mechanisms, which are the basis of the suggested health-promoting properties, are currently becoming an emerging theme in research.

Healthy, non-clinical populations have increasingly utilised products containing functional foods for ‘self-promotion of health’ during the last decade, giving rise to the global functional food market being worth around $173.26 billion in 2019 (Precedence Research, 2020). The most established functional food market is for products associated with alterations to mood and cognitive performance (Zamora-Ros et al., 2013). There are alleged *in vivo* effects of these products, however, there is a distinct lack of scientifically reproducible data that provide credible evidence for their efficacy. The upsurge in consumer demand has emphasised the requirements for quantifiable

information, with *in vivo* effects for health preservation, disease prevention, and alterations in cognitive functions. One such functional food is the date fruit. Various functional foods demonstrate cognitive changes in animal and human models, with outcomes most frequently observed in memory and attention (see sections 1.7, 1.8 and 1.9). These cognitive changes are noticeable for other fruits which are habitually consumed such as blueberries, blackcurrant and grapes as illustrated in Table 4. Research has also been conducted on some beverages such as green coffee and regular coffee (see Table 4).

An example of this scientific evidence is the demonstrated statistically significant effects of the phenolic content of green coffee blend on some cognitive and mood measures like sustained attention, decision in the choice reaction time, and alertness (Camfield et al., 2013). Camfield et al. (2013) investigated the effect of the consumption of a single dose of a green coffee blend containing 530 mg of chlorogenic acids (CGAs)/cup against a placebo and a similar dose of 530 mg of pure CGAs on sixty healthy participants following a double-blind acute, crossover study design. However, the significant effect was only observed after the consumption of green coffee. Another example of the statistically significant effect but on different cognitive and mood measures like; attention and calmness rates has been reported following the administration of a single dose of 138.3 mg of anthocyanin contained in 200 ml of purple grape juice compared to placebo (Haskell et al., 2017). Both studies followed a randomised, placebo-controlled study design. To date, there have been no such randomised placebo-controlled studies conducted on either date fruit or date seed to the best of our knowledge. However, a single publication of a human intervention indicated a significant enhancement of the reaction time in the Stroop, 1-back and 2-back working memory tests following the consumption of Ajwah dates (Abdullah et al., 2019). The study followed a one-group, repeated measures design to investigate the chronic effect of consuming seven Ajwah dates per day for six weeks with no control on twenty healthy young volunteers. Cognitive functions were assessed using the Stroop and N-block tasks, while the mood was assessed using the Profile of Mood States (POMS) and the Depression, Anxiety and Stress Scale-21 (DASS-21). However, the study results were not reliable due to the risk of bias.

The popularity of “functional foods”, evolving evidence of enhancing effects of flavonoid-rich foods, the absence of controlled human trials on both date fruit and date seeds and their influences on cognitive performance give rise to many questions. The initial question for this thesis to address is whether the intake of a standardised date flesh or seed extract could cause an alteration to human cognitive functions. The thesis will comprise studies which incorporate developed treatments with either date fruit or date seeds and matched placebos, which are examined for palatability and organoleptic aspects. The created treatments will be investigated in terms of cognitive-enhancing outcomes.

The subsequent sections of this chapter provide an appraisal of the literature and focus on functional foods which are flavonoid-rich, in addition to date fruit and date seeds. Usually, the first chapter of a thesis covers all aspects of the literature review for the research topic, however, to improve readability, some of the literature review has been embedded in the other chapters for several reasons. Firstly, because the literature is not directly relevant to the main aim of the thesis and secondly, because it has been included to justify or to support the rationale of a chosen approach. The following Figure 1 provides a clearer illustration.

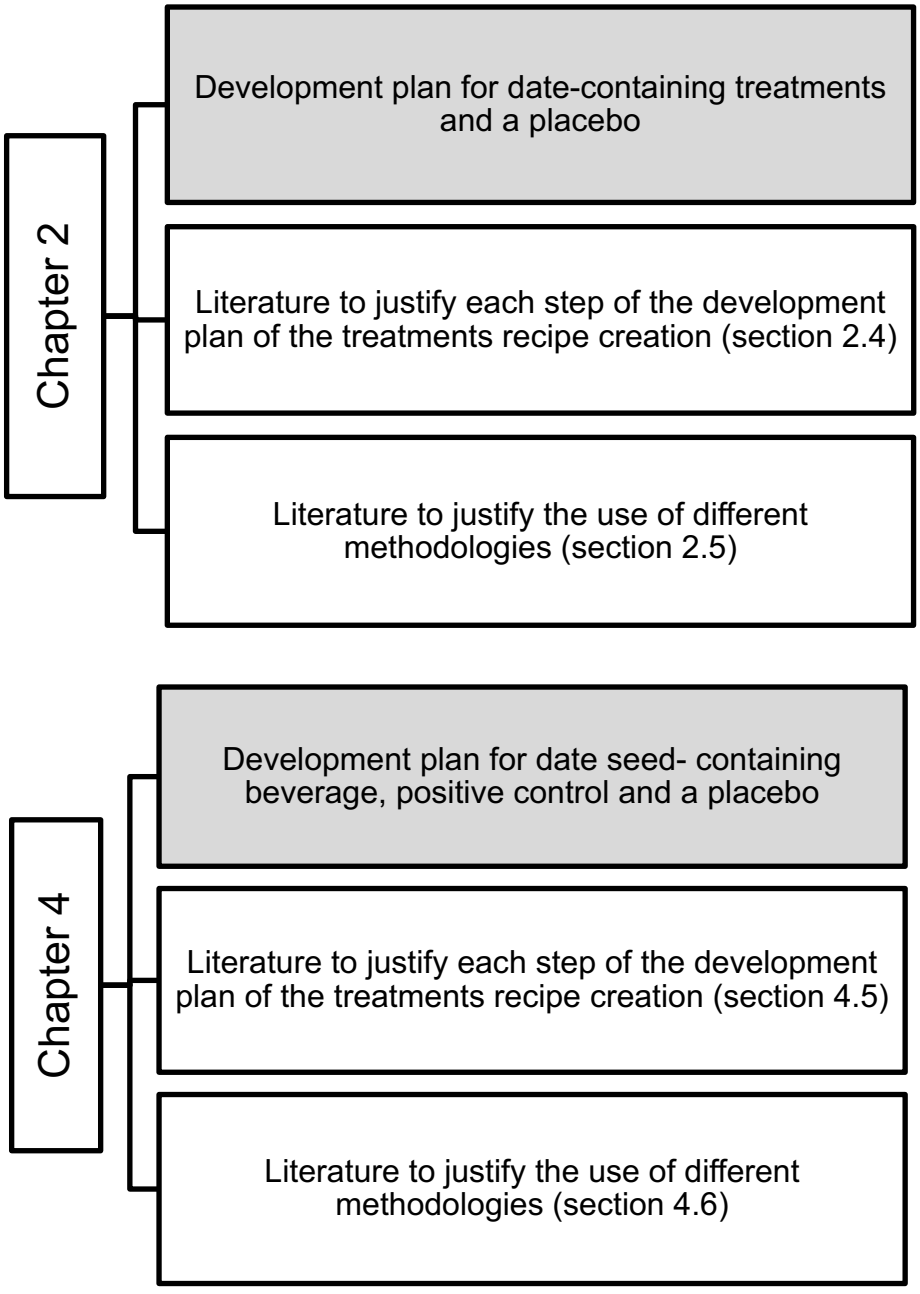

Figure 1. Flowchart to identify the locations of the literature reviews within the thesis.

1.2 Cognitive functions areas and domains

In clinical neuropsychology, cognitive performance is typically characterised by the referral to domains of cognitive performance (Harvey, 2019). Different component abilities within each domain can be measured by cognitive tests (Al-Aidroos et al., 2012). These domains vary from simple processing speed to more complex like the executive function domain. Tests of cognition are generally administered as a battery of standardised tests that tap into different, well-defined cognitive domains. By testing a range of cognitive domains, test batteries may allow researchers to detect changes in humans' cognitive performance (Harvey, 2019).

Some cognitive tests have been developed and implemented to assess the effects of nutritional experiments on cognitive function (Vogel et al., 2010), administered either with paper and pencil or on computers (Lieberman, 2007). For commercially available automated, computerised cognitive test systems, there are several programmes available that present computerised versions of validated cognitive tests. These programmes include the Cambridge Neuropsychological Test Automated Battery (CANTAB) (Robbins et al., 1994), the Cognitive Drug Research (CDR) Computerised Assessment System (Simpson et al., 1989), CogState (Maruff et al., 2009), the Computerised Mental Performance Assessment System (COMPASS) and CogTrackTM. In principle, these programmes are similar, however, there is a large number of cognitive tests, and an estimated total of eighty different types of cognitive tests were applied in twenty-eight different studies reviewed by Lamport et al. (2012). The diversity of cognitive tests may cause difficulties drawing a clear correlation between specific nutritional interventions and the cognitive effects, as some cognitive tests may be more sensitive in detecting specific cognitive changes, which might make some of them more suitable to use in specific age groups than others.

Investigating cognitive functions associated with specific interventions requires that test batteries target a spectrum of domains reported to be sensitive to this specific intervention. To further our understanding of the structure of cognitive functions in humans, we should look at the usually utilised specific domains within cognitive test

batteries in the literature (Burkart et al., 2017). The most typical group of domains commonly utilised in test battery are verbal, spatial, memory and processing speed. Episodic memory, attention and global cognitive function were repeatedly reported to be enhanced by the consumption of phenolics and believed to have more evidence of positive effects compared to other cognitive domains (de Jager et al., 2014).

The selection of cognitive domains and the domains sensitive to phenolic manipulation has been a topic of an overview by Lamport and Williams (2020) and a review by (de Jager et al., 2014). Since so many cognitive tests are available, the authors recommended avoiding the “Scatter gun” manner in selecting the domains and providing a supportive rationale for their test selection, which should assess domains that only match their hypothesis as this may obstruct comparisons between studies. However, the diversity of phenolic compounds may challenge the prediction of the cognitive domains in new research and eventually affect the statistical approach, since the use of many independent tests in the same intervention can lead to an increase in the number of comparisons and eventually increase the probability of obtaining a significant result (de Jager et al., 2014). Although utilising composite scores instead of individual tests can provide a focused number of outcomes and minimise the statistical comparisons needed for analysis, the key to an appropriate cognitive test choice is to be driven by previous evidence showing the task to be sensitive to phenolics manipulations (Adolphus et al., 2017).

There are different ways to classify cognitive domains, including classification by the process associated with motor skills, perception, memory, attention, executive function, processing speed and languages—alternatively, the classification is based on the regional brain functions linked to specific brain areas (Harvey, 2019). Definitions for each of the previously mentioned domains are available in Table 1.

Table 1. Domains of cognitive functioning extracted from Harvey (2019).

Motor skills	
These include several different basic elements of motor activity such as fine motor abilities including manual dexterity and motor speed, as well as reaction time, and more global skills such as balance.	
Perception	
The ability to recognise objects, sounds, or the identification of previously experienced objects from sensory information.	
Attention	
Attention is a multifaceted construct and is generally divided into two global subdomains: selective attention and sustained attention (or vigilance).	
Selective attention	**Sustained attention (vigilance)**
The process of attending to information that is relevant and important and ignoring other nonrelevant information.	The ability to sustain attention over time has been referred to as vigilance.
Memory	
Memory functioning is the most complex and multifaceted of the cognitive domains.	
Working memory	**Episodic memory**
This is the ability to hold information in consciousness for adaptive use. This can include information from all sensory modalities as well as verbal and nonverbal information.	This component of the memory system interacts with working memory storage processes to encode, maintain, and retrieve information into and out of longer-term storage.
Executive functioning	
This cognitive domain is also referred to commonly as reasoning and problem-solving.	
Processing speed	
Processing speed refers to cognitive processing assessments that require rapid performance of tasks that range from very simple to complex.	
Language skills	
Language skills include receptive and productive abilities and the ability to understand language, access semantic memory, identify objects with a name, and respond to verbal instructions with behavioural acts.	

The origin of these domains was originally linked or localised to the areas of the brain in which these processes were seen to be occurring, a perspective that is translated from animal research (Darcet et al., 2016), and studies looking at the physiological effect of nutritional manipulations (Spencer, 2009). Generally, animals learn, remember and process information to make decisions and perform accordingly (Shaw and Schmelz, 2017). Therefore, scientists typically used cognitive paradigms in the laboratory involving prolonged animal training to complete tasks to study these performance changes (Darcet et al., 2016).

Table 2. Extracted examples of cognitive tests used to measure different domains in animal research.

Examples from animal research	
Cognitive domain	Example of cognitive test
Attention	5-choice reaction time task (5-CSRTT)
Executive function	Attentional set-shifting task (ASST), Reversal Morris water maze and Prepulse inhibition (PPI)
Working memory	Delayed attention Y-maze and Delayed attention T-maze
Episodic memory	Novel object recognition, Object location recognition, Passive avoidance place
Spatial memory	Morris water maze, Barnes maze and Radial arm water maze
Motor function	Open field test, Rota-road test, and Hot plate test
Examples from Human research	
Cognitive domain	Example of Cognitive test
Immediate verbal memory	Hong Kong List Learning Test, Word list recall, Word presentation (CDR system), Word recognition (CDR system)
Delayed verbal memory	Benton Visual Retention Test, Colour matching and Corsi Block Tapping Test
Spatial memory	Rey Complex Figure Test, Spatial Pattern Recognition and Long-term episodic memory
Executive function	Trail Making test A&B, Cube comparison Test, Visual scanning, Word fragmentation completion and Mazes
Working memory	Digit ordering, Numeric working memory, Serial subtraction by 3's and Serial subtraction by 7's
Attention and information processing speed	Attention switching, Stroop Colour Test, Simple reaction time, Choice reaction time and Digit Vigilance Test
Psychomotor skills	Finger Tapping Test, Grooved Pegboard Test and Movement Assessment Battery

*As mentioned in Darcet et al. (2016) and extracted examples of cognitive tests utilised to measure different domains in human research as mentioned in de Jager et al. (2014).

Although there is a significant difference in cognitive capacity between humans and animals, there are some similarities in cognitive domains in both research fields, as illustrated in Table 2.

1.3 Increasing interest in investigating the therapeutic uses of dates

Up to the year 1969, there was a lack of scientific research available on the date palm, with investigations of the botanical, horticultural and pharmacological properties of the date palm commencing thereafter due to the interest of both the Food and Agricultural Organisation and policy creators within Arabian Countries (Anwar, 2006). Information on pharmacological studies comprising in vitro studies, animal studies and clinical studies can be obtained from scientific databases such as Scopus. Data were obtained using the keywords “date fruit” AND/OR “date seed” AND “Therapeutic uses” and demonstrated an intense growth in the volume of published studies in connection to the therapeutic applications of date palm products, including both the date fruit and seeds. There were 0 to 20 articles published per year between 2000 and 2013, and 40 to 80 articles published per year between 2014 to 2020, as illustrated in Figure 2.

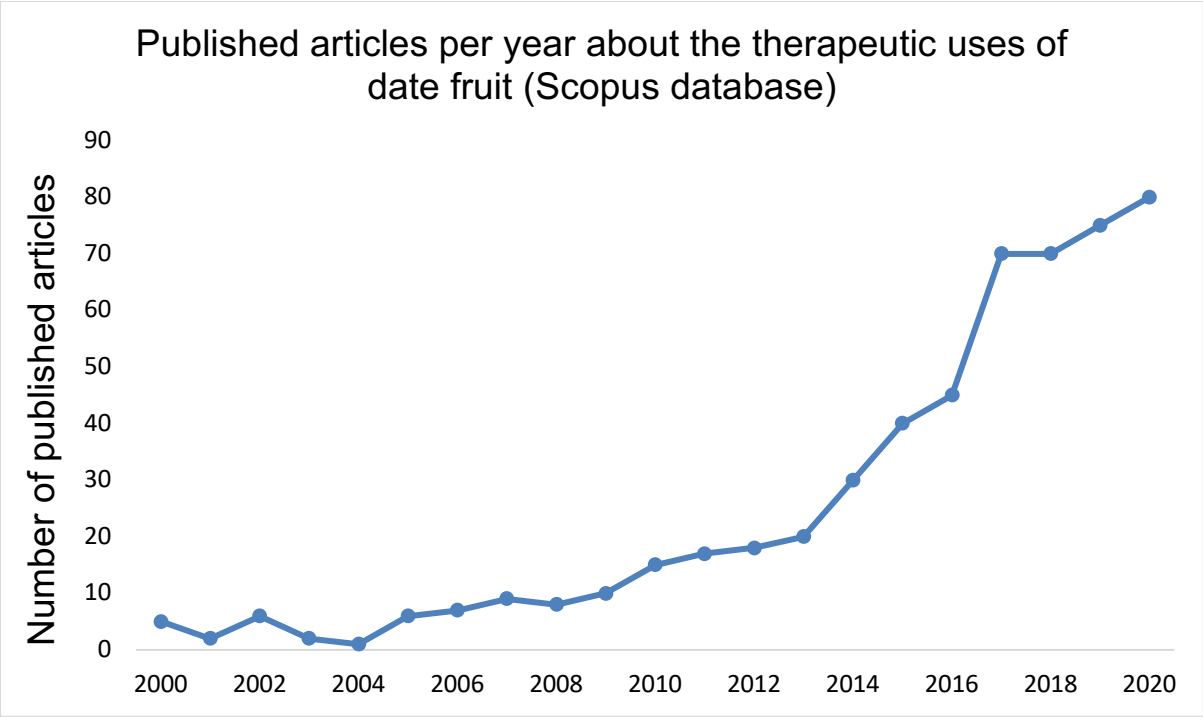

Figure 2. The number of published articles per year about the therapeutic uses and benefits of date fruit and seeds (obtained from the Scopus database).

There were 0 to 20 articles published per year between 2000 and 2013, and 40 to 80 articles published per year between 2014 to 2020, as illustrated in Figure 2.

1.4 An overview of date palm

The date palm is an important product, both commercially and culturally, in the Middle Eastern and Northern African countries that have an arid or semi-arid climate. The date palm can endure intense drought while still supplying nutritious fruit. Recently, the worldwide demand for date fruit has increased, thus the agricultural production of the date palm has become more widespread with other locations such as California in the United States effectively cultivating a variety of commercial types of high-quality date palm such as Deglet, Nour and Medjool (Chao and Krueger, 2007). The world production of date fruit has gradually increased with over 1.1 million tonnes produced in 2019, and yet Saudi Arabia continues to be the most prominent producer with a 20% share of global production.

The date palm, *Phoenix dactylifera L.* (Arecaceae), produces fruits which also offer social and economic functions for the inhabitants of the oases of the Middle East due to their nutritional and pharmacological properties (Baliga et al., 2011). They are a substantial source of nutrition in a region which is unsuitable for the cultivation of most plants. Traditionally, date fruits have constituted a significant part of the Arab diet from ancient times. Moreover, date fruits and date palm trees acquire special attention in the daily routine of the Middle Eastern and African countries due to their religious practices (Terral et al., 2012). Socially, dates resample hospitality and generosity for Bedouin people (Barreveld, 1993) and are valued in Islamic populations and the first food to be consumed by fasting Muslims in the holy month of Ramadan to break their fasting (Benmeziane-Derradji, 2019).

The date fruit is generally eaten when in three palatable phases of maturation. These are based on customary Arabic practice and acknowledged international vocabulary: the mature but unripe *Khalal* or *Bisr* (50% moisture), ripened *Rutab* (30–35% moisture), and mature *Tamr* (10–30 % moisture) (Baliga et al., 2011). However, soft and semi-dry selections of dates are often stored before partial drying, where moisture levels are <25%, as these demonstrate an acceptable shelf-life (Baliga et al., 2011). The maturation stages of dates are presented in Figure 3.

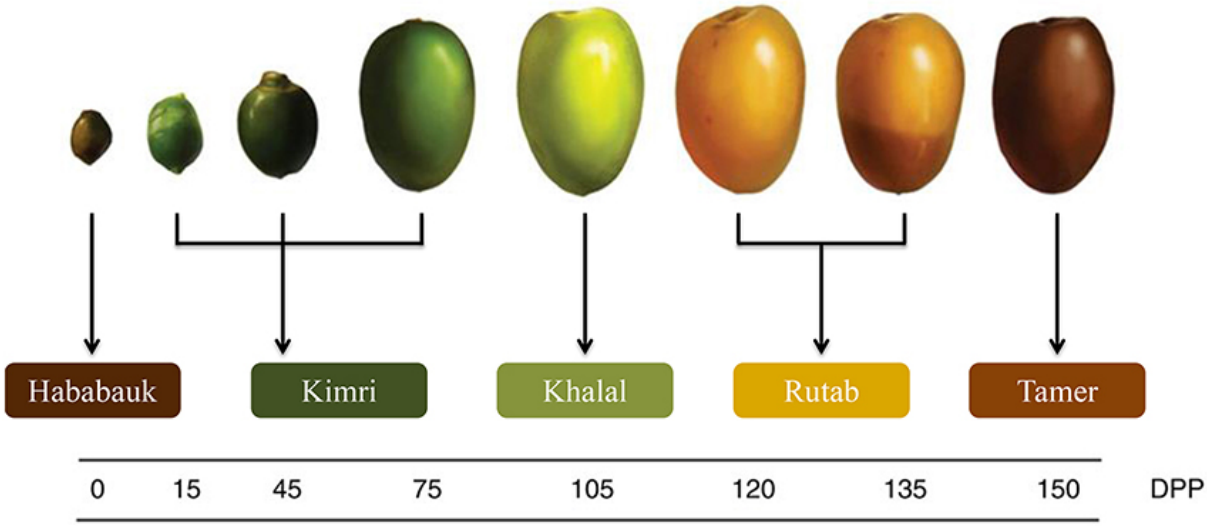

Figure 3. The five date ripening stages according to the number of days post pollination (DPP).

Hababauk is the first stage of development after pollination (inedible hard whitish-cream colour), Kimiri is the second stage (inedible hard greenish colour), Khalal is the third stage (edible less hard yellowish or purplish colour), Rutab is the fourth stage (edible soften yellowish or purplish-brown colour), and Tamar the fifth stage (edible soft dark brown colour) (obtained from (Al-Mssallem et al., 2013).

Many different date fruit varieties are grown within the Kingdom of Saudi Arabia, with at least 3000 distinctive types (Asif et al., 1983). A cultivar as a taxon is 'an assemblage of plants that (a) has been selected for a particular character and (b) remains distinct, uniform, and stable in these characters when propagated by an appropriate method' (Brickell et al., 2016). Each variety has a preferential maturation stage when it is normally consumed, thus the variation among dates on the market is a combination of genetic and maturity variation. Generally, the variety name is used to indicate the corresponding maturity stage unless otherwise specified. Consumers usually preferred to consume Khassab and Barhi in the Khalal stage, Sukkari and Majdool in the Rutab stage, and Khalas and Ajwah in the Tamar stage.

The characteristics of the studied date varieties include the ripening stage, colour, shape, size, texture, the percentage of the moisture and the origin of where each variety is cultivated in the Kingdom of Saudi Arabia (KSA) (see Table 3).

Table 3. Characteristics of studied date varieties extracted from Hamad et al. (2015)

Date variety	*Preferred ripening stage*	*Colour*	*Shape*	*Size*	*Texture*	*Moisture (%)*	*Origin in KSA*
Sukkari	*Rutab*	Golden brown	Cone	Medium or small	Chewy flesh	30-35 %	Qassim
Barhi	*Khalal*	Bright yellow	Oval	Medium	Soft/ crunchy	50 %	Qassim
Ajwah	*Tamer*	Black/ white wrinkles	Round	Small	Soft	10 %	Almadinah
Khassab	*Khalal*	Dark red	Long	Large	Crunchy	50 %	Almadinah
Khalas	*Tamer*	Reddish/ dark brown	Round	Medium	Soft and sticky	10 %	Al Ahsa

1.5 Phytochemicals in date fruits and seeds

Phytochemicals are biochemical composites which are innately found in plants and plant-based products. A sizeable quantity of these phytochemicals, which are habitually eaten in the human diet, impact well-being via an array of mechanisms (Kennedy and Wightman, 2011). The principal subclasses of phytochemicals include thiols, alkaloids, isoprenoids and phenols. Phenols are the most prevalent and the following sections will focus on polyphenols and phenolic acids, both of which are abundant in date fruit and seeds. These phytochemicals are postulated as the central compounds behind the physiological and psychological effects.

1.5.1 Phenols

Polyphenols contain more than one phenol group in their structure and are organic compounds originating from plants, with thousands of molecules in plants having a

polyphenolic structure (Manach et al., 2005). Natural phenols vary from simple molecules like phenolic acids to more complex compounds like tannins (Bravo, 1998). There are four chief subcategories of plant phenolics: tannins, flavonoids, phenolic acids, and coumarins. Within these groups, flavonoids are split into the subdivisions of flavonols, flavones, flavanones flavanols and anthocyanins (Beecher, 2003), and phenolic acids including hydroxycinnamic acids and hydroxybenzoic acids, as illustrated in Figure 4. These are considered “secondary metabolites” and are found in different forms in most plant families. The principal phenolic compounds in date fruit are flavonoids and phenolic acids (Al-Farsi and Lee, 2008). Accordingly, the prospective benefits of phenolic compounds in mammalian biological systems could be correlated with the known bioactive activities of plants; including their antioxidant, anti-allergenic, anti-inflammatory and antiviral effects observed in in vitro studies (Kennedy and Wightman, 2011). Many features of phenolic compounds, such as molecular dimensions and structures, influence their absorption and bioactivity. The structure of flavonoids and phenolic acids are now presented according to their concentrations in date fruit and seeds.

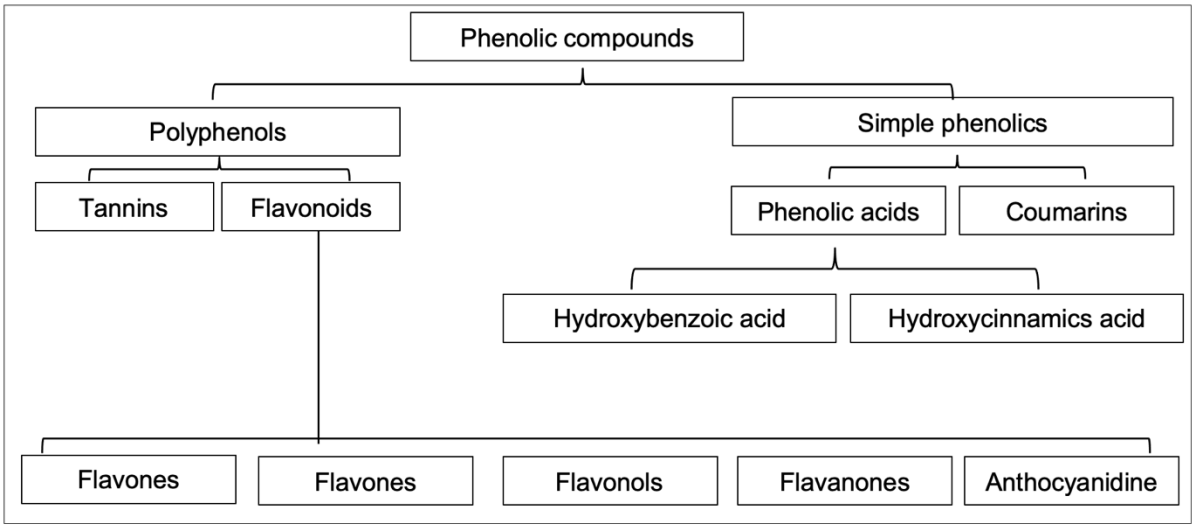

Figure 4. Chemical classification of phenolic compounds (Magnani et al., 2014)

1.5.2 Flavonoids

Flavonoids are secondary metabolites, including the pigments providing some plant coloration. Obtained from 2-phenylchromen-4-one (McNaught and Wilkinson, 1997), flavonoids are a range of phenylbenzopyrone arrangements based on a standard three-ring nucleus. The foremost subclasses of flavonoids are flavonols, flavanols, flavones, flavanones and anthocyanidins (McNaught and Wilkinson, 1997).

1.5.3 Phenolic acids

Dates contain two main subclasses of phenolic acids: hydroxycinnamic acids and hydroxybenzoic acids, which are distinguished by the number of carbons connecting the ring and the acid; hydroxycinnamic acids have two carbons, while benzoic acids have none (Al-Farsi and Lee, 2008). Within these groups, hydroxycinnamic acids include ferulic, p-coumaric, sinapic and caffeic acids, and the hydroxybenzoic acids include p-hydroxybenzoic, protocatechuic, vanillic, and syringic acids (Mansouri et al., 2005). The contents of ferulic, sinapic acids and certain cinnamic acid derivatives can vary between cultivars or as a result of soil environments and agronomic procedures (Al-Farsi and Lee, 2008).

Date fruit varieties originating from Saudi Arabia (Al Sagey, Helwat Al Jouf, and Al Sour) exhibit antioxidant abilities (Hamad, 2014), possibly due to their phenolic constituents, phenolic acids (caffeic acid, ferulic acid, protocatechuic acid, catechin, gallic acid, *p*-coumaric acid, resorcinol, chlorogenic acid, and syringic acid) and flavonoid glucosides (quercetin, luteolin, apigenin, isoquercitrin, and rutin). Furthermore, of the premium Saudi Arabian dates (Khalas, Sukkari, and Ajwa), Ajwa has the highest content of phenolics and antioxidant capacity. Additionally, Sukkari dates contain the highest quantity of flavonoids in the form of rutin. The concentrations of flavanols like catechins are comparable between the Sukkari and Ajwa dates, but the highest concentrations of phenolic acids like caffeic acid were found in Khalas dates. Most notable, the major phenolic compounds in date seeds are flavan-3-ols, in particular, flavanols like epicatechins and catechins. The method employed to acquire this data used depolymerisation (Habib et al., 2014a).

1.6 The rationale for including literature about other foods rich in phenols

As briefly mentioned in section 1.1, a few studies indicate that phenol-rich foods may modulate human physiological processes, such as cognition. However, there is a distinct lack of peer-reviewed studies which have investigated such effects following the consumption of date fruit and/or seeds. Therefore, details about berries and cocoa are shown as examples of flavanol and flavanol-rich food/drinks, respectively, and coffee as a phenolic acid-rich drink to cover the range of foods with different types of phenolics as dominant constituents to also represent several other foods/drinks that contain similar or the same constituents. Other phenol-rich foods, drinks and phenolic supplements such as apple, orange, tea, matcha green tea and supplements like soy, resveratrol, isoflavone, EGCG and others were also included to provide background information for the work presented in this thesis (see Table 4). Furthermore, Table 4 below shows the phenolic content of different phenol-rich foods which have been utilised in studies investigating the effect of these foods on mood and cognitive function. Although this is not an exhaustive list, it should help when comparing different phenol-rich foods.

Table 4. Phenolic content of different foods classified as rich in phenols

*The rows highlighted in grey signify the foods that have already been utilised in studies to test their efficacy on mood and cognitive function regardless of the study outcomes.

Type of food	***Reference***	***Phenolic content***
Grapes	*(J. Hendrickson and D. Mattes, 2008)*	*2.1 mg GAE[1]/ml*
Blackcurrant	*(Watson et al., 2019)*	*5.17 mg GAE/ml FW[2]*
Blackcurrant	*(Watson et al., 2015)*	*3.7 mg GAE/ml FW*
Blueberry	*(Dodd et al., 2019)*	*2.9 mg flavonoids/g FW*
Grapes	*(Haskell et al., 2017)*	*0.65 mg GAE/ml FW*
Cocoa	*(Field et al., 2011)*	*22 mg TF[3]/g FW*
Cocoa	*(Francis et al., 2006)*	*172 mg TF/portion*
Cocoa	*(Scholey et al., 2010)*	*2.6 mg TF/ml* *4.97 mg TF/ml*
Cocoa	*(Pase et al., 2013)*	*24 mg TF/ml* *12.5 mg TF/ml*
Cocoa	*(Massee et al., 2015)*	*250 mg TF/capsule*
Cocoa	*(Brickman et al., 2014)*	*37.5 mg TF + 5.75 mg (-)-Epicatechin/ml*
Orange juice with pomace fibre	*(Alharbi et al., 2016)*	*1.13 mg TF/ml FW*
Coffee	*(Cropley et al., 2012)*	*86.82 mg GAE/ml DW[4]*
Green coffee blend	*(Camfield et al., 2013)*	*88.7 mg GAE/ml FW*
Matcha green tea *Matcha green bars*	*(Dietz et al., 2017)* *(Dietz et al., 2017)*	*Tea 70 mg EGCG[5]/g FW* *Bar 70 mg EGCG/ml FW*
Apple	*(Bondonno et al., 2014)*	*1.82 mg GAE/g FW*
Isoflavone supplement (4 capsules)	*(Pipingas et al., 2008)*	*120 or 960 mg isoflavone/capsule*
Soy supplement (4 capsules)	*(Thorp et al., 2009)*	*116 mg isoflavone /capsule*
Green oat supplement	*(Kennedy et al., 2020)*	*1.4 mg/g DW*
Resveratrol (2 capsules)	*(Kennedy et al., 2010)*	*500 or 250 mg resveratrol/capsule*
Flavonoid supplement (3 tablets)	*(Ryan et al., 2008)*	*150 mg flavonoid/tablet*
EGCG supplement (2 capsules)	*(Wightman et al., 2012)*	*135 mg or 270 mg EGCG/capsule*
Greek Mountain Tea	*(Wightman et al., 2018)*	*475 mg or 950 mg/capsule*
Apricot	*(USDA, 2004)*	*1.6 mg GAE/g FW*
Peach	*(USDA, 2004)*	*1.63 mg GAE/g FW*
Banana	*(Faller and Fialho, 2010)*	*0.91 mg GAE/g FW*

1.6.1 Cocoa

The *Theobroma cacao* plant produces a cocoa pod, which encompasses around 20-60 seeds (cocoa beans) when ripe and weighs approximately 500 g. These cocoa beans are between 40-60% fat and are very rich in polyphenols, particularly flavan-3-ols. Flavan-3-ols are a subclass of flavonoids and are extensively acknowledged to be, along with theobromine and alkaloids, one of the principal active components which account for the health benefits of cocoa. However, there are some variations in the total quantity of flavan-3-ols due to differing cultivars and conditions for growing, harvesting and processing. These compounds usually contribute around 10% of cocoa powder (Hammerstone et al., 2000), with the monomer (+)-catechin and (-)-epicatechin being predominant. The monomers comprise around 12% of the total procyanidin quantity in cocoa-based products.

1.6.2 Berries

Berries are fleshy fruits which contain seeds (Allaby, 1996) and there is a wide variety including grapes, blueberries, cranberries, redcurrants and blackcurrants. They are phenol-rich but the total polyphenol quantity and type are dependent on the individual species, as well as growth conditions and location (Kähkönen et al., 2001). Commonly, the principal polyphenols in berries are flavonoid anthocyanins, which can contribute up to 3090 mg/100 g of fruit (Kähkönen et al., 2001) and phenolic acids (Mattila et al., 2006).

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[1] GAE - Gallic acid equivalent
[2] FW - Fresh Weight
[3] TF -Total Flavonoids
[4] DW - Dry weight
[5] EGCG - Epigallocatechin gallate

1.7 Phenols' mechanisms of action

There is increasing evidence to support the beneficial outcome for memory and learning subsequent to the consumption of fruit-derived phytochemicals, most prominently flavonoids (Watson et al., 2019, Watson et al., 2015, Williams et al., 2008, Shukitt-Hale et al., 2006). One possible explanation for their influence on memory is the physiological effects directly on the innate architecture for memory in the brain (Williams et al., 2008). The aforementioned architecture has been established to weaken during the ageing process as neuronal populations or synaptic connections are reduced over time, thereby reducing efficiency in both the processing and storage of data. A review of the impact on this architecture from flavonoids or flavonoid-rich fruits is presented in the following sections, providing information on how these flavonoids may impact processing and therefore have consequences on memory and other outcomes.

1.7.1 Interaction with neural signalling and synaptic function

The capacity of flavonoids appears to be connected to their capability to interact with both the molecular and physiological apparatus employed in the standard memory processing (Rendeiro et al., 2015, Spencer, 2009, Spencer, 2007). The quantity of flavonoids and subsequent metabolites believed to reach the brain after dietary consumption is approximately 10-300 nmol, sufficient to induce pharmacological activity in receptors, kinases and transcription factors (Williams et al., 2004). However, the specific site of activity remains uncertain, although a variety of methods of action have been suggested including (1) attaching to the ATP locations on enzymes and receptors; (2) regulating the action of kinases directly, i.e. MAPKKK, MAPKK or MAPK; (3) influencing the function of central phosphatases which act in opposition to the kinases; (4) regulating transcription factor initiation and binding to the promoter sequences, i.e. cyclic AMP-response element-binding protein reviewed in Figueira et al. (2017), Spencer (2009) and Rendeiro et al. (2015) and increasing the blood flow and neurogenesis.

Foods rich in flavonoids have been demonstrated to counteract cerebrovascular disease, which incorporates both stroke and dementia (Commenges et al., 2000, Dai et al., 2006), possibly via the beneficial effects on endothelial function and peripheral blood flow (Schroeter et al., 2006). The augmentation in cerebrovascular function can assist neurogenesis within the hippocampus (Gage, 2000), with hippocampal cells clustered in close proximity to blood vessels and proliferating in response to vascular growth factors, thus potentially enhancing memory (Palmer et al., 2000). Optimal brain functioning is dependent on a variety of factors, including a proficient cerebral blood flow (CBF). A variety of studies have displayed a reduction in CBF in dementia (Nagahama et al., 2003, Ruitenberg et al., 2005), and an association has been observed between CBF and cognitive outcomes in human subjects using functional magnetic resonance (MRI) and transcranial Doppler ultrasound (Ruitenberg et al., 2005). These brain imaging methods have observed a reduced CBF rate in Alzheimer's patients and as markers of dementia, with a reduced risk of dementia progression in individuals with a higher CBF (Iadecola, 2013).

Moreover, foods rich in flavanols have been observed to considerably increase CBF in human participants around 1-2 hours post-supplementation (Francis et al., 2006, Fisher et al., 2006). A cocoa drink containing 400–900 mg of flavanols acutely increased blood flow 2 hours post-consumption, with alterations observed in the blood oxygen level-dependent response of participants undergoing a 'task switching' test. In addition, arterial spin-labelling sequence MRI also showed an increase in CBF for up to 2 hours post-consumption of a cocoa drink rich in flavanols (Wang et al., 2008). Furthermore, the use of trans-cranial Doppler ultrasound also displayed an increase in CBF within the middle cerebral artery post-supplementation of cocoa (Fisher et al., 2006). However, this area still requires further examination as these studies are relatively old. Another study by (Decroix et al., 2016) reported that 903 mg of cocoa flavanol significantly benefits cerebral oxygenation at rest but this randomised, double-blind, crossover study was relatively small (12 participants) compared to the norm (24–36 participants), for example, (Dodd et al., 2019, Watson et al., 2019, Dietz et al., 2017, Haskell-Ramsay et al., 2017, Watson et al., 2015). Also, the participants were

asked to perform a 30 min duration of strong exercise which may have induced the increase in cerebral infusion and oxygenation when measured by NIR.

1.7.2 Inhibition of neurodegeneration and neuroinflammation

Neurodegeneration in Parkinson's, Alzheimer's and additional neurodegenerative diseases is considered as being elicited by multi-factorial routes and is inclusive of neuroinflammation (Yamakawa et al., 2016, McGeer and McGeer, 2003), glutamatergic excitotoxicity and, either an increase of Fe and/or reduction of endogenous antioxidants (Barzilai and Melamed, 2003, Spires and Hannan, 2005). There is some evidence of a counteracting effect of this neural injury by flavonoids and foods rich in flavonoids resulting in delayed disease progression (Spencer, 2008, Vauzour et al., 2008b), and protecting cortical primary neurons against glutamate neurotoxicity (Mikami and Yamazawa, 2015). Nigral neuronal death in Parkinson's disease includes the formation of endogenous neurotoxins, 5-*S*-cysteinyl-dopamine, and synthesis of the oxidation product of dihydrobenzothiazine-1(Vauzour et al., 2008a). Yet, this neuronal damage caused by 5-*S*-cysteinyl-dopamine production (Spencer et al., 2001b, Spires and Hannan, 2005) can be efficiently counteracted by a variety of Spencer et al., 2001b flavonoids and alternative polyphenols naturally occurring within fruits such as oranges, berries, apples and grapes (Vauzour et al., 2008a). Furthermore, these flavonols and metabolites are potential inhibitors of oxidant-induced neuronal injury (Spencer et al., 2001a, Spencer et al., 2001b). This data has been gathered at amounts appropriate to those detected *in vivo* and the brain (around 10–300 nmol), and they may act by moderating PI3 kinase (PI3K)/Akt as well as mitogen-activated protein kinase signalling (Figueira et al., 2017, Spencer, 2007, Williams et al., 2004).

1.8 The long-term impact of phenols from other sources on mood and cognitive function

Kennedy et al. (2020) investigated the effect of the phenolic content of green oat (*Avena sativa*) capsules in three different doses of 430 mg, 860 mg and 1290 mg of

cognitaven® and a placebo in a double-blind, randomised, parallel groups study design on a total of 132 healthy adults aged between 35 to 65 years. The three doses were equivalent to 300 mg, 600 mg, and 900 mg of native green oat extract, respectively. The study assessed both the acute and the chronic effects of the phenolic content of the green oat capsules consumed as a capsule/day for 29 days. The 1290 mg dose significantly improved performance in the Corsi Blocks working memory task and the verbal serial substructions and computerised tracking. Also, four weeks of daily consumption of the 430 mg and 1290 mg doses significantly improved the same tasks but there was no information about the phenolic content of the treatment.

In a similar acute/chronic, double-blind, placebo-controlled, parallel study design, Wightman et al. (2018) investigated the effect of the daily consumption of two doses of 475 mg and 950 mg of *Sideritis scardica* (Greek Mountain Tea) extract in capsule form for 28 days on mood, cognitive function and cerebral blood flow of 155 healthy elderly adults between 50 to 70 years. After 28 days, the higher dose of 950 mg significantly improved the participants' performance in choice reaction time and rapid visual information processing tasks, while both doses increased the oxygenated haemoglobin and oxygen saturation in the prefrontal cortex during the completion of the high intensity cognitive tasks on day 1.

Significant improvements in the speed of spatial working memory and word recognition within an elderly population were observed after chronic consumption of a daily dose of 960 mg of flavonoids, as a capsule of Enzogenol® for 5 weeks (Pipingas et al., 2008). Similar improvements were also noted after the daily consumption of 150 mg of flavonoids as capsules of Pycnogenol® for 3 months (Ryan et al., 2008). In addition, a lower dose of 116 mg of soy isoflavones significantly improved spatial working memory after 6 weeks (Thorp et al. (2009). However, Basaria et al. (2009) reported no significant effects of a 160 mg dose of flavonoids for 12 weeks. Although this study involved a higher dose for a longer time, this study was a parallel-group, double-blind, placebo-controlled design.

Drawing conclusions regarding the associations between differing polyphenol doses and effects are particularly challenging due to contradictory results. Supplementation

of a daily low 116 mg dose of soy isoflavones demonstrated positive results (Thorp et al., 2009), whereas a higher dose of 1470 mg of polyphenols from grape juice exhibited no acute effects (Hendrickson and Mattes, 2008). Although similar cognitive assessment tools were utilised in both studies, there was a difference between the participants' age groups, with healthy elderly adults recruited by Thorp et al. (2009) in contrast to young volunteers by (Hendrickson and Mattes, 2008).

1.9 The impact of cocoa and berries on mood and cognitive function in humans

As presented in section 1.6, there is evidence of the potential alterations in cognitive function after the consumption of cocoa and berries and a variety of human interventions which have examined the effects of cocoa or berries on mood and cognitive function are considered in the following sections and summarised in Table 5. These are included in support of the thesis rationale. Even though the doses ranged from 116 to 1470 mg, there is no obvious dose-effect relation, with the different studies reporting a variety of effects.

Table 5. A summary of the most relevant studies of polyphenols in different plant foods or supplements and their effects on cognitive function

Type of food	*Study*	*Type of phenolics*	*Design*	*Experimental treatment*	*Quantity*	*TPC dose*	*Significant effect*	*Participant age*
Berries	Hendrickson & Mattes, 2008	Anthocyanin	Acute	Welch's® juice	NA	1470 mg	No effect	Young adults
Blackcurrant	Watson et al., 2019	Anthocyanin	Acute	Ben Hope juice	96.96 ml	500 mg	Better speed responses during the choice reaction task	Young adults
Blackcurrant	Watson et al., 2015	Anthocyanin	Acute	DelCyan™ drink or Blackadder Juice	1.66 g or from 142 ml	524±5 mg per 60 kg of body weight	Better rapid visual information processing task accuracy	Young adults
Blueberry	Dodd et al., 2019	Anthocyanin + procyanidin	Acute	300 ml of skimmed milk + 30 g of freeze-dried blueberry drink	30.1 g of blueberry extract	578.82 mg	Better performance on a variety of tasks of the global cognitive function including immediate word recognition and digit switch task	Healthy elderly volunteers

Type of food	*Study*	*Type of phenolics*	*Design*	*Experimental treatment*	*Quantity*	*TPC dose*	*Significant effect*	*Participant age*
Grapes	Haskell et al., 2017	Anthocyanin	Acute	Welch's® + Purple grape juice	200 ml of Welch's® + 30 ml of Schweppes	150.4 mg	Better reaction time for attention tasks	Young adults
Cocoa	(Pase et al., 2013)	Cocoa flavanols	Acute/ chronic	Chocolate drink	20 g of dark chocolate drink mix in 200 ml water	500 mg 250 mg	Increased self-related calmness and contentedness	Healthy middle-aged
Cocoa	(Massee et al., 2015)	Cocoa flavanols	Acute/ chronic	Tablet Wellness Pty. Ltd.	3038 mg of *T*. cacao	250 mg	Improved self-reported mental fatigue and performance on the serial sevens task	Young adults
Cocoa	(Brickman et al., 2014)	Cocoa flavanols and (-)-epicatechin	Chronic	chocolate packet	2 12 g packets	900 mg of cocoa flavanols & 138mg of (-)-epicatechin	Enhanced reaction time of the ModBent task	Healthy elderly

Type of food	*Study*	*Type of phenolics*	*Design*	*Experimental treatment*	*Quantity*	*TPC dose*	*Significant effect*	*Participant age*
Cocoa	Scholey et al., 2010	Flavan-3-ols	Acute	Chocolate drink from Mars® dry cocoa blind drink	NA	520 mg and 994 mg	Better performance on serial three tasks after both doses, with more correct responses at all time points after the 520 mg dose	Young adults
Cocoa	Field et al., 2011	Flavan-3-ols	Acute	Commercial chocolate bars	35 g of commercial chocolate	773 mg	Better spatial working memory and choice reaction time	Young adults
Cocoa	Francis et al., 2006	Flavan-3-ols	Chronic	Mars® chocolate drinks		172 mg	No effect	Young adults
Orange	Alharbi et al., 2016	Hesperidin and narirutin	Acute	Flavonoid-rich orange juice Tropicana®	NA	272.14 mg	Better performance in executive function tests and psychomotor speed	Healthy middle-aged males
Coffee	Cropley et al., 2012	Chlorogenic acid	Acute	Special blind of green and roasted coffee drink	6 g of enriched instant coffee with CGA	520.89 mg	Enhanced alertness (Bond-Lader VASs)	Healthy elderly

Type of food	*Study*	*Type of phenolics*	*Design*	*Experimental treatment*	*Quantity*	*TPC dose*	*Significant effect*	*Participant age*
Green coffee blend	(Camfield et al., 2013)	Chlorogenic acid	Acute	NESCAFE Green Blend coffee	6 g of green coffee blend	530 mg	Improved sustained attention in N-back task and decision time in 2-choice reaction time task	Healthy elderly
Green tea Matcha	(Dietz et al., 2017)	Epigallocatechin gallate	Acute	Aiya Europe GmbH in two formats: tea and bars	4 g of matcha powder	280 mg EGCG	Small effects on basic attention abilities and psychomotor speed response	Healthy elderly
Apple	Bondonno et al., 2014	Quercetin glycoside and (-) epicatechin	Acute	Flesh and skin of Pink Lady® juice	80 g of skin and 120 g of apple flesh	364 mg	No effects	Healthy elderly
Flavonoid supplement	Pipingas et al., 2008	Flavonoids	Chronic	Enzogenol® capsules	4 capsules/ day	960 mg	Significant improvements in the speed of spatial working memory and word recognition	Healthy elderly

Type of food	*Study*	*Type of phenolics*	*Design*	*Experimental treatment*	*Quantity*	*TPC dose*	*Significant effect*	*Participant age*
Soy supplement	Thorp et al., 2009	Isoflavones	Chronic	Life® capsules	2 capsules/ day	116 mg	Significant improvements on the Novel Spatial Working Memory task following isoflavones. No significant effects for other tests	Healthy elderly
Green oat supplement	(Kennedy et al., 2020)	Unknown phenolic content	Acute/ Chronic	Cognitaven® capsules	1 capsule/ day	430 mg 860 mg 1290 mg	Improved performance on Corsi Blocks working memory task, the verbal serial substructions and computerised tracking.	Healthy elderly
Resveratrol supplement	Kennedy et al., 2010b	Resveratrol – a phytoalexin polyphenol	Acute	(Biotivia Bioceuticals) capsules	2 capsules/ visit	250 or 500 mg	No significant effects	Young adults
Flavonoid supplement	Ryan et al., 2008	Flavonoid	Chronic	Pycnogenol® capsules	2 capsules/ day	150 mg	Faster spatial working memory and faster word recognition	Healthy elderly

Type of food	*Study*	*Type of phenolics*	*Design*	*Experimental treatment*	*Quantity*	*TPC dose*	*Significant effect*	*Participant age*
Greek Mountain Tea	(Wightman et al., 2018)	Hydroxycinnamic acid and flavonoids	Acute/chronic	Sideritis scardica capsules	1 capsule/ day	475 mg 950 mg	Improved performance in choice reaction time and rapid visual information processing tasks	Healthy elderly
Epigallocatechin gallate supplement	(Wightman et al., 2012)	EGCG	Acute	Capsules	2 capsules/ visit	135 mg 270 mg	No effect	Young adults

1.9.1 Cocoa

The results regarding the effect of cocoa on cognitive augmentations have been both inconsistent and contradictory between peer-reviewed interventions. A significant increase in cognitive performance and reduction in mental fatigue were observed after supplementation of a drink containing both 520 and 994 mg of cocoa flavanols in comparison to the matched control of healthy adults in an acute randomised, controlled, double-blinded, balanced, crossover study. Furthermore, a significant enhancement was observed in sustained attention, with decreased reaction times in tasks that required rapid information processing after supplementation with 994 mg treatments. However, this treatment increase the likelihood of errors in the serial sevens subtractions task. In addition, participants self-reported a higher level of mental fatigue post-consumption of the 520 mg treatment (Scholey et al., 2010). Interestingly, an 'inverted U-shaped curve of effect was observed, with results most prominent post-supplementation of the lower flavanol quantity, possibly due to the other dose being too high.

Administering cocoa in the form of tablets instead of chocolate bars or drinks eliminated participants' expectations and excluded the effect of any other potentially active ingredients such as fat and sugar in the study by Massee et al. (2015). The cocoa flavanols dose in Massee et al. (2015) was 250 mg or 0 mg for the placebo and the study followed a randomised placebo-controlled, double-blind study design of forty healthy volunteers, investigating the effects of both acute and sub-chronic (once/day for four weeks) consumption of the study treatments. There was a significant improving effect of the acute administration of the cocoa flavanols on self-reported mental fatigue and performance in serial seven tasks in the first cycle of the cognitive demand battery (CDB) compared to placebo. In contrast, Pase et al. (2013) utilised three doses, 500 mg, 250 mg and 0 mg of cocoa flavanols in a randomised placebo-controlled, double-blind, parallel-group study of seventy-two healthy middle-aged volunteers examining the effects of both acute and sub-chronic (once/day for 30 days) treatment, showing no significant effect on cognitive function. However, the high dose of cocoa flavanols

(CF) after 30 days significantly increased self-rated mood factors of both calmness and contentment relative to the placebo.

Francis et al., (2006) observed the effects of daily supplementation of a flavan-3-ol-rich cocoa treatment (172 mg) against a matched control (13 mg) over five days in healthy young females, demonstrating significant attenuation of blood oxygenation in active brain regions measured by fMRI BOLD signal intensity after cocoa consumption in comparison to the control. However, no significant differences were observed on the fifth day during the letter-pair switching task which was conducted 90 minutes post-consumption, indicating no variance in attention switching performance between the treatment and control at this point. Brickman et al. (2014) conducted a controlled, randomised parallel-group study to investigate the effect of cocoa flavanol and aerobic exercise on cognitive and neuroimaging measures of regions of the hippocampal (dentate gyrus) functions in thirty-seven healthy elderly adults. The high-flavanol intervention of 900 mg of cocoa flavanols and 138 mg of (-)-epicatechin per day for three months significantly enhanced dentate gyrus function during the completion of cognitive tasks. Also, the high-flavanol treatment significantly enhanced the reaction time of the ModBent task independent of exercise. However, the number of participants (eight) in the high flavanols and exercise arm was relatively small.

Significant enhancements in visual contrast sensitivity and motion direction recognition were observed 90-minutes post-supplementation of 720 mg cocoa flavan-3-ols and 38 mg caffeine from dark chocolate compared to white chocolate (Field et al., 2011). This was evidenced by the enhanced visual-spatial memory task following acute supplementation of dark chocolate. However, the placebo did not match the chocolate treatment which may have introduced bias. By comparison, the six-week supplementation of a similar daily dose of 754 mg of cocoa proanthocyanins in 101 healthy adults had no significant effect on cognitive paradigms (Crews et al., 2008).

Camfield et al. (2012) measured the cognitive outcomes after chronic supplementation of cocoa in adults aged forty to sixty-five years. The participants consumed daily doses of cocoa: low (~0 mg), medium (~250 mg) or high (~500 mg) flavan-3-ol levels for 30 days. At both baseline and after 30 days of consumption, the spatial working memory

task was completed during which the participants' Steady State Visually Evoked Potentials (SSVEPs) were documented using electrophysiological brain imaging (Steady State probe Topography, SST) (Silberstein, 1990). Post-consumption of the medium flavan-3-ol dose significantly reduced the posterior-parietal SSVEP amplitude compared with the control, whereas the latency, which indicates the time before the effect starts in seconds, decreased in the same region after consuming both the medium and high doses, which could be attributed to enhanced neural processing speeds. This study suggested that chronic supplementation of cocoa flavanols for 30 days may enhance spatial working memory, as evident by the SSVEP results, even when no significant effects were observed regarding working memory. The authors concluded that these improvements were due to improved vascular function and antioxidant processes within the brain associated with cocoa flavonols consumption. The absence of outcomes on cognitive performance but the reduction in latency was proposed as participants having the ability to accomplish a similar level of performance while employing a lower sum of neuronal activation, and therefore an enhanced neuronal efficacy.

One reason for the absence of a conclusive outcome post-supplementation of cocoa has been proposed as being due to participants performing adjacent to the ceiling of benefits, and this results in the improbability of outcomes being detected. Additionally, the neuropsychological tasks employed within these studies were not sensitive enough to identify cognitive alterations, or that any effect was too small to make any meaningful difference. Scholey et al. (2010) reported enhancements in healthy, young adults on performance and fatigue post-supplementation of 520 and 994 mg flavan-3-ols in comparison to the matched control (46 mg). These enhancements were observed 90-mins post-consumption during an intense 60-minute cognitive demand battery, suggesting that the results may only be observed through prolonged cognitive demand.

Separately, while studies control flavonoid intake in the presented interventions, methylxanthines like caffeine (Smit et al., 2004) are often not matched between

groups. Therefore, this could have an influence and result in contradictory outcomes in the literature.

1.9.2 Berries

A small number of studies have observed human physiological processes which could influence human cognition post-consumption of berries. For example, significant positive outcomes on verbal learning, spatial memory and delayed verbal recall were observed after consumption of 532 ml of concord grape juice per day for 12-weeks. Krikorian et al. (2010a) employed a participant group of twelve adults who were 78.2 ± 5 years old, all of whom showed age-related memory deterioration. However, neither the phytochemical composition of the experimental treatments nor the placebo were specified. Another study reported a significant increase this time in accuracy on the Californian verbal learning task after daily consumption of blueberry juice containing an average of 1.26 g of polyphenols per day for 12 weeks compared to the baseline measurements (Krikorian et al., 2010b). This study also used adults with age-related memory deterioration, involving a group of nine adults with an age of 76.2 ± 5.2 years. However, the small sample size utilised and the variation between age groups may have affected the validity of the results. Furthermore, the study was an add-on to the previous grape study and therefore employed the same placebo arm which resulted in an absence of a suitable control and most importantly, affected the blinding of the study design, therefore the results should be interpreted with caution.

However, the same author published another intervention study involving the consumption of grape juice which was determined by body weight in the range of 6.3 – 7.8 mL/kg, this equated on average to 209 mg of polyphenols (96.2 mg anthocyanins, 60.61 mg phenolic acids, 20.9 mg procyanidins) per day and was examined against a placebo which was matched for sugar. In Krikorian et al. (2012), a total of twenty-one healthy adults aged 68 years or older with mild memory deterioration, which was associated with the natural ageing process, were included in the 16-week intervention. There was a significant decline in the interference through

the recognition memory task post-consumption of the grape treatment, which displays a greater ability in distinguishing material from decoy stimuli.

Separate studies using shorter interventions have been conducted. A six-week study reported no significant effects on outcomes on various memory and central executive tasks. No information was provided on phytochemical composition but the study employed a double-blind, placebo-controlled design, requiring participants to consume two 8 oz (226.8 g) cranberry treatments per day, each containing 27% cranberry juice. The participants were older adults between 50-60 years with no cognitive decline (Crews Jr et al., 2005).

Interventions which examine the effects of berry consumption on healthy, young volunteers are lacking. A study observed the acute consumption of grape juice on implicit memory or mood but presented no significant outcomes (Hendrickson and Mattes, 2008). However, the authors suggested that the supplementation alongside the ingestion of lunch could have obscured the outcomes due to alterations in pharmacodynamics, and therefore influences the active compounds in the treatments. Furthermore, the method utilised implicit memory tasks, therefore it is problematic to draw conclusions and appraisals against alternative studies which often employ explicit memory tasks. Explicit memory is defined as a process memory, where information needs effort to be remembered consciously, while implicit memory is a memory process information that needs no effort to be remembered unconsciously. Alternatively, it has been demonstrated in more relevant studies on healthy elderly populations, which have shown significant effects of blueberries treatments on working memory (Bowtell et al., 2017) and word recognition delay (Whyte et al., 2018).

An acute intervention encompassing a dose of 368–968 mg of polyphenols based on 524 ± 5 mg per 60 kg of bodyweight using an anthocyanin-enriched blackcurrant drink (DelCyan™) was conducted by (Watson et al., 2015). This study used thirty-six young, healthy adults aged between 18-35 years and employed a control in the methodology of a double-blind design. The results presented statistically significant improvements in the accuracy of the rapid visual information processing task post-consumption of the blackcurrant treatment. In another acute intervention by Watson et al. (2019), a drink

containing 500 mg of anthocyanin significantly improved the speed of responses of nine young healthy volunteers during the choice reaction time. However, the number of participants in this study was relatively small.

Dodd et al. (2019) demonstrated that the consumption of blueberry extract (578.82 mg of antho-and pro-cyanidin/30.1 g dose of blueberry powder) in comparison to control has no statistically significant difference in the composite measure of global cognitive function following the blueberry drink. However, the analysis of the individual cognitive tasks exhibited a statistically significant improvement in many of the global cognitive function tasks, such as immediate word recognition and the digital switch tasks, post-consumption of the control in comparison to the blueberry extract. It is important to note that this data was obtained from eighteen healthy adults aged 68.72 ± 3.30 years.

1.10 General reported and purported therapeutic benefits of dates

In addition to its uses as a principal food source (see section 1.4), ethnobotanical research has reported that dates are used to treat liver disorders (Edeoga et al., 2005), diabetes (Ziyyat et al., 1997), constipation, and diarrhoea (Maatalah et al., 2012), and are considered an aphrodisiac (Zaid and De Wet, 2002). Additionally, date fruit has been applied to ease asthma (Zaid and De Wet, 2002), reduce skin wrinkling (Proksch et al., 2014), for coughs, bronchitis, and respiratory disorders, as well as to relieve headaches, aid sexual debility and positively influence immunity (Zaid and De Wet, 2002). With a large number of applications and list of uses, dates have also shown the following capabilities of raw dates, date products and/or constituents from dates: antioxidant, antimutagenic (Vayalil, 2002), antihaemolytic (Periyathambi et al., 2019), antiviral (Jassim and Naji, 2010), antifungal (Shraideh and Khaled), anti-inflammatory (Taleb et al., 2016), antihyperlipidemic (Baliga et al., 2011) hepato-protective (Al-Qarawi et al., 2004) nephroprotective (Al-Qarawi et al., 2008) gastroprotective (Al-Qarawi et al., 2005), anticancer (Ishurd and Kennedy, 2005), and as an immunostimulant (Puri et al., 2000). Dates contain a range of phytochemicals which have been proposed to be involved in these effects, such as phenolics (notably p-hydroxy benzoic acid, protocatechuic acid, gallic acid, vanillic acid, syringic acid),

phenylpropanoids (prominently cinnamic acid, caffeic acid, o-caffeoyl shikimic acid, ferulic acid, sinapic acid, o-coumaric acid, p-coumaric acid) (Mansouri et al., 2005), carotenoids (β-carotene, lutein), sterols (including cholesterol, campesterol, stigmasterol, β- sitosterol, isofucosterol) (Kikuchi and Miki, 1978), flavonoids and their glycosides (such as catechin, epi-catechin, quercetin, luteolin, apigenin) (Hong et al., 2006b), procyaninidins (Hong et al., 2006a), and anthocyanins (Al-Farsi et al., 2005).

Dates have also been used to manage disorders such as psychosis, anxiety, cognitive dysfunction, and the nervous system (Shanmugapriya and Patwardhan, 2012). Furthermore, it is associated with treatments to aid sciatica, headache, and hemicranias, or as an external treatment for inflammatory illnesses such as an abscess, boil or ulcer (Shanmugapriya and Patwardhan, 2012). Dates have also been used within Chinese and Japanese herbal preparations for sleep disorders (Sheikh et al., 2016). In addition, while considering acute toxicity in the date fruit extract, results have displayed a prolonged period of sleep in test animals (Fakhri et al., 2018)

Although there is a growing interest in investigating the therapeutic uses of dates as shown in section 1.3, and although most published articles have referred to the phenolic content in dates as the main cause of such effects, many questions have been raised considering the phenolics' contribution to any of the reported effects. For example, for those studies not using standard dietary doses of dates as food, what is the evidence that their results are relevant for actual date consumption regarding both composition and amount? Additionally, what does this frequently repeated word "significant" in most of the published research mean in this context? Would it be a significant contribution to the daily intake for people with a normal date-rich diet? Or significantly different from being undetectable when measured using sensitive chemical analysis? Reflecting on the phenolic content of date fruit, a review by Rahmani et al. (2014) considered date fruit as a good antioxidant source with notable carotenoid and phenolic content compared to other fruits and vegetables regarded as functional foods, e.g. grapes and carrots (Hasler and Brown, 2009). In addition to the difficulty of making comparisons between different types of polyphenol-rich foods as explained in 1.6, the quantification of the phenolic compounds of the used varieties of

both date fruits and seeds was conducted using different methodologies as detailed in chapter 2, section: 2.7.2.1 and in chapter 4, section: 4.6.6.

1.11 Date fruit's potential properties as a cognitive performance enhancer

1.11.1 Phenolic related effects

While the therapeutic attributes of date fruit have been appraised comprehensively over the last decade, the physical and psychological aspects of the brain and possible associated effects have not been extensively considered. The antioxidant effects of date fruit on the brain have been reviewed in a systematic review to demonstrate the advantages (Nurlaily et al., 2016), and were considered as a contributing benefit to the brain, referred to as the neuroprotective or cerebroprotective effect. Commonly, this shields the brain from the damaging action of reactive oxygen species (ROS) generated either endogenously from cell metabolism or the interaction with an exogenous source like xenobiotic compounds such as pesticides, chemicals and environmental pollutants (Nurlaily et al., 2016). The neuroprotective effects promote a comparable concept, alongside a further extension, to the formerly identified antioxidant effect. In contrast, demonstrating this association between antioxidant and neuroprotection was unsuccessful in much research. Therefore, this repetitive attribution may occur due to speculation rather than demonstrated fact by research. However, there are multiple ways that a phytochemical can provide a neuroprotective effect. Antioxidant activity is usually a relatively unlikely mechanism for several reasons, for example, it would require an unreasonably high constant concentration at the site of action (Vauzour, 2012).

Most investigations have examined the physical effects on animal brains, and this might impact the psychological effects of date fruit consumption. Many researchers interpret the results as an indication of antioxidant effects (Pujari et al., 2011). Study theories have focused on the restriction of blood flow to the brain through the constraint of bilateral common carotid arteries (BCCAO) or middle cerebral artery occlusion (MCAO) to produce cerebral ischaemia, an insufficient quantity of oxygen due to the obstruction of these arteries which causes damage, this is provoked by

ROS activity on the brain in rats. Subsequently, the obstruction is removed but this causes the possibility of neuronal death due to reactions between cellular macromolecules and ROS (Pujari et al., 2011). However, others argue that this does not necessarily prove that antioxidant activity is the underpinning mechanism of action in which phenolics play a role in neuroprotection. Any mechanism that reduces cell damage during ischaemia may show this effect whether it affects ROS directly or not (Sanderson et al., 2013).

Most published articles which provide links between date administration and mammalian brains, to the best of our knowledge, are summarised in only one systematic review about date administration to animal models of neurological diseases (Nurlaily et al., 2016), a review about the benefits of date fruit to the brain (Ismail and Radzi, 2013) and a further review about the beneficial effects of dates on neurodegenerative diseases (Essa et al., 2016). The research available centred around the brain is limited, so it is imperative to highlight any cognitive benefits which can be measured by tests such as motor coordination, locomotor activity and response latency. Currently, the only available data is about the neuroprotective effect, and this was important to consider when seeking literature for the rationale of the current study to understand that even the most relevant study of cognitive benefits has limitations.

Most studies centring around dates and mammalian cognition concentrate on the deceleration or reversal of the natural cognitive decline in controlled animal experiments using ischaemic stroke models (Kalantaripour et al., 2012a, Majid et al., 2008a, Pujari et al., 2011, Pujari et al., 2014, Pujari et al., 2013), in Alzheimer's models (Subash et al., 2015, Subash et al., 2014, Essa et al., 2015) and on a substance-induced brain damage model (Agbon et al., 2014, Joseph et al., 2014). Both anti-inflammatory and antioxidant responses, as well as improvements in neural signalling, have been considered as mechanisms to improve memory in animal models.

The biomarkers tested in these studies to demonstrate the postulated mechanism of actions are a significant area of debate and require careful interpretation. The two studies by Agbon et al. (2014) and Joseph et al. (2014) examined the effects of aqueous date extracts on an animal model of brain damage using histological

observations to assess the neuronal changes and damages. This was after a short chronic trial where the rats were fed diets containing different doses of dates once for eight days. Lead acetate was utilised in the first study (Joseph et al., 2014) to provoke neuronal degeneration, and 350 mg/kg/ of body weight of date extract vs control was implemented. By contrast, artesunate, an anti-malarial drug, was employed in the second study to induce cerebellar damage in Wistar rats. In this second study, three groups of rats (male and female) consumed a date diet: 500, 1000 and 1500 mg/kg. After histological observation evaluating the neuronal changes and damages caused, date fruit was associated with the decreased neuronal disruption in the occipital region of the rats with lead acetate-induced brain damage (Joseph et al., 2014). There was less cellular hypertrophy and perineural spaces observed in the brain damage-induced rats which were supplemented with dates in comparison to the non-treated group (Joseph et al., 2014). Furthermore, the simultaneous treatment of dates in rats supplemented with artesunate (to induce degenerative changes due to its toxicity) showed less neuronal damage compared to the control (Agbon et al., 2014). Additionally, amplified quantities of endogenous antioxidants were observed in the brains of rats which were fed with three different doses of date extracted in methanol (Pujari et al., 2011, 2013, 2014). The aforementioned studies define a neuroprotective effect of date extract and promotes a prospect for this as a therapeutic agent for treating brain ischaemia and further neurodegenerative diseases. A decrease in the malondialdehyde (MDA) levels, which is a marker of oxidative stress, was observed with the date methanolic extract treatment. Furthermore, there was an enhancement in the antioxidant status due to an elevation in the content of low molecular weight antioxidants. After treatment with date fruit extract, the neuronal damage in rats with induced ischaemic stroke was significantly reduced. However, no information about whether the effect was dose dependent or not was included in the study results.

The decrease in animal brain damage has been linked to systemic antioxidant enzymes and free radical scavengers. Enhanced levels of a group of small molecule antioxidants including catalase (CAT), reduced glutathione (GSH) and glutathione reductase (GR) but not glutathione peroxidase (GPx) and glutathione-S-transferase (GST) in rats treated with methanolic extract of date was found in studies by Pujari et

al. (2011), (2013, 2014). Results from the three studies indicate that the reduction in oxidative stress levels and improvements in antioxidant status in these rats with induced ischaemic stroke was due to the phenolic compounds of the date extracts (Nasir et al., 2015); however, the design of these three studies did not elucidate such attribution.

Other biomarkers measured after supplementation with date-containing diets showed a reduced Aβ1 protein level in both the plasma (Subash et al., 2015) and the brain tissue of the transgenic mice model of AD (Essa et al., 2015). In addition, the quantity of Aβ1–40 and Aβ1–42 proteins in the cortex and hippocampus were significantly reduced in the group of transgenic mice model of Alzheimer's disease supplemented with the 4% date diet compared to the control (Essa et al., 2015). The reduction in beta-amyloid proteins means reducing plaques around the brain cells and tangles within brain cells, improving neuron functions and possibly providing protection against Alzheimer's disease. The transgenic AD mice exhibited an elevated abundance of IL-1β, TNF-α, and IL-6 within the brain. However, these brain inflammatory markers were significantly diminished in the 4% date diet group compared to transgenic mice consuming the control diet (Essa et al., 2015).

Oxidative stress was diminished as measured by reduced levels of lipid peroxidation (LPO) and protein carbonyl in the brain regions of transgenic mice after date diet consumption (both 2% and 4%) (Subash et al., 2014). After supplementation, the action of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione, and glutathione reductase significantly increased in comparison to the control (Subash et al., 2014). However, since neither of these studies (Nasir et al., 2015, Subash et al., 2014) included wild-type control mice (not transgenic), the observed effect of phenolics might have been specific to this strain of transgenic mice.

1.11.2 Glucose related effects

Dates are considered a good energy source due to their high carbohydrate content. The primary source of carbohydrates is sugar, such as glucose, fructose, and sucrose. The average of each type of sugar of ten different fresh date varieties has been estimated by Al-Farsi et al. (2007) to be 19.4 g of glucose, 22.8 g of fructose and 4.03 g of sucrose /100 g of dates. At the same time, the average of each type of sugar of nineteen different dry date varieties has been estimated to be 29.4 g of glucose, 30.4 g of fructose and 11.6 g of sucrose (Al-Farsi et al., 2007). The high sugar content in dates was discussed in the literature as an energy source (Siddiqi et al., 2020) from a glycaemic response (Miller et al., 2003), and is of concern to people with diabetes (Chaudhary and Pankaj, 2018). However, the effect of the sugar content, primarily glucose and cognitive functions, was not discussed in the literature. Since glucose contributes toward glycemia acutely more than fructose (Bantle, 2006), and since the sucrose content in dates is low, the impact of glucose in enhancing cognitive function is discussed in this section.

Human studies have shown that the performance of difficult tasks requiring intensive cognitive resources results in a measurable decline in peripheral blood glucose (BG) concentration, which is suggested to be due to increased neural energy expenditure (Donohoe and Benton, 1999, Scholey et al., 2006). In animals, it has been shown that at a high cognitive load, hippocampal glucose demand exceeds supply, whereas exogenous glucose supply enhances performance (McNay et al., 2000). This is also supported by several human studies that have shown that acute glucose consumption compared to placebo or breakfast omission enhances cognitive performance both in healthy participants and in participants with memory deficits and those with poor glucose regulation (Korol and Gold, 1998, Smith et al., 1994). The optimal glucose dose for enhancing verbal episodic memory relative to placebo in elderly participants was found to be 25 g (Parsons and Gold, 1992), whereas in healthy young women the optimal glucose dose for mood and cognitive performance was found to be 18 g of glucose (Messier et al., 1998). Owen et al. (2012) in a double-blind, placebo-controlled, balanced, study design with six crossover experimental conditions

investigated the effect of two doses of glucose (25 and 60 g) with two different durations of fasting on a total of thirty young healthy volunteers. The six experimental conditions were as follows: (a) 2-h fast and 0 g of glucose, (b) 2-h fast and 25 g of glucose, (c) 2-h fast and 60 g of glucose, (d) 12-h fast and 0 g of glucose, (e) 12-h fast and 25 g of glucose, and (f) 12-h fast and 60 g of glucose. There was a significant enhancing effect on the high demanding working memory tasks such as serial seven tasks, which were conducted following the 2-h fast and 25 g of glucose condition. In comparison, there was no significant enhancing effect on the less demanding working memory task: serial three tasks were conducted following the 12-h fast and 60 g of glucose condition compared to placebo (Owen et al., 2012).

It is worth noting that the glucose-enhancing effect on memory is more consistent in healthy elderly participants (Manning et al., 1997) and patients with Alzheimer's disease (Manning et al., 1993). In the latter group, glucose reliably facilitates memory when the cognitive demand of the task is high or under conditions of divided attention (Smith et al., 2011). In addition, it is now well established that poor glucose regulation is a risk factor for impaired CF, as shown in patients with diabetes mellitus and those with poor glucose regulation (Rebelos et al., 2021). The effect of postprandial glycaemia on cognitive measure outcomes has been inconsistent, as demonstrated by a systematic review of glycaemic manipulation and cognitive function (Hoyland et al., 2008). Moreover, the shape of the glycaemic response to cognition has been inconsistent. A rapid decline in glycaemia may affect cognition as a better result for memory was found following a low compared to a high glycaemic index GI test beverage during a rapid glycaemic decline in people with good glucose tolerance (Young and Benton, 2015). However, Benton et al. (2003) found no difference in memory following a period of more rapid decline (30–90 min) in glycaemia between a low- and high-GI test. Also, Marchand et al. (2020) found no difference in the cognitive function outcomes following the consumption of two trifles made with sugars of different GI tested in a total of sixty-five young, healthy volunteers in a double-blind, placebo-controlled, crossover study design. The absence of a glucose effect on cognitive performance was regardless of the difference in postprandial glycaemia

observed between the two trifles made with sugars of different GI (high GI = 65 and low GI = 34).

Given the high sugar content of dates and despite the inconsistency regarding the effect of the GI on cognitive function, a consideration of the GI of the date-containing treatments in this thesis was taken into account when creating and developing the treatments.

1.12 Date seeds' potential properties as a cognitive performance enhancer

Date seeds are a major by-product in the date fruit industry, generated at various post-harvest processing levels including sorting (according to size and colour) and quality control (Najjar et al., 2020). At this stage, lower quality dates are usually separated and used to manufacture date syrup or date paste and the seeds are discarded, while at a later stage, some high-quality dates are also deseeded before packaging, which also generates date seeds as a by-product (Oladzad et al., 2021). Date seeds have been extensively studied for their nutritional and functional properties in recent times. Valorisation and proper recycling of date seeds can benefit farmers by adding value to an otherwise discarded waste product. Additionally, as date seeds are rich in various bioactive compounds and dietary fibres, they can provide the food and pharmaceutical industries with a sustainable and low-cost source of functional ingredients (Najjar et al., 2020). The major class of bioactive compounds in date seeds, in addition to dietary fibre, are polyphenols (Warnasih et al., 2020). Several advances have been made in incorporating date seed powders into food products (Najjar et al., 2020). Date seed powder in roasted or unroasted form is commercially produced for niche markets as a caffeine-free 'date seed drink'(Ghnimi et al., 2015b).

There are no assessments of the effect of date seed extracts on human cognitive function but there is some evidence from animal models. An extract made from extracting ground raw date seeds in water has been tested for antioxidant and free radical scavenger effects in animals. Habib and Ibrahim (2011) investigated the antioxidant effect of date seeds in vivo by feeding Wistar rats with a diet containing 70 or 140 g/kg date seeds. The date seeds significantly reduced malondialdehyde MDA

(lipid peroxidation product) in both tissues. In vivo lipid peroxidation can be influenced by several factors, including the type of dietary fat (saturated or unsaturated), as well as exogenous and endogenous antioxidants. Although the mechanisms resulting in reduced lipid peroxidation are not clear, it is conceivable that the antioxidants found in date seeds, especially phenolics and flavonoids, are the primary contributors to the ameliorating effect of date seeds on lipid peroxidation.

Table 4 provides a comparison of date seed's phenolic content and other fruit which are considered rich in phenolic content. For the dose of date seeds in the diet of 70 g and 140 g/kg, the TPC and the TF were 1722 and 3444 mg GAE/100 g for TPC and 4457.18 and 8914.36 mg RE/100 g for TF respectively.

Sekeroglu et al. (2012) examined and compared date seed drinks to three other different herbal alternative drinks (tumble thistle, black cumin and carbo) consumed in Turkey. The date seed extract was the most active in the antioxidant assays and also the richest in terms of total phenolic content (Sekeroglu et al., 2012). Moreover, the inhibition of the activities of the enzymes acetylcholinesterase and butyrylcholinesterase and the antioxidant activities of the four extracts were assessed showing that the highest inhibition was caused by the date seed coffee. Acetylcholinesterase is a cholinergic enzyme primarily found at postsynaptic neuromuscular junctions, especially in muscles and nerves, whereas butyrylcholinesterase is a serine hydrolase related to acetylcholinesterase that catalyses the hydrolysis of choline esters, including acetylcholine**,** widely distributed in the nervous system. These compounds are also used in the treatment of Alzheimer's disease based on the hypothesis that increasing the availability of acetylcholine (a neurotransmitter) at acetylcholine receptors in the brain will result in improvements in neuron communications and eventually will enhance cognitive functions (Pohanka, 2014). The ferric reducing antioxidant power (FRAP) and phosphomolybdenum-reducing antioxidant power (PRAP) tests were used to estimate the absorbance indicative of antioxidant power. Also, the radical scavenging effect was determined among the five extracts using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. The percentage of DPPH radical scavenging activities of date

seed extract was 93.80± 0.09 at 800 µg/ml, which is equivalent to the DPPH radical scavenging activities of vitamin C of 92.70 ± 0.17 as estimated in (Olugbami et al., 2015). Therefore, the high antioxidant activity of the date seed extract could be attributed to the rich phenol content, which has been quantified to be 7630 ± 4.59 mg GAE/100 g (Sekeroglu et al., 2012).

Another linkage between the antioxidant and anti-inflammatory capacities of date seeds due to their rich phenolic content is the ability to enhance the body's defence and provide protection against metal toxicity, such as mercury (Abdel-Salam et al., 2018). The toxic effect of mercury on animals has revealed some alterations to neural architecture and integrity, which leads to decreases in cognitive functioning and reproductivity (Abbott, 2017). Aqueous date seeds, in comparison to coriander, dandelion and a mixture of all of them, showed the highest TPC of 6375 mg of GAE/100 g and the highest antioxidant capacity of 3009.1 µmol Trolox equivalent/dL (Abdel-Salam et al., 2018). Histological examination showed that among rats exposed to mercury, those treated with date seeds had the most significant number of totally recovered neurons. Yin et al. (2011) suggested that this attenuation of deterioration may be attributed to decreased oxidative stress.

Kalantaripour et al. (2012) investigated the cerebroprotective effects of aqueous date seed extract on rats. All rats were healthy, however, the experimental group suffered 30 minutes of MCAO, and were treated with the date seeds dose after 30 minutes, followed by 48 hours of reperfusion. The administration of a dose of 80 mg/kg significantly decreased the neural damage (30.33%) and decreased the MDA level (which is one of the final products of polyunsaturated fatty acids peroxidation in the cells and considered an oxidative marker) in comparison to the control group. A significant increase was observed in the antioxidant enzyme activity (SOD, an important antioxidant defence against oxidative stress in the body) and antioxidant levels. Although the mechanistic insight by which date seed induces a cerebroprotective effect remains unknown, it has been attributed to the phenolic compounds in the date seed. However, no information was available about the phenolic content of the administered date seed treatment (Kalantaripour et al., 2012a).

Furthermore, an acute (150 mg/kg single dose) and subacute (150 mg/kg/day) for 7 days study was performed to evaluate the neuropharmacological effects of roasted date seeds drink against a regular commercial coffee extract as a control. The neuropharmacological activity was evaluated using the open field test (locomotive activity) and phenobarbital sodium (a popular hypnotic and sedative drug) induced sleeping time test (Farag et al., 2019). The results showed that the group of animals which were pre-treated with date seeds drink DSD had a statistically significant reduction in their sleeping time and prolonged duration of alertness compared to both the control group pre-treated with phenobarbital sodium and the group pre-treated with commercial DSD (Farag et al., 2019). Hence, DSD had an alertness effect on the experimental animal. However, pre-treatment with DSD and the commercial DSD showed no significant stimulation of the locomotive activity (which reflects alertness and wakefulness of mental activity) compared to the group pre-treated with RC, possibly due to the absence of caffeine in DSD (Farag et al., 2019).

Saleh et al. (2020) examined the dual prophylactic/therapeutic potential of a nutraceutical formula based on an aqueous extract of roasted date seeds, nigella and virgin-olive oils against experimentally induced Alzheimer's disease in rats. Alzheimer's disease-like pathology was induced in male Wistar rats using oral $CuSO_4$ (200 mg/kg/day for two months). It is important to mention that the main pathological indications of Alzheimer's disease are the accumulation of formatted extracellular amyloid-β plaques and intracellular neurofibrillary tangle. The accumulation of amyloid-β aggregates in the brain leads to oxidative stress and inflammation (Syarifah-Noratiqah et al., 2018). In the study by Saleh et al. (2020), the nutraceutical formula was given orally to experimental animals (10 mL/kg/d) for 14 days before (as prophylaxis) and after Alzheimer's disease induction, and its therapeutic effect in both cases was tested in comparison to the anti-dementia drug donepezil (0.5 mg/kg/d). The nutraceutical formula ameliorated the $CuSO_4$-induced neuronal damage and regenerated the affected hippocampus tissue, significantly improving learning ability. The formula was also effective in decreasing brain amyloid-β, tau protein, TNF-α, and iNOS levels in the hippocampus, oxidative stress, and inhibiting acetylcholinesterase activity and expression in the brain and hippocampus, respectively. Furthermore, an

increase in GSH levels, activities of superoxide glutathione, and glutamines-S-transferase and levels of hippocampus a disintegrin and metalloprotease 17 (ADAM 17), and brain phospholipids was observed. The used roasted date seed was prepared by washing with distilled water, sun dried and then roasted in an oven at 100°C for 5 h. The dried pits were ground into a fine powder and an aqueous extract (200 g/L) was prepared. No quantification of TPC was provided.

Although the aforementioned study examined the effect of date seeds in combination with nigella and olive oil, it included several other measures to illustrate the underpinning mechanism of action. Taking into account that the psychoactive ingredient postulated to have an enhancing effect in treating or mitigating the effect of the induced AD in the treatment formula of date and olive oil was their high phenolic content.

1.13 General conclusion and summary of the objectives of the thesis

The existing knowledge about the effects of phytochemicals in date fruits and seeds on mood and cognitive functioning, and additionally the effects of alternative fruits which are high in flavonoids have been comprehensively considered in the preceding literature review. This has included some information that could go beyond the focus of this thesis but may display similar mechanisms of cognitive benefits to those in date fruit and date seeds.

There is a sufficient quantity of data which supports the hypothesis of modulation or reversal of cognitive decline after dietary supplementation with a food high in flavonoids in rodent models. It is important to distinguish between testing for the effects of isolated flavonoids or a food rich in flavonoids. Further data showing a beneficial effect in aged humans are also being published. The cognitive domains which have been observed to be sensitive to date fruit supplementation in animal models are motor coordination, locomotor activity, response latency, spatial memory, and alertness.

Furthermore, research provides observations of the behavioural effects post-supplementation of flavonoids in both animal models and cognitive functions of aged humans. Many flavonoids shown to have an effect are found in date fruit, even though in different concentrations. Therefore, it is possible that some date varieties with high phenolic contents could provide a physiological effect which modulates human cognitive functioning. Although the phenolic content of date fruit is considered “high” according to the authors of several publications, which has encouraged the conduction of this research, it is important to note that the researcher is aware that their classification may have overrated the phenolic content of dates as shown in

Table 4, and thus estimations for date fruit and seeds used in the research reported in this thesis are further clarified in chapters 2 and 4.

With the general research of the effect of foods rich in polyphenols considered, a useful point for research within this field would be to integrate the following standards: well-defined extracts, objective computer-based measures for cognitive functioning and a robust methodological design which incorporates paradigms that have been demonstrated as sensitive to comparable nutritional interventions in both animal models and human trials.

Considering the first criteria of well-defined extracts, the first treatments employed two extracts from date fruits in a standardised, freeze-dried and powdered form from the two most popular date fruits in KSA. These were original treatments which have been specifically developed. Next, the second treatments incorporated roasted date seeds. These treatments were, likewise, original and developed specifically. The developed treatments and placebos in each study were employed in human interventions. The total polyphenol doses and any additional psychoactive ingredients such as glucose or caffeine were quantified.

A software application, CogTrack, which was created for the flexible delivery of randomly generated parallel versions of standard and novel cognitive assessment tasks was employed to assess cognitive functioning. This has previously exhibited sensitivity, reliability and validity within an assortment of nutritional interventions,

including in an energy drink study (Wesnes et al., 2017b) and for polyphenols in blackcurrant (Watson et al., 2019). CogTrack is a platform which allows for the delivery of standardised cognitive tests assessing aspects of attention, information processing, as well as working and episodic memory. The selected nine tests are comparable to the outcome measures used in the standard battery of cognitive tests in the CDR system, including immediate/delayed word recall, word recognition, picture recognition, simple reaction time, digit vigilance, choice reaction time, numeric working memory and spatial working memory. Considering the similarity between the CogTrack and other cognitive batteries (as described in section 1.2) and the collaborations between CogTrak and Newcastle University, it was deemed suitable to utilise in both studies. The CogTrack is an online assessment tool that allows the researcher to test an unlimited number of participants on the same testing day, facilitating participation and providing another factor for the use of CogTrack. Moreover, the eight indices of core measures on CogTrack combine the same task measures as used in the CDR system to form the factor scores: the power of attention, continuity of attention, quality of memory and speed of memory etc. The calculations and the descriptions of the composite scores used within this thesis are described in detail in Chapter 3, section 3.3.4.11. It is important to mention that due to the absence of previously published research on the acute effect of either date fruit or date seeds on mood and cognitive performance, it was difficult to define a primary outcome in advance. Therefore, a more cautious approach was performed, analysing the data across multiple outcomes, in addition to assessing individual outcomes as done in most studies on this topic.

The use of paradigms which have shown sensitivity in animal models and humans post-consumption of flavonoids has enhanced the rigorous methodological design. This includes attention, psychomotor performance, mood, central executive tasks and a variety of features of memory. Multiple time points were utilised within the studies to ensure active elements were present in the peripheral circulatory system and to integrate robustness for the biphasic nature of absorption for flavonoids and phenolic acids.

It is important to mention that the design of the studies included within this thesis followed double-blind, placebo-controlled, crossover interventions as it was found that within-person comparisons were appraised as most appropriate due to the subjectiveness of such functions in comparison to the parallel design. It is widely known that using crossover design minimises the risk of confounding, as all interventions are evaluated on the same volunteer. Furthermore, the human interventions included in this thesis are not proposing to answer the entirety of the questions surrounding the consumption of date fruits and date seeds. Also, the acute study design and the young participants' cohort utilised in this thesis were chosen to be the starting point of such novel research; a younger population and acute study design were deemed faster within one PhD project.

Although chapter one highlighted the antioxidant capacity of both date fruit and date seeds, which makes the adoption of the chronic study design and elderly cohort sound more beneficial, considering the inconsistency in study designs, duration and sensitivity of specific populations mentioned in a systematic review of the effect of polyphenols on cognitive functions by Ammar et al. (2020), and the overview of the polyphenols and cognition in humans by Lamport and Williams (2020), it was decided to employ an acute design with young volunteers.

Furthermore, the studies did not anticipate exclusively providing the mechanism of actions underpinning any observed effects. The data quantified any acute effects but did not consider or assess any chronic or accumulative effects of supplementation. The studies do, however, provide an establishment for basic acute effects which can aid future research to investigate these in more advanced and long-term research.

The main objective of the two studies that make up this thesis was to examine the possible consequences of acute consumption of standardised date fruit and/or date seed extracts.

The principal aims were:

1. To assess the effect of a standardised acute date fruit treatment on the cognitive performance and mood of healthy adults.
2. To assess the effect of a standardised acute treatment, made as a drink with roasted date seeds, on the cognitive functioning and mood of healthy adults.

The objectives of the thesis were as follows:

1. To develop original date-containing treatments and a placebo to investigate the acute effects of date fruit supplementation in healthy adults.
2. To develop a date seed beverage and placebo to investigate the acute effects of date seed supplementation in healthy adults.
3. To recruit the desired number of participants for each human intervention, determined according to the power calculation.
4. To train and manage the participants to perform the tests.
5. To analyse and assess the test results.

Chapter 2. A Product Development Plan: Designing Three Treatments for the Investigation of the Acute Effect of Date Fruit Consumption on Mood and Cognitive Performance in Healthy Young Volunteers

2.1 Introduction

As discussed in chapter 1, established procedures for how to make a date-containing treatment and a matched placebo are not yet available, therefore, a framework was established to develop the human intervention trial. This framework incorporates different methodologies to develop these procedures according to established guidelines for the design, conduct and reporting of human intervention studies to evaluate the health benefits of foods (Welch et al., 2011). The postulated phenolic content in date fruit was quantified using validated methods, Folin-Ciocalteu and HPLC. The main reason for the use of two different methods is that the Folin-Ciocalteu (or spectrophotometric method) lacks specificity which may cause overestimations in most of the published articles quantifying the TPC of dates using such a method (Escarpa and González, 2001).

This framework included the following:

- Data from the literature regarding a well-known polyphenol-containing food (blackcurrant) with a reported significant acute enhancing effect on cognitive performance and/or mood was used to calculate the sample size required to detect a significant effect of the experimental product (date fruit) on healthy young volunteers.
- Formulation and standardisation of a date-containing experimental treatment, based on quantification of the polyphenols as postulated active compounds to match a negative placebo to achieve sufficient organoleptic consistency to attain a "double-blind placebo-controlled" study design.

2.2 Aim

To develop a recipe for a date-based product with quantified and justified levels of phytochemicals and similar sensory characteristics to a placebo.

2.3 Objectives

1. Select two date fruit varieties for the development of the treatments.
2. Quantify the TPC for product development.
3. Determine how to prepare and execute the study treatments.
4. Create recipes for date-containing treatments and a placebo.
5. Conduct a sensory test to assess the palatability and blinding of all created recipes.
6. Determine the glycaemic index and the glycaemic load of the date-containing treatments.
7. Set up a HACCP plan for treatment preparation.
8. Determine the sample size (power calculation).

2.4 The rationale of treatment recipe creation steps

At the commencement of the recipe creation stage, it was critically important to define the treatment characteristics of the study in respect of psychoactive ingredients for cognitive performance. The phenolic content (mainly phenolic acids in dates) (Mohamed et al., 2016, Farag et al., 2016) was hypothesised to be the potential psychoactive compounds which could have an acute enhancing effect on mood and cognitive function as previously mentioned in Chapter 1, section 1.11.1.

To fulfil the experimental aim and design, it was decided that two different date varieties with different TPC values would be investigated against an inert placebo which contained no constituents with postulated psychoactive properties (phenolic compounds).

The treatment recipes were formulated according to the quantification of the phenolic content obtained by the Folin-Ciocalteu method, then confirmed and identified by HPLC in parallel with the results from the sensory evaluation panels to ensure the flavour masking process.

Therefore, the development of the recipe included the following steps to compensate for the lack of published information and to reflect the study objectives in section 2.3 regarding how to create or develop a date-based treatment.

- Step 1: Identification of five of the most consumed date varieties in the KSA.
- Step 2: Converting the date fruit into a standardisable edible form.
- Step 3: Quantifying the phenolic content of the five nominated date varieties.
- Step 4: Selecting two varieties of the five with the highest TPC.
- Step 5: Determining the “experimental dose”.
- Step 6: Import the quantity needed from KSA.
- Step 7: Quantifying the phenolic content of the two chosen date varieties.
- Step 8: Chemical composition analysis of the two chosen date varieties.
- Step 9: Choosing a suitable execution method for treatments.
- Step 10: Creating recipes.
- Step 11: Conducting a sensory evaluation panel.
- Step12: Modifying recipes where needed.

Due to the multiple methodologies used in this chapter and to facilitate the transition between the steps of this development plan, all steps are summarised in a flow chart in Figure 5. The rationale and the aim of each phase of the recipe creation steps will be illustrated in detail in the following subheadings.

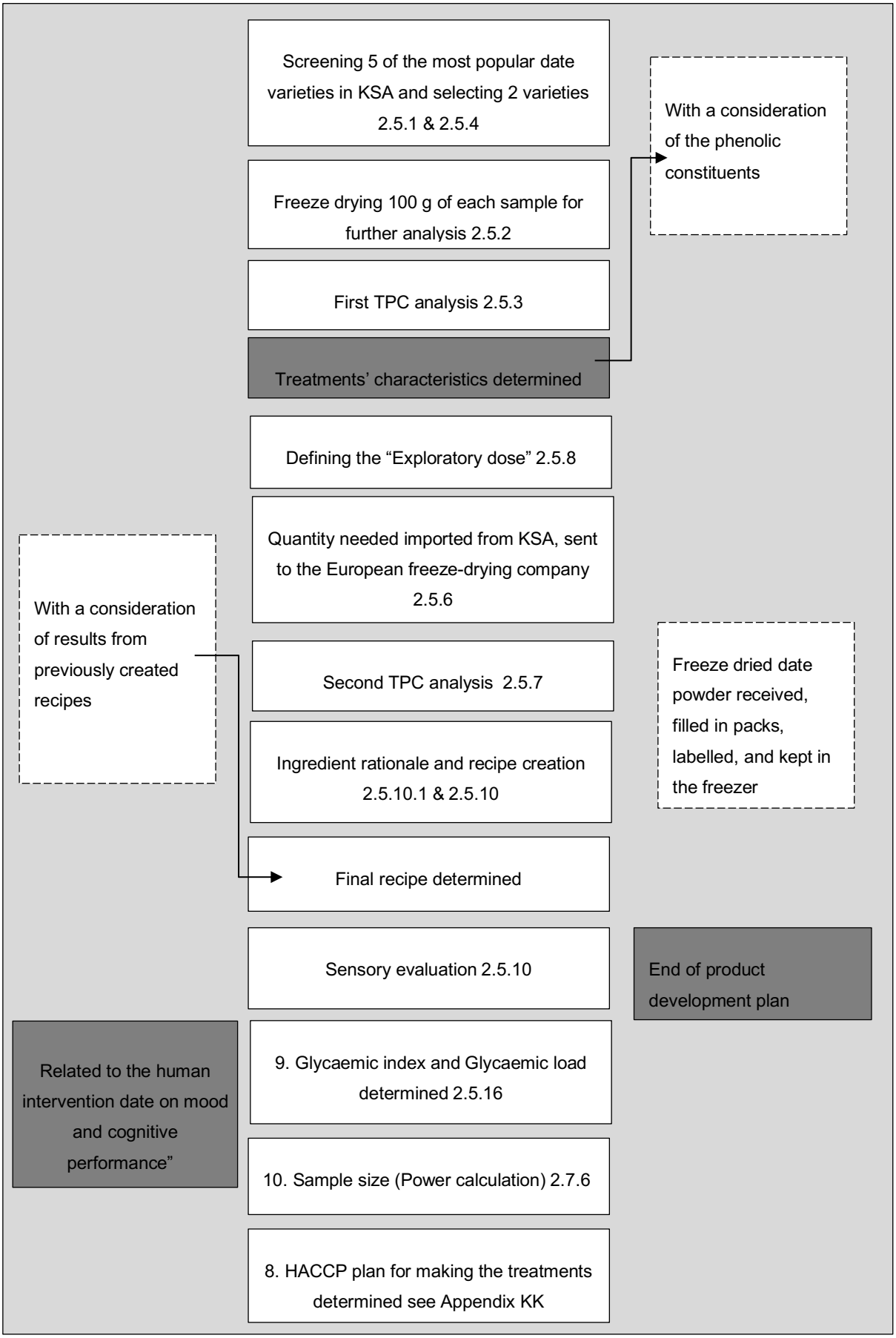


Figure 5. Flow diagram of the study timeline with the order of steps corresponding to the development plan steps and the study objectives.

KSA: Kingdom of Saudi Arabia. *TPC*: Total Phenolic Content. Numbers in boxes (eg. 2.5.1) referes to the corrosponding subheading in the thesis.

2.5 Materials and methods

The materials and the methods utilised in every step are explained under each subheading listed below.

2.5.1 Step 1: Nominating five of the most consumed date varieties in the Kingdom of Saudi Arabia (KSA)

Among the 300 date palm varieties which are cultivated in the Kingdom of Saudi Arabia (Asif et al., 1983), five different cultivars of date fruit were nominated to be tested in the first major human intervention in this thesis. The nomination of date cultivars in this study was made according to the following criteria: popularity, representation of the different maturation stages, and representation of the different colours.

The maturation stage of the date has a great influence on the quantity of phenolic compounds within the date fruit. Therefore, to screen a range of date varieties that cover all edible maturation stages, the following five cultivars were selected: Khassab and Barhi dates were chosen to represent the Khalal stage, Sukkari dates to represent the *Rutab* stage, while Ajwah and Khalas dates were chosen to represent the *Tamer* stage. More information about the maturation stages of date fruit is available in section 1.4. It may be more consistent to test different maturation stages in the same cultivar rather than in different cultivars or different cultivars with the same maturation stage. However, each cultivar was nominated with the most favourable and most common maturation stage for Saudi consumers applicable to this cultivar. It is also important to mention that these cultivars occupy the top list of major shares of commerce in the most important regions of date production in the KSA (Aleid et al., 2015). Ajwah and Khassab are two of the most popular cultivars of the western region, Sukkari and Barhi are important cultivars in the central region of KSA, while Khalas is the best-selling variety in the Eastern region (see Figure 6).

Figure 6. The five nominated date varieties.

(Images obtained (FeeDo, 2016, BuyMassry, 2021, Krish International, 2016, Ajwati, 2020, Al Barakah Dates, 2020).

2.5.2 Step 2: Converting the date fruit into a standardised edible form

Since the nature of the nominated date varieties varied in some features such as maturation stage, colour, texture, and sugar content, as illustrated earlier in Table 3 in chapter 1, it was important to find a technique that could preserve the phytochemical content of the date cultivars. Date fruit can be dried effectively with three drying methods, convective, microwave and freeze-drying (İZLİ (2017).

A standardised and homogenous form of date fruit with a longer self-life was essential for this study to preserve the phytochemical components, therefore, freeze-drying was selected.

Initially, 100 g of each of the five nominated cultivars was freeze-dried using the freeze dryer at Newcastle University.

The dates were weighed (100 g) and chopped up into small pieces before homogenisation in 10 ml of distilled water. Each date homogenate was placed onto a foil plate, labelled and freeze-dried at -25°C for 120 h using the freeze dryer (Freeze dryer Alpha 1–2 Christ - Martin Christ, Osterode am Harz, Germany) in the agriculture building of Newcastle University. The yield of the five nominated date cultivars is shown in Figure 7.

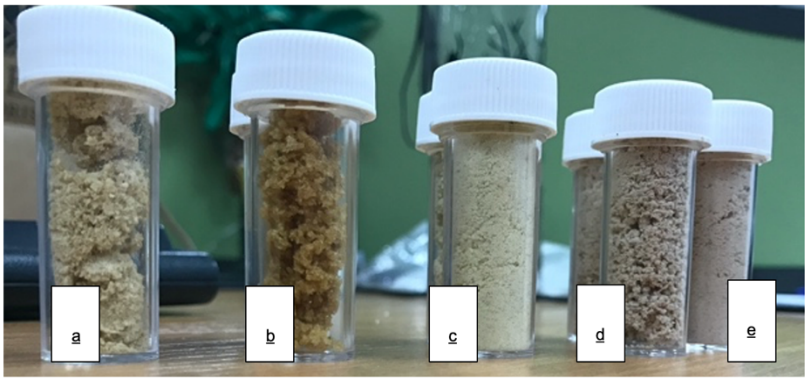


Figure 7. The freeze-dried yield of the five nominated date cultivars.

a: Sukkari, b: Ajwah, c: Barhi, d: Khassab

2.5.3 Step 3: Quantifying the total phenolic content (TPC) of the five nominated date varieties

The purpose of the initial TPC quantification of the nominated date varieties was to determine the top two dates with the highest amount of TPC for this study. The Folin-Ciocalteu method was used, detailed information regarding the used method, sample preparations and analysis can be found in section 2.5.13.

2.5.4 Step 4: Selecting two varieties with the highest TPC

Phenolics were postulated to be the psychoactive ingredient in the date fruit, so the results informed the recipe creation in step three. Therefore, based on the initial TPC quantification results using Folin-Ciocalteu, two date varieties, Barhi and Khassab were selected to prepare the active treatments. Each variety had a different phenolic profile according to the HPLC results, as described later in this chapter, in section 2.7.3.1.

2.5.5 Step 5: Determining "experimental dose"

As demonstrated in chapter 1, there are three approximate ranges of doses of phenolic compounds used in the twenty-three studies summarised in Table 5: small (116-364

mg), medium (500-773 mg) and high (960-1470 mg) regardless of the unit used to measure the phenolic compounds. The gallic acid equivalent GAE is commonly used as a calibration curve standard when using the Folin-Ciocalteu method to quantify the phenolic content (GAE/g), while any phenolic compound that is predicted to be predominant can be used as a standard and a representative unit in the HPLC method. However, among the three ranges of doses, there is little correlation with the associated results, and inconsistent significant results were obtained from different studies that utilised similar cognitive tasks. For example, as mentioned in Table 5, three effective single doses were reported to enhance cognitive function: 773 mg, 520 mg and 994 mg of cocoa flavanol. However, the TPC of the dates tested was too low to achieve the lowest effective dose of flavanol, 520 mg. The HPLC analysis showed that Barhi and Khassab have epicatechin equivalent content of 88 g and 291 g/100 of FW, respectively, as detailed in Table 14, therefore unfeasible quantities of Barhi and Khassab (approximately 590 g and 178g, respectively) would be required to achieve this dose (Figure 12). Therefore, taking into consideration the lack or even the absence of an investigation into the effect of the consumption of date fruit on mood and cognition in the literature, an “Experimental dose” based on the normal consumption of fresh date fruit among people in Middle Eastern countries, such as KSA and UAE, was used in this experiment. One hundred and fifteen grams of date fruit was reported by Ismail et al. (2006) to be the normal daily fresh date consumption.

2.5.6 Step 6: Importing the quantity needed from KSA

Based on the TPC quantification of the five nominated dates, Barhi and Khasab with the highest phenolic content were chosen to make the study treatment (see 2.7.2.1 for explanations). Also, the quantity of fruit required was calculated based on the dry weight yield calculated for the five date samples after freeze-drying (see section 2.7.1) and the calculated sample size (see section 2.7.6). A total of 40 kg of the two chosen varieties, Barhi and Khassab, were imported from the date farm Nabu Alnakeel Dates EST located in Onaizah city in the central region (Al-Qasim district) of Saudi Arabia. Twenty kilos of each variety were shipped to the University of Newcastle.

The required quantity of fruit for freeze-drying was sent to an external freeze dryer, European Freeze Dry.

As soon as the required quantity of both Barhi and Khassab cultivars arrived from KSA at the University of Newcastle, the dates were individually washed, selected, destoned and packed in the food safe pilot kitchen at the NU-Food Research Facility and sent to the European Freeze Dry Company (London, UK), as shown in Figure 8.

Figure 8. Final freeze-drying steps of the Barhi and Khassab dates

Steps included washing the imported dates, selecting the matured dates with good conditions, destoning the dates, preparing dates by loading them into separate bags, and freezing and sealing them to be transferred to the European Freeze Dry Company.

The fruits were prepared by the European Freeze Dry Company s follows: the dates were loaded separately onto aluminium trays with plastic liners at a pre-determined weight per tray. The trays were then placed into a blast freezer and frozen overnight to -25°C. Once frozen, the dates were placed into the dryer and the temperature was reduced to ≤10°C and the vacuum down to 1 Mb. The primary drying stage (sublimation

of ice to vapour) was run for 6.5 hrs, then the secondary drying (evaporation of bound water) commenced and ran for 8-10 hrs with the temperature reaching 40-45°C until the dates were dry.

At this point, it was determined it would be better to powder the dates as soon as possible to ensure that they did not absorb moisture as they are quite hydroscopic, more so once powdered. The dates were powdered while warm to <2 mm in size and a moisture test was performed using an Ohaus moisture analyser, showing less than 1% moisture. The powdered dates were then bagged and heat sealed.

2.5.7 Step 7: Quantifying the phenolic content of the date samples

After freeze-drying, a sample of each variety was analysed for TPC to (a) firstly confirm the TPC of the chosen varieties obtained by the initial TPC analysis of both date varieties, and (b) secondly, to identify the phenolic profile of powder and as a prepared treatment utilising HPLC. Detailed information regarding the used method, sample preparation and analysis can be found in section 2.5.14.2.

2.5.8 Step 8: Choosing a suitable execution method for treatment

The main challenge was finding a carrier or media for the date powders. Therefore, specific standards were created to help when evaluating any suggested nutritional substance to be used as a medium for the three treatments (Barhi, Khassab and placebo) as follows:

- Contains no phenolic content such as chlorogenic acids, catechins and flavanols
- Facilitates the masking procedure of treatments' organoleptic properties
- Does not involve any heating processes
- Feasible in terms of execution
- Has an acceptable level of palatability

- Provides an element of homogeneity, contributing to an acceptable level of participants' uncertainty to discriminate one treatment from the others

Therefore, three ideas were proposed and discussed as follows:

- Date capsules

The idea of filling the freeze-dried date powder into capsules has been used in some nutritional research, such as Egert et al. (2012). There are many advantages of using capsules, however, the most important advantage is facilitating the blinding design, and therefore the practicality of minimising the need for a third party when conducting human trials. The capsules are usually affordable and made of gelatine which has no phenolic content.

A review of typical capsules currently on the market indicated that the largest capacity of capsules was "000" and holds approximately 1000 mg. However, for48 g and 34 g of freeze-dried Barhi and Khassab, respectively, around 48 and 34 capsules would need to be filled, which is unrealistic for a participant to swallow once daily, so encapsulating the powder was deemed infeasible.

- Date juice

An attempt was made to prepare date juice by dissolving the date powder in 250 ml of water but the product was very gritty and unpalatable due to the high amount of soluble and insoluble fibres in the date powder.

- Date yoghurt

Fruit yoghurt is among the most frequently consumed fermented dairy products throughout the world (Saint-Eve et al., 2006), and is manufactured through coagulating milk with acid without drainage (Sodini et al., 2004). The functionality and antioxidant capacity of such products are frequently enhanced by supplementation of ingredients such as strawberry fruit (Coïsson et al., 2005, Trigueros et al., 2011).

Preliminary testing of incorporating the date powder in yoghurt showed potential, with this media also providing a favourable ability to regulate sensory characteristics such as smell, colour and homogeneity.

Initial concerns regarding decreases in TPC due to the formation of soluble complexes owing to the affinity of the phenolic content in dates to affect the proteins in the yoghurt were resolved after reviewing various models. This association has been indicated as a surface phenomenon formed by amino acid side chains and polyphenol ring complexes established by multiple weak interactions (mainly hydrophobic). Polyphenols in the form of strawberries were incorporated into low-fat yoghurt by Oliveira et al. (2015). Across the shelf-life of the product, the antioxidant capacity, TPC and anthocyanin content was observed to distinguish the dairy protein effects. The most affected compounds were (+)-catechin (60%), (-)-epicatechin (60 %) after 24 hours and no data about the immediate supplementation of the yoghurt with strawberry. However, the yoghurt and date powder combination utilised in the trial treatments would be prepared just before consumption, thus this may not be representative.

The possible interaction between the yoghurt proteins like tryptophan or tyrosine and the F-C reagent when quantifying phenolic content using the Folin-Ciocalteu assay, as mentioned in Prior et al. (2005), was concerning. This reaction may increase the TPC, therefore, the phenolic content of the placebo, which consisted of yoghurt, sugars and added fibres, was quantified using HPLC.

Furthermore, the alteration in solubility does not necessarily alter the bioavailability (Ferruzzi et al., 2012). An observation of polyphenol-protein complexes produced in milk in the presence of cocoa chocolate in the gut showed that these were efficiently disrupted by habitual digestion, with in vivo bioaccessibility of flavan-3-ols. Therefore, it was decided to use yoghurt as the carrier media for the date powder.

2.5.9 Step 9: Chemical composition analysis of the treatments

2.5.9.1 Chemical composition analysis of the samples of those two date varieties

A total of 10 g of each date variety was sent to the Huson & Hardwick Laboratories (Alex Stewart Agriculture, Liverpool, UK) for chemical composition analysis including the quantification of sugar content, soluble and insoluble fibre content, starch, total carbohydrates, salt and energy (see results in Table 6).

This quantification of chemical components aimed to aid placebo replication and match the sugar content, energy and fibre while maintaining the phenolic content. Since the calorie content of the treatments is important, the naturally occurring sugars in both date varieties were quantified using the variety with the highest sugar levels, Barhi, as a reference. Both Khassab and placebo were supplemented with the desired amount of sugars (see Table 6 for sugar content and Table 9 for treatment sugar supplementation).

Table 6. The nutritional composition of Khassab and Barhi dates[6].

Nutritional composition	*In 100 g of FW of Khassab*	*In 100 g of FW of Barhi*
Energy	344 Kcal/1450 KJ	361 Kcal/1528 KJ
Protein	4.41 g	4.21 g
Ash	3.33 g	2.96 g
Moisture	0.75 g	1.59 g
Available Carbohydrate	69.45 g	79.02 g
Total Sugars	61.42 g	78.11 g
Glucose	36.32 g	*38.49 g*
Fructose	27.04 g	*34.24 g*
Sucrose [7]	0 g	*1.6 g*
Maltose [6]	0 g	*1.73 g*
Insoluble Dietary Fibre	18.91 g	9.37 g
Soluble Dietary Fibre	2.53 g	2.38 g
Total dietary Fibre	21.44 g	11.75 g
Fat:	0.62 g	0.56 g
saturates	0.12 g	< 0.1 g
monounsaturates	0.17 g	< 0.1 g
polyunsaturates	0.3 g	0.36 g
Starch	0.67 %	1.41 %

[6] Analysed by Huson & Hardwick Laboratories in triplicate.
[7] The amount of sucrose and maltose was low in Barhi corresponding to less than 1 g when calculated for the experimental dose of 48 g, therefore they were excluded from the treatment recipe.

2.5.9.2 Chemical composition of the yoghurt

The chemical composition of the 0% fat yoghurt was obtained from the yoghurt nutritional information on the company website (Yeo Valley, 2018) and is shown in Table 7.

Table 7. The nutritional composition of the fat-free natural yoghurt (Yeo Valley, 2018)

Typical values	*Per 100 g of FW*
Energy	59 kcal/ 249 kJ
Fat	0 g
Carbohydrates	8.5 g
Of which is sugars	8.5 g
Protein	5.9 g
Salt	0.23 g
Calcium	172 mg

2.5.10 Step 10: Creating recipes

2.5.10.1 Treatment characteristics and ingredients

Three treatment recipes were created, two of which contained dates and the third recipe was the placebo. Each date-containing treatment had a different phenolic content profile as shown in section 2.7.3.1. Since glucose has an established enhancing effect on mood and cognitive performance for humans and animals (Kaplan et al., 2000, Ragozzino et al., 1996) and all types of sugars in both date varieties had been quantified, it was important to match all treatments for sugar content. According to the chemical composition analysis conducted by Huson & Hardwick Laboratories, the Barhi variety contained a high content of glucose and fructose of 38.49 g and 34.24 g/100 of FW respectively (see Table 6 and the HPLC chromatograms in Appendix PP). The higher content of each sugar type was set as a standard, and any diminution was supplemented to meet the standards.

In summary, the study treatments were:

- Khassab
- Placebo
- Barhi

The treatment characteristics are shown in Figure 9.

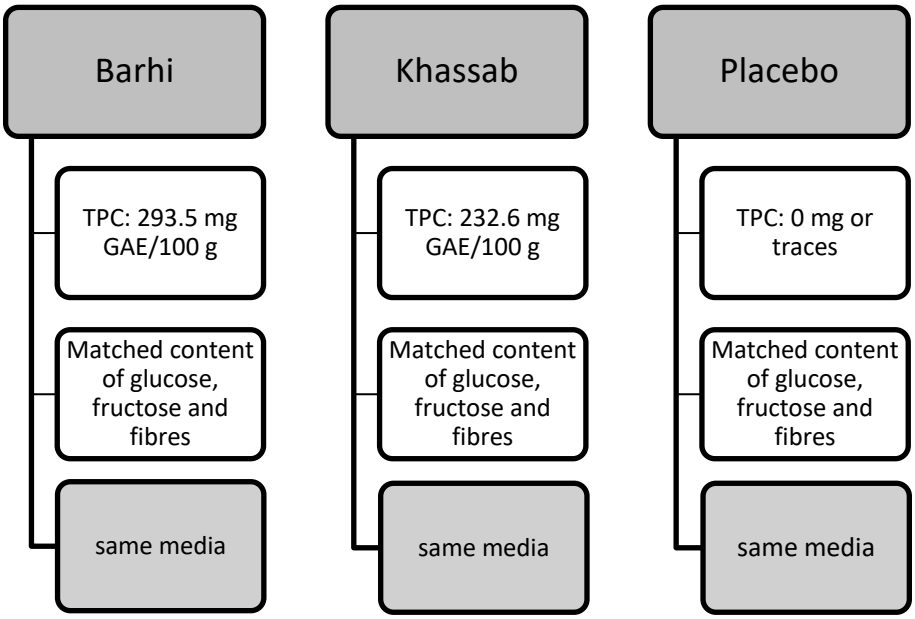


Figure 9. Treatment characteristics

The phenolic content (TPC) was quantified using the Folin-Ciocalteu method and presented as gallic acid equivalent (GAE).

In line with the treatment characteristics, the ingredients used were:

- Barhi: Nabu Alnakeel Dates EST, KSA
- Khassab: Nabu Alnakeel Dates EST, KSA
- 0 % fat yoghurt: Yeo Valley Family Farm
- Glucose: Dextrose, Bulk powders.com
- Fructose: Fructose, Bulk powders.com
- Soluble fibres: VITAFIBER™ powder, Bulk powders.com

- Insoluble fibres: Purified Powdered Psyllium Husk, Holland & Barrett, Newcastle, UK
- Sugar-free Strawberry flavour: LIQUIFLAV™, BulkTM
- Food colouring (red, orange and blue): The Vanilla Valley, Cardiff, UK

2.5.11 Step 11: Sensory evaluation of the treatments

All the treatments were subjected to sensory evaluation to ensure that they were sufficiently similar to each other in most sensory features including appearance, smell and taste. This was intended to provide a sufficient level of blinding so that the participants were unable to identify the treatment which contained the date powder. The recipes produced three different shades of red treatments and placebo and a weak flavour (Table 8 and Figure 10).

Table 8. The initial recipes for the treatments made using Barhi and Khassab dates and the placebo

Ingredients	*Barhi treatment*	*Khassab treatment*	*Placebo*
Date powder	48 g	34.5 g	0 g
Water	75 ml	90 ml	0 ml
0% fat yoghurt	150 g	150 g	150 g
Glucose	0 g	4.94 g	16.78 g
Fructose	0 g	6.48 g	15.84 g
Soluble fibre	0 g	0 g	1.04 g
Insoluble fibre	2.46 g	0 g	6.54 g
Strawberry flavour	5 drops	5 drops	5 drops
Red food colouring	3 drops	3 drops	3 drops
Orange food colouring	2 drops	2 drops	2 drops
Total portion weight	275.46 g	285.92 g	190 g

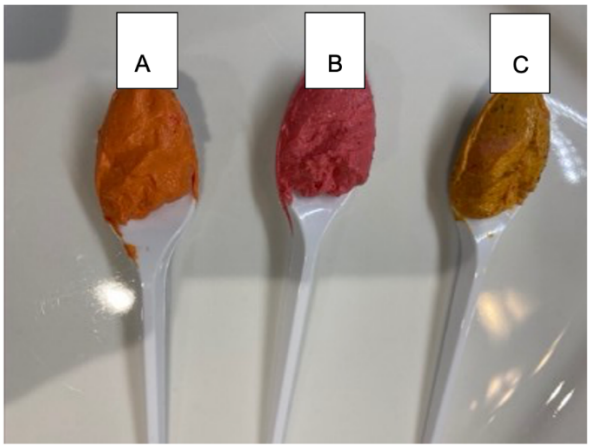


Figure 10. A photo of the A: placebo and two treatments B: Khassab and C: Barhi

2.5.12 Step 12: Modification of recipes

The initial recipes were modified to improve the taste and the colour, thus, the number of strawberry flavour drops was increased to eight to enhance the flavour and mask any distinguishable differences. Additionally, the reddish colour was replaced with blue to overcome any variations caused by the contrast between the yellowish base and the red colour drops to obtain a greenish homogenised colour for all treatments, with all other ingredients remaining the same. The modified recipes are detailed in Table 9 and photos of these are in Figure 11.

Table 9. The final treatment and placebo recipes with the modifications highlighted in grey.

Ingredients	*Barhi treatment*	*Khassab treatment*	*Placebo*
Date powder	48 g	34.5 g	0 g
Water	75 ml	90 ml	0 ml
0% fat yoghurt	150 g	150 g	150 g
Glucose	0 g	4.94 g	16.78 g
Fructose	0 g	6.48 g	15.84 g
Soluble fibre	0 g	0 g	1.04 g
Insoluble fibre	2.46 g	0 g	6.54 g
Strawberry flavour	8 drops	8 drops	8 drops
Blue food colouring	5 drops	5 drops	5 drops
Total portion weight	275.46 g	285.92 g	190 g

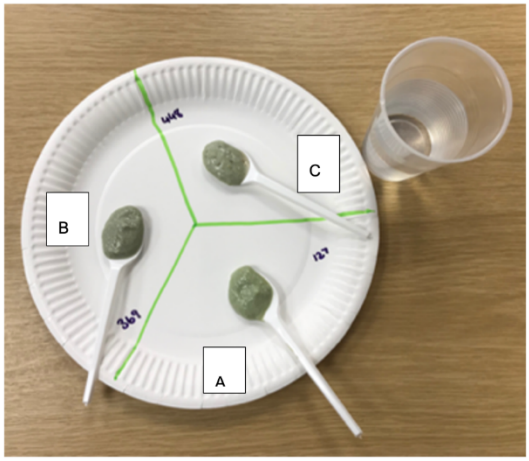


Figure 11. A photo of the A: placebo and two treatments B: Khassab and C: Barhi after recipe modification

A flow diagram showing the critical analysis adopted to create the treatments from first principles is shown in Figure 12.

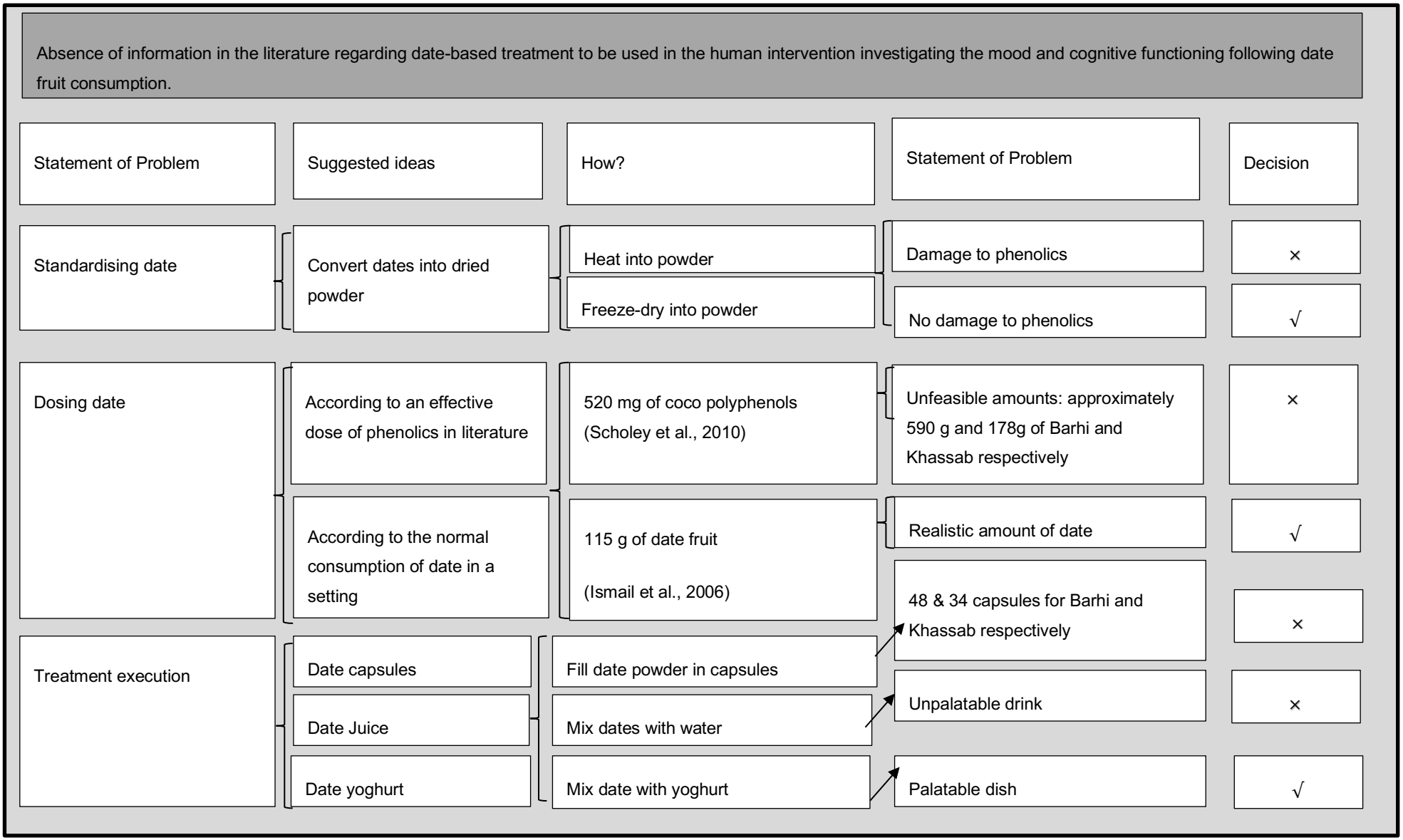


Figure 12. The critical analysis adopted in creating the treatment from first principles

2.5.13 Determination and quantification of the total phenolic content (TPC)

The total phenolic content was determined using the Folin-Ciocalteu method modified from (Zhang et al., 2006). Before the assay, the Folin-Ciocalteu reagent (1: 10, v/v) and 7.5% sodium carbonate anhydrous solution were prepared with deionised water.

This assay was conducted as follows:

- All five nominated date cultivars were included to aid the selection of the richest date cultivar in TPC.
- To confirm the TPC for the selected and imported two date cultivars.
- To confirm our expectation of a very low TPC For the placebo.

2.5.13.1 Chemicals and reagents

Sodium carbonate (Na_2CO_3; Lot # SZBD150AV), Folin-Ciocalteu reagent (Lot # BCBM8482V), gallic acid anhydrous (Lot # SLBF8212V) and calcium chloride (Lot # MKBP63497) were purchased from Sigma-Aldrich®.

2.5.13.2 Determination of dry matter content

Triplicate samples of the date cultivars were accurately weighed before and after freeze-drying. The dry matter content was calculated using the equation:

$$Dry\ matter\ content\ (\%) = \frac{Weight\ after\ freeze\ drying}{Weight\ before\ freeze\ drying} \times 100$$

The dry matter content was expressed as % of fresh weight.

2.5.13.3 Samples preparation for TPC

All freeze-dried date and placebo samples were prepared by dissolving 200 mg of extract powder in 20 ml of water using the homogeniser. The samples were centrifuged for 10 min at 4000 rpm and 20 µl of supernatant was transferred into a separate cuvette, then 1.58 ml of deionised water and 100 µl of Folin-Ciocalteu reagent were added and mixed well. After 5 minutes, 300 µl of 7.5% sodium carbonate

anhydrous solution was added and mixed, then the samples were incubated at 20°C for 2 hours. The absorbance was measured at 750 nm using a spectrophotometer. A calibration curve (R^2 = 0.9992) of serial dilutions of gallic acid (100, 50, 25, 12.5, and 6.25 ug/ml) was prepared using deionised water, with the results expressed as mg of GAE/100 g dry matter or dry extract.

2.5.14 Sample extraction for phenolic profile determination using HPLC

The method used was as described by Alarcón-Flores et al. (2013) and modified by Qadir et al. (2017). The samples (100 mg) of freeze-dried Barhi and Khassab date extracts and Barhi and Khassab extracts mixed with yoghurt were weighed into a 1.5 ml polypropylene centrifuge tube and 1 ml of methanol: water (80:20, v/v) was added. The mixture was agitated for 30 min on a shaker and centrifuged for 10 min at 4000 rpm, then 300 µl of the supernatant was transferred into an HPLC vial. The chemicals and solvents used for HPLC are detailed in Table 10 and Table 11.

Table 10. List of authentic standard compounds.

Standards	***CAS No***	***Sourced***
Epicatechin 98% pure by HPLC Green Tea Extract	123219700415	eBay
Caffeic Acid > 89.0% (HPLC)	331-3	Sigma-Aldrich®
Chlorogenic acid crystalline 89.0% (HPLC)	327-97-9	Scientific Laboratory Supplies
Gallic acid 89.0% (HPLC)	149-91-7	Sigma-Aldrich®

*Compounds were selected based on Alarcón-Flores et al. (2016) and Llorach et al. (2008) and used to compare and identify the phenolics in date fruits. Serial dilutions of each standard were made to make standard curves.

Table 11. List of solvents for the HPLC analysis.

Solvents	***CAS No***	***Sourced***
Acetonitrile > 98.0 % (HPLC)	75-05-08	Sigma-Aldrich®
Trifluoroacetic acid > 89.0 % (HPLC) (TFA)	76-05-1	Sigma-Aldrich®
Methanol > 98.0 %(HPLC)	67-56-1	Fisher Chemical®

2.5.14.1 Phenolic acid composition and flavonoids analysis

According to the method described by Alarcón-Flores et al. (2013), the HPLC column was a Kinetex EVO Reverse phase (C18, 100A, 250 × 4.6 mm, 5 um), and the column oven was set at 25°C. The injection volume was 10 µl, and the HPLC system was equipped with a Shimadzu 2 LC-20AD pump, SIL-20A system Autosampler, SPD-M 20A photodiode array UV−vis detector set to collect all data from 200 to 600 nm, and a CTO-20AD column oven (Shimadzu Corp. Kyoto, Japan). The mobile phase was 0.1% v/v trifluoroacetic acid in ultrapure water (solvent A), 0.1% v/v trifluoroacetic acid in HPLC-grade acetonitrile (solvent B) with a flow rate of 1 ml/min. The solvent gradient (A:B) was 0 (100:0), 5 (100:0), 15 (83:17), 17 (83:17), 22 (75:25), 30 (65:35), 35 (50:50), 40 (0:100), and 50 min (0:100) followed by re-equilibration as 55 (100:0) and 65 min (100:0).

2.5.14.2 Quantification and identification of phenolic content and compounds

Identification and quantification of phenolic compounds were based on the retention times and absorption spectra of authentic standards selected based on Alarcón-Flores et al. (2016) and Llorach et al. (2008). The diode-array detector was set at 320 nm for the quantification of phenolic acids and 280 nm for flavanols. For the calculation of total phenolic compounds in each sample, a range of absorption maximum wavelengths (lambda max) (within 10 nm) of each standard was selected to calculate the total equivalent of the standard as follows:

270–278 nm for GAE

279–288 nm for Epicatechin Equivalent (EPE)

320–330 nm for Chlorogenic Acid Equivalent (CAE)

An example of the standard chromatograms is shown in Figure 13 and the sample chromatograms are shown in

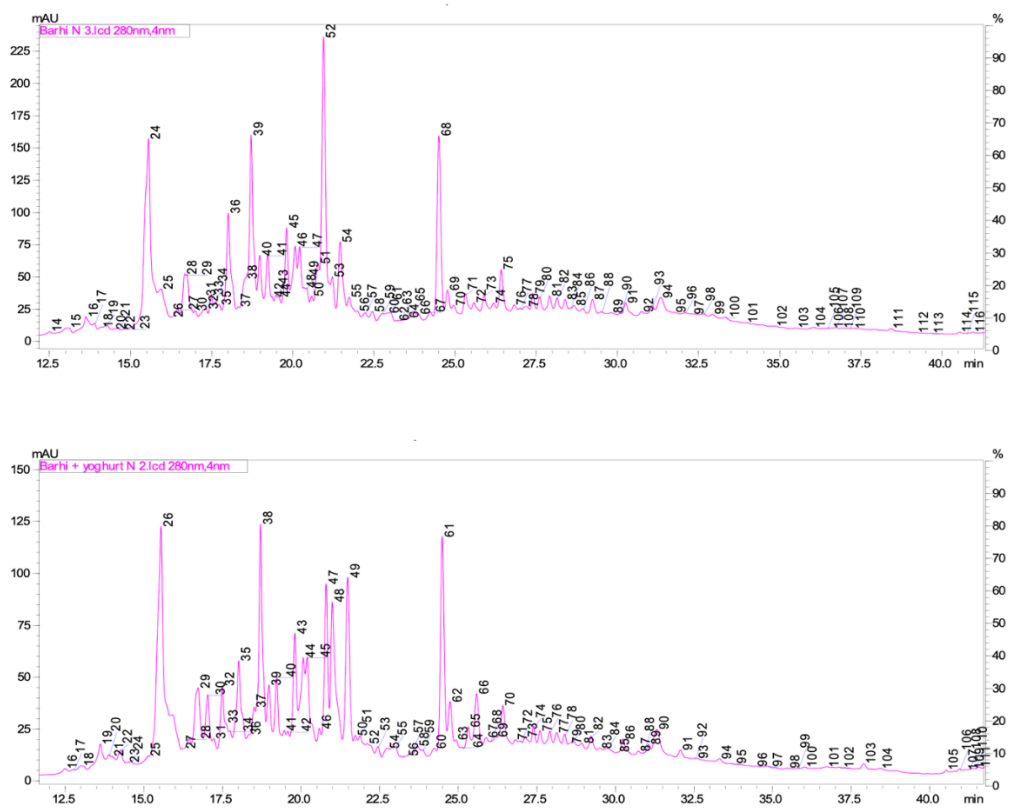


Figure 14 and Figure 15.

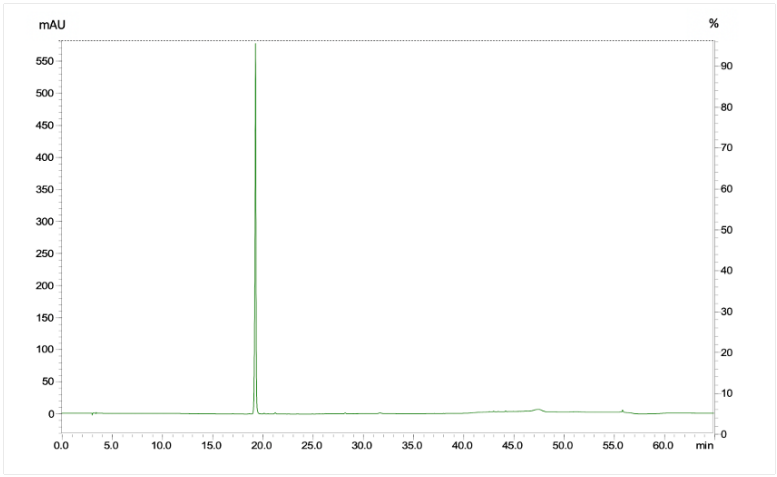


Figure 13. Chlorogenic acid chromatogram @ 320 nm.

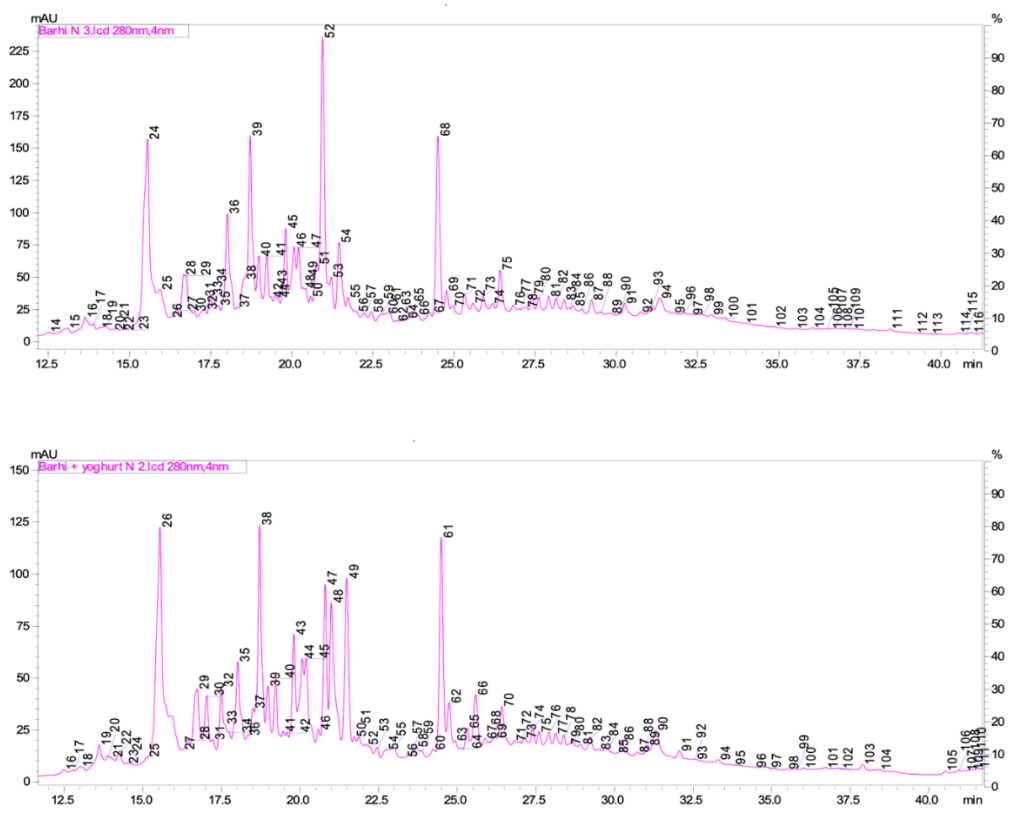


Figure 14. HPLC chromatogram of phenolic compounds of Barhi without yoghurt, and for Barhi with yoghurt as an example @ 280 nm wavelength.

*****The first chromatogram is for Barhi: peak 24 is gallic acid (GA), peaks 25 to 36 are gallic acid equivalents (GAE), peak 39 is epicatechin (EP), and peaks 40 to 70 are epicatechin equivalents (EPE). The second chromatogram is for Barhi and yoghurt: peak 26 is GA, peaks 27 to 37 are GAE, peak 38 is EP, and peaks 39 to 70 are EPE.

*Peaks identified by comparison with authentic standards as follows: GA, GAE, EP and EPE.

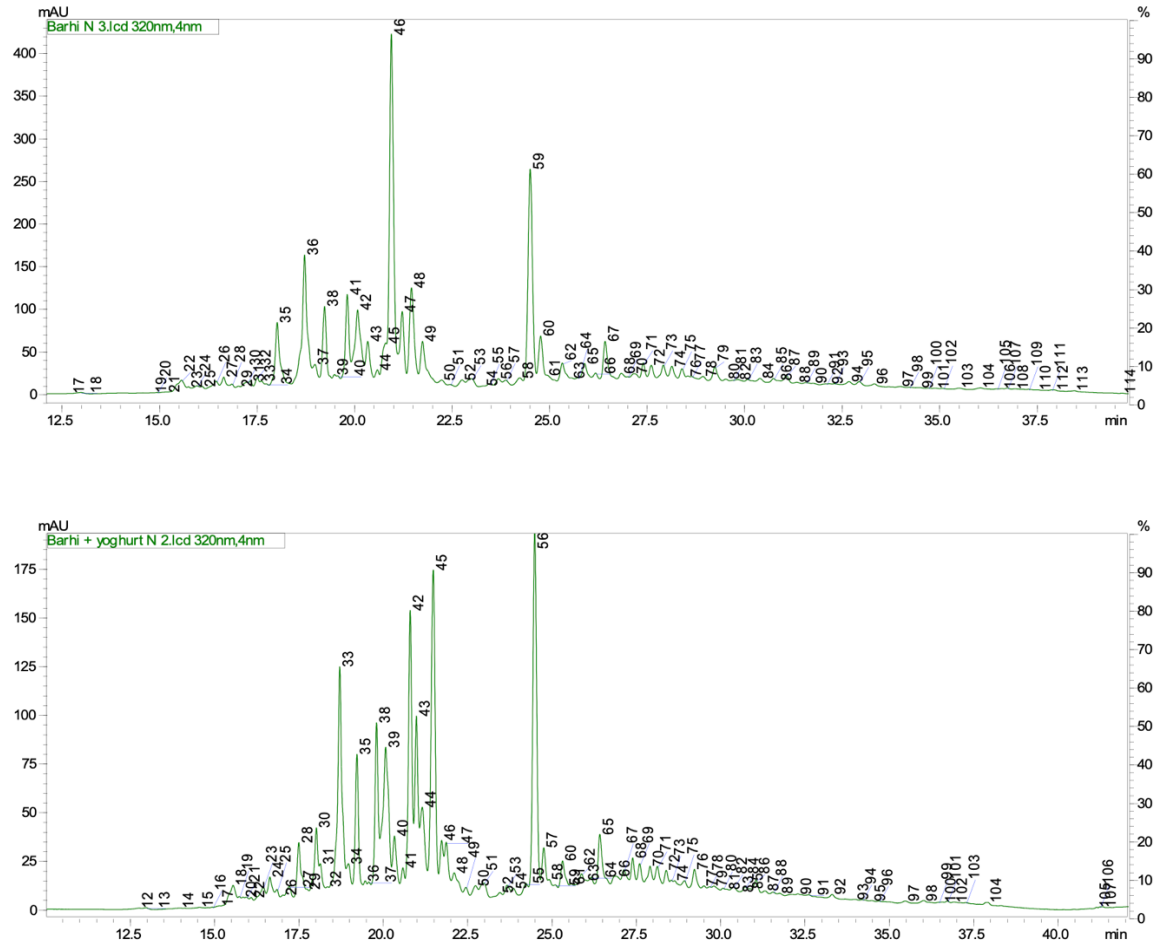


Figure 15. HPLC chromatogram of phenolic compounds of Barhi without yoghurt and for Barhi with yoghurt as an example @ 320 nm wavelength.

*****The first chromatogram is for Barhi: peak 41 is chlorogenic acid (CA), peaks 42 to 51 are chlorogenic acid equivalents (CAE). The second chromatogram is for Barhi and yoghurt: peak 38 is CA, peaks 39 to 49 are CAE.

*Peaks identified by comparison with authentic standards CA and CAE.

2.5.15 Sensory evaluation of treatments

It was essential to create treatments with comparable sensory qualities, such as appearance, smell and taste, to ensure participants' inability to differentiate between the date-containing treatments and the placebo. To assess the fulfilment of this

masking procedure from the formulated treatments and placebo, a small sensory evaluation panel test was conducted.

Approximately 3 g of each treatment sample was presented on disposable teaspoons to the panel, and each teaspoon was positioned on a paper plate which had been divided into three sectors and labelled with the treatment codes. These randomisation codes were generated using an online tool for sample randomisation (https://www.random.org/lists/). The treatments were prepared in line with the recipe in section 2.5.10 (Table 9) and were served at room temperature. The plate containing all treatments and a sensory evaluation form (see Appendix G) were provided to participants seated in individual booths at the NU-Food facilities at Newcastle University.

2.5.15.1 Participants

Ten colleagues from the Department of Natural and Environmental Sciences at Newcastle University participated, and no previous training was provided. The participants were adults aged between 25 and 55 years old (mean 33.5 ± 7.60) and comprised seven males and three females. All participants reported being healthy, with no allergies or intolerances to food including dairy products and dates. The participants were verbally briefed on the procedure before being seated in the sensory booths prior to the sensory evaluation commencing.

2.5.15.2 Procedure

The sensory evaluation form (Appendix G) contained seven unmarked visual analogue scales in the form of a line each measuring an attribute: smell, sweetness, texture, flavour, colour, aftertaste and overall acceptance. The participants were required to write each sample code next to the line to score each attribute.

Instructions were provided to panellists on the evaluation form as follows: “Taste the samples on your plate and answer questions below by writing the sample code on the line as indicated in the below example. If you think two samples are exactly the same, you can write them at the same place”.

Participants were provided with water as a palate cleanser between samples.

2.5.16 Determination of the glycaemic index (GI) and the glycaemic load (GL) of the date treatments

2.5.16.1 Rationale

The brain requires a continual energy source to function, with the central source being glucose (Amiel, 1994). Glucose is also fundamental to providing various organs with the required energy and is usually acquired by the breakdown of carbohydrate-containing foods into glucose, as opposed to being consumed directly in the diet.

The rate of glucose release from carbohydrate-based foods is used to classify these foods and is referred to as the glycaemic index (GI). This is measured by comparing the blood glucose concentration 2 hours post-consumption of a 50 g carbohydrate portion of food against blood glucose quantities 2 hours after ingestion of 50 g of glucose (Jenkins et al., 1981). GI stipulates an indication of carbohydrate quality (Wolever et al., 2003), with low-GI (LGI) foods absorbed slower equating to a slower release of glucose, and high-GI (HGI) foods resulting in a fast absorption. The glycaemic load (GL) can be used if both carbohydrate quality and quantity are equally required and is calculated as GL = (GI X amount of carbohydrate /100) (Salmeron et al., 1997).

The rate of glucose release corresponding to the GI of carbohydrates has been hypothesised to affect cognitive function. A systematic review by Philippou and Constantinou (2014) indicated that the cognitive performance of healthy adults might benefit from LGI meals; however, the findings are inconclusive. Additionally, the eleven studies included in Philippou and Constantinou (2014) systematic review comprised a variety of inconsistent methodologies, which could explain the inconsistency of the findings. Kaplan et al. (2000) determined no variation between high and low glycaemic index meals on cognitive functioning in elderly adults with normal glucose tolerance, using an acute repeated measure, placebo control, crossover study design. Furthermore, the effects of consuming a high or low glycaemic

index evening meal on cognitive functioning the subsequent morning presented no significant difference, however, did display a statistical trend favouring an HGI (Lamport et al., 2011). In contrast, Benton et al. (2003) reported an enhancement of verbal recall of abstract words after consumption of an LGI breakfast and increased concrete word recall in the delayed postprandial phase (210 min) after the LGI meal in young women. Similarly, Nilsson et al. (2009) exhibited results of enriched performance from ingestion of LGI compared to HGI during the late postprandial time frame. However, this was exclusive to selective attention and precluded any significance in cognitive reaction time or working memory.

No available information relating to the date powder and yoghurt treatments created during product development is available in the literature, therefore the GI and GL were measured against 50 g of glucose in young, healthy participants.

2.5.16.2 Study design

Figure 16 displays the timeline for the GI measurement.

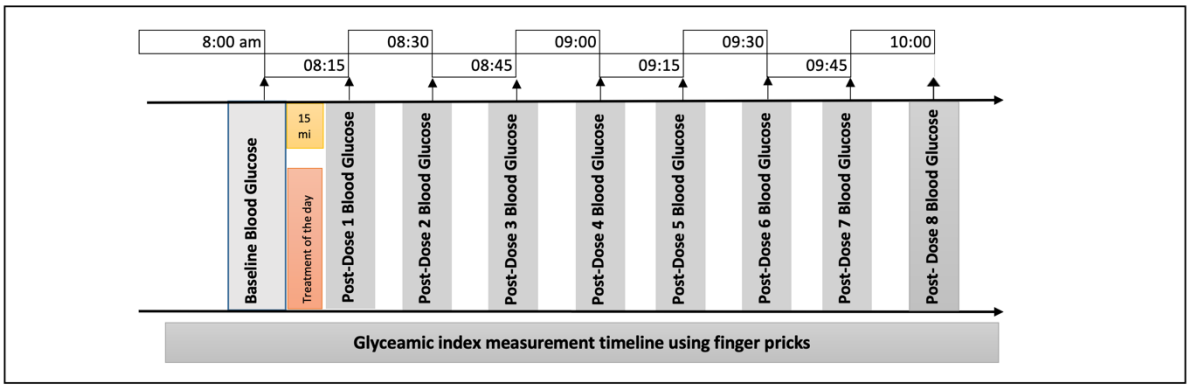


Figure 16. GI determination study timeline

2.5.16.3 Treatments

Fifty (50) grams of glucose (dextrose) purchased from Bulk Powder® dissolved in 120 mL water was used as the reference food. Yoghurts containing date powder were

prepared on the day of testing following the recipe in section 2.5.12 by a third party who had no connection to the experiment (see Figure 17).

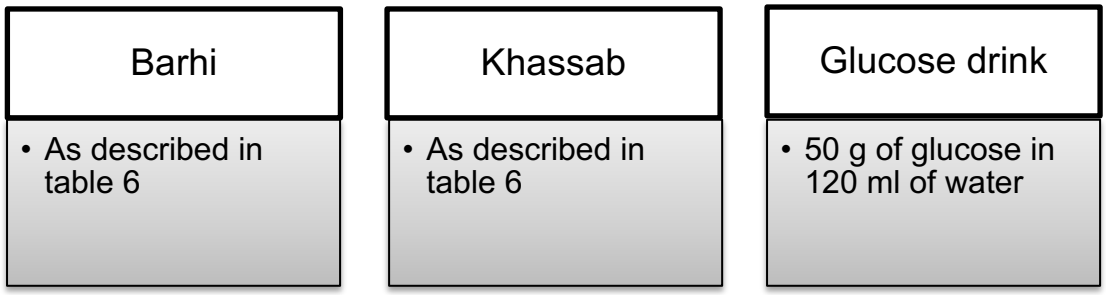


Figure 17. The treatments and the glucose drink reference recipes

Both date treatments were tested in portions containing almost equivalent available carbohydrate amounts (50 g).

2.5.16.4 Glucose measuring instruments and materials

- Blood glucose concentrations were measured in the capillary whole blood obtained by finger prick (On a Call® EZ Glucometer meter system)
- Using Call® Plus, Blood strips, purchased from valuemed.co.uk
- Safety Lancet blade blue, and penetration depth 2 mm, purchased from valuemed.co.uk
- Glucose (dextrose) purchased from Bulk Powder®

2.5.16.5 Participants

Ten healthy young participants (3 males, 7 females) were screened for any contraindications before the study, briefed about the study protocol and signed a written informed consent document (see Appendix D and Appendix E). Anthropometric measurements and their fasting blood glucose were recorded (4.3–5.7 mmol/L, mean 4.98±0.39). All participants reported being with no history of any food allergies, including milk, with both immediate and delayed symptoms. There was no history of any metabolic diseases such as Type 1 or Type 2 diabetes. At the end of the visit, participants were given a snack and a drink. See Figure 18 for a visual summary.

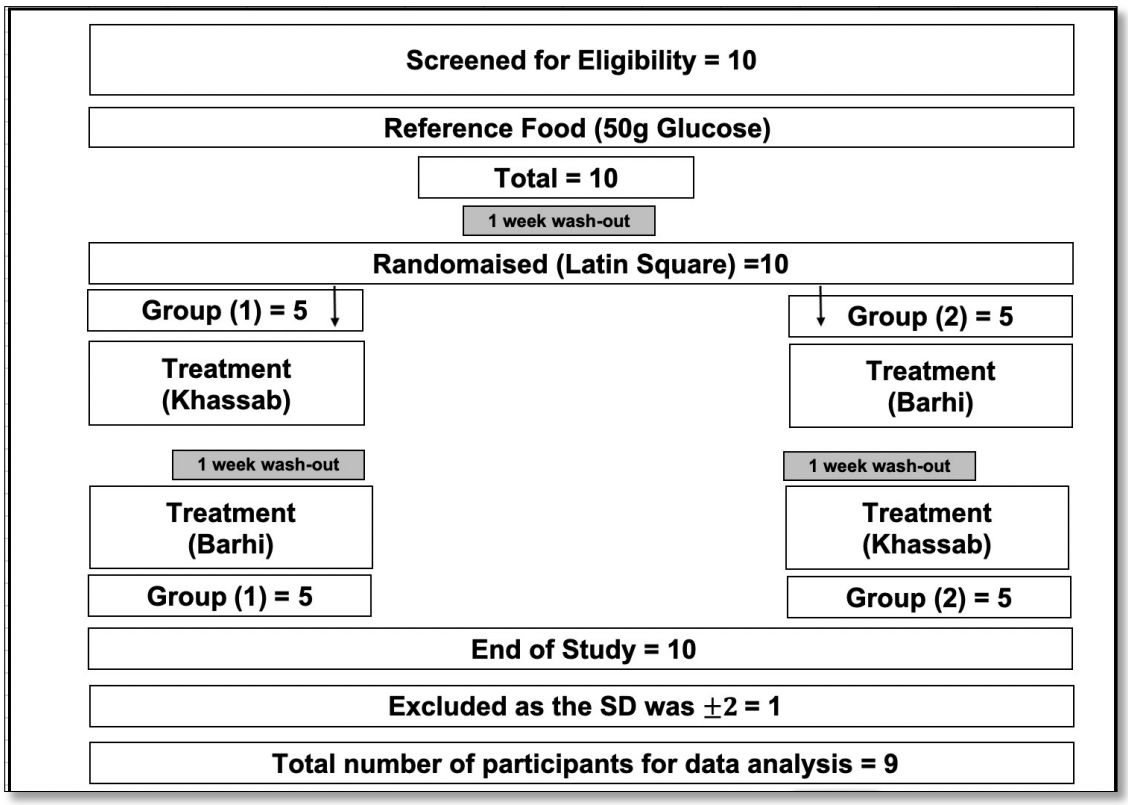


Figure 18. Study design and participant allocations for treatments

The study was approved by the Ethical Committee of Newcastle University (application number 1490/14474/2018) and registered on the ClinicalTrial.gov website (see Appendix B).

2.5.16.6 Procedure

The participants arrived at the NU-Food research centre at about 9:30 am after a 12 hour overnight fast. A baseline blood glucose measurement was taken before the participants consumed their daily treatment which was prepared by a third party who had no further involvement in the study. A maximum of 10 minutes was allocated for treatment consumption and they were then asked to move to the assessment room for blood glucose measurements at 15 min, 30 min, 45 min, 60 min, 75 min, 90 min, 105 min and 120 min post-dose (see study timeline).

Each of the three treatment study visits lasted approximately 2 hours 15 minutes, with a one-week washout between each visit. Participants were required to stay in the

research centre during any breaks between each test/blood sample and they had access to a comfortable waiting room.

The study was performed according to the international standard GI testing protocol, in line with procedures recommended by the FAO/WHO1 Expert Consultation (Organization, 2003). The order of test foods was randomised using a Latin square randomisation allocation.

2.5.16.7 Calculation of glycemic index (GI) and glycemic load (GL)

The GI and the GL calculations were obtained according to the food, glycaemic response and health published by the International Life Sciences Institute (ILSI) (SERIES). The incremental area under the postprandial blood glucose curve (iAUC), ignoring the area beneath the baseline, was calculated geometrically for each treatment and the GI was evaluated as a percentage of the mean iAUC of the reference glucose solution consumed by the same subject

GI = [iAUC of test food/ individual subject's average iAUC of the reference food] × 100

When the individual GI values for any subject fell outside the range of values calculated as mean ± SD (standard deviation), this result was considered an outlier and was thus excluded from the mean GI calculation. The GL of a specific serving of each date treatment was calculated using the formula:

GL = [GI × total carbohydrate in food portion] /100 (SERIES).

2.6 Statistical analysis

All data were recorded in Microsoft Excel. The GI and GL data were also analysed using Microsoft Excel, while the results of the dry matter calculation, the sensory evaluation panel, TPC and HPLC were handled using IBM SPSS package 25. One-way analysis of variance (ANOVA) and Tukey's HSD test were used to detect any significant differences among the three treatments: BY Barhi and yoghurt, KY Khassab and yoghurt and placebo P. Also, one-way ANOVA was used with the Tukey test to

detect any significant differences among the TPC of the date varieties, between the two selected date varieties and between date-containing treatments measured by Folin-Ciocalteu and HPLC. Finally, for the sensory evaluation test, Bonferroni correction was performed to protect from Type I errors arising from the multiple ANOVAs. As there were seven different attributes measured, the alpha level of 0.05 was divided by seven to be P = 0.007.

2.7 Results

2.7.1 Dry matter calculation

The dry matter content of the freeze-dried samples was determined (Table 12) and compared to the fresh samples. Fully matured date varieties Ajwah, Khalas and Sukkari contained the highest dry matter content of 90.5%, 90.7% and 86.4% respectively, while the early ripened date varieties Barhi and Khassab contained the lowest dry matter of 42.3% and 30% respectively. Additionally, the three treatments of Barhi and yoghurt, Khassab and yoghurt and the placebo had a low dry matter content of 30.53%, 24.70% and 30%, respectively, as shown in Table 13.

Table 12. The percentage of dry matter content in the five date varieties

Date variety	*Dry matter content %*
Ajwah	90.50
Khalas	90.70
Barhi	42.30
Sukkari	86.40
Khassab	30.00

Table 13. The percentage of dry matter content in treatments made according to the finalised recipes for Barhi + yoghurt, Khassab + yoghurt and Placebo

Date variety	*Dry matter content %*
Placebo	33.12
B + yoghurt	30.53
K + yoghurt	24.70

2.7.2 Total phenolic content (TPC) determined using the Folic-Ciocalteu method

2.7.2.1 TPC of the five date varieties

The total phenolic content of the five date varieties is shown in Figure 19 and the gallic acid standard curve is presented in Figure 20. The one-way ANOVA showed that there was a significant difference among the five date varieties in TPC, ($F(4,10) = 465.81$, $p = 0.001$), with Barhi and Khassab having the highest TPC of 294 ± 14 and 233 ± 10 of GAE mg/100 g of FW, respectively. A Tukey post hoc test for multiple comparisons revealed that the total phenolic content TPC of Barhi was significantly higher than that of Khassab.

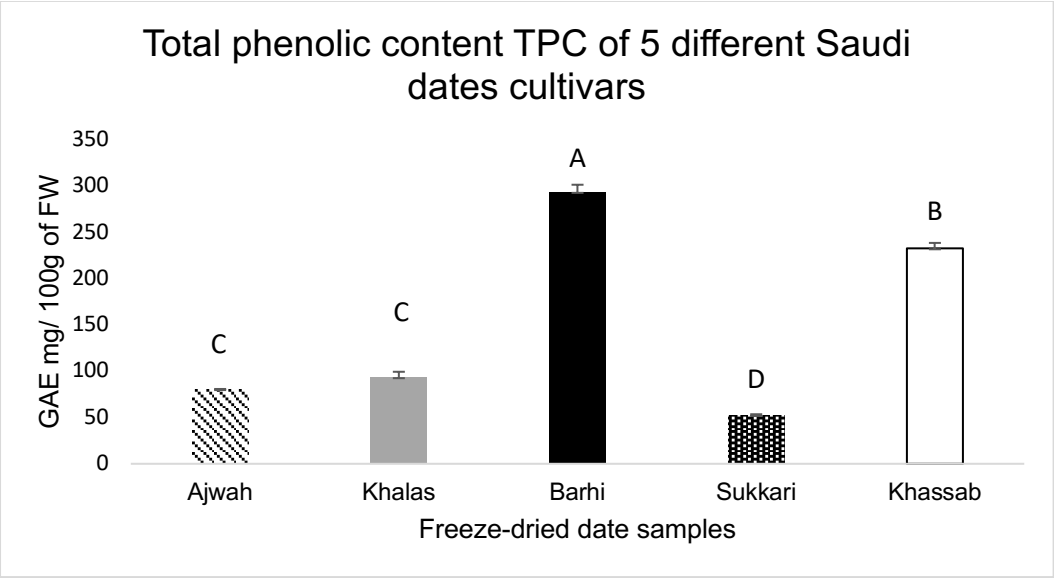


Figure 19. Total phenolic content (TPC) of the five date cultivars measured by the Folin-Ciocalteu method.

*TPC is expressed as GAE (mg/100) in fresh weight. Each bar represents the mean ± standard error calculated from triplicates. The bars denoted with different letters indicate significant differences at $P < 0.05$ according to Tukey's HSD test.

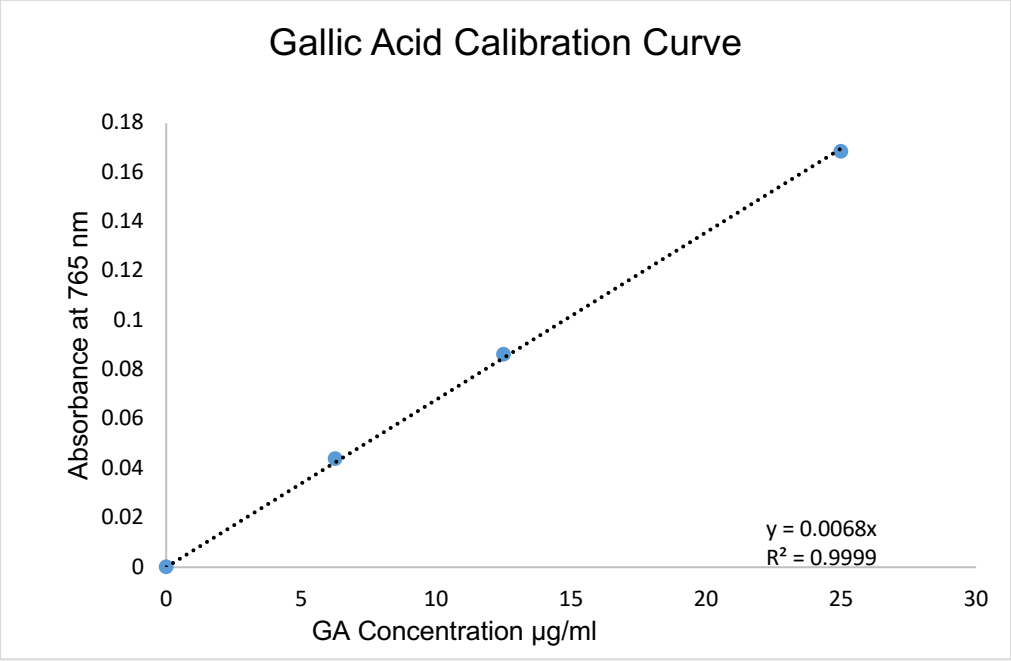


Figure 20. **Gallic acid calibration curve**

2.7.2.2 Total phenolic content (TPC) of Barhi and Khassab

The total phenolic content TPC of Barhi was significantly higher than that of Khassab according to the Tukey test and as shown in Figure 21. The TPC of Barhi was significantly higher than that for Khassab.

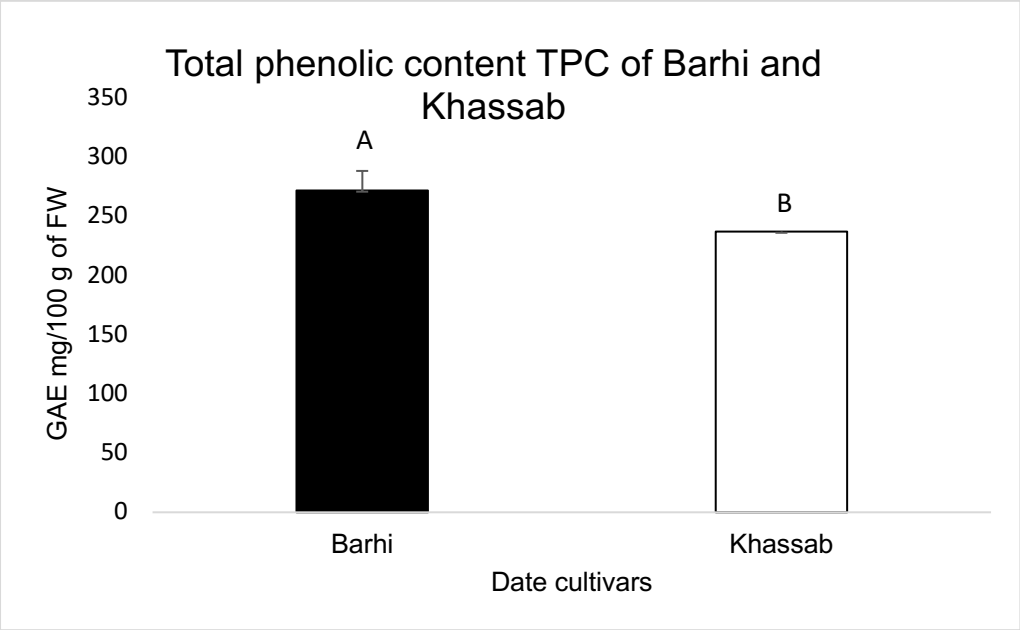


Figure 21. Total phenolic content (TPC) in Barhi and Khassab of the second batch imported from KSA measured by the Folin-Ciocalteu method.

*TPC was expressed as GAE (mg/100) in fresh weight. Each bar represents the mean ± standard error calculated from triplicates. The bars denoted with different letters indicate significant differences at $P < 0.05$ according to the Tukey test.

2.7.2.3 Total phenolic content (TPC) of placebo

The TPC of the placebo was 11 ± 4 mg/100 g FW, therefore, the TPC for the 150 g placebo portion was calculated to be 16 ± 6 mg/150 g FW. It is important to mention that the quantified TPC of the placebo was not added to the contribution from each of the dates. This low amount presumably was detected due to either the food colouring added to the placebo or from the yoghurt but both ingredients were also added to the date treatments.

2.7.3 Phenolic profile identification and total phenolic content (TPC) quantification using the HPLC method

2.7.3.1 Phenolic profile of Barhi, Khassab, Barhi with yoghurt and Khassab with yoghurt and TPC calculations

One-way ANOVA showed a significant difference in TPC between Barhi and Khassab ($P = 0.001$). A Tukey post hoc test for multiple comparisons revealed that the phenolic content of Barhi was significantly lower than Khassab ($P = 0.001$), and a lower TPC than Khassab with yoghurt ($P = .008 < 0.05$). However, there was no statistical difference between Barhi and Barhi with yoghurt, nor between Barhi with yoghurt and Khassab with yoghurt (Table 14). The output of ANOVA can be found in Appendix H. Chromatograms of the date samples conducted by the HPLC are available in Appendix I.

Table 14. The phenolic profile of the various treatments quantified by HPLC

Treatment	*GAE (mg/100 g FW)*	*CAE (mg/100 g FW)*	*EPE (mg/100 g FW)*	*TPC[8] concentration (mg/100 g FW)*	*TPC[2] dose used (mg/115 g FW)*
Barhi	31 ± 3	39 ± 1	88 ± 1	159 ± 18[A]	184 ± 21[A]
Khassab	72 ± 5	5 ± 2	291 ± 15	369 ± 12[B]	424 ± 14[B]
Barhi + Y[9]	27 ± 2	18 ± 2	76 ± 2	121 ± 2[A]	139 ± 3[A]
Khassab +Y[10]	39 ± 1	6 ± 1	161 ± 7	207 ± 9[B]	238 ± 10[B]

*Gallic acid equivalent (GAE), chlorogenic acid equivalent (CAE), epicatechin equivalent (EPE) and total phenolic content (TPC) of Barhi, Khassab, Barhi with yoghurt, Khassab with yoghurt measured by HPLC method, and the TPC in the experimental dose of 115 g of the fresh weight of the same varieties. All values are the means ± SD of triplicates.

2.7.4 Sensory evaluation

The one-way ANOVA revealed that in the sensory evaluation test (Table 15 and Figure 22), there was a significant difference among the three treatments in two of the attributes as follows: sweetness ($F(2, 27) = 3.45$, $P = 0.046 < 0.05$) and texture (F (2,

[8] The TPC shown here is the sum of the estimate of the three different types of phenolic compounds.

[9] Barhi and yoghurt treatment
[10] Khassab and Yoghurt treatmen
[A B] The means±SD denoted with different letters indicate significant differences at $P < 0.05$ according to 2-sample 2-sided t-test conducted on the TPC concetration and used dose.

27) = 13.86, P < 0.001 < 0.05). However, after the Bonferroni correction to the alpha level, only texture remained significant (P= 0.007).

Table 15. Sensory attribute measures of the three treatments Barhi yoghurt BY, Khassab yoghurt KY and placebo P.

Attribute	*Sample*[11]	*Mean*	*SD*	*P-value*
Smell	BY	7.1	2.3	0.14
	P	5.7	3.3	
	KY	8.1	1.9	
Colour	BY	6.0	2.0	0.324
	P	7.3	2.6	
	KY	5.8	2.3	
Flavour	BY	6.0	2.8	0.765
	P	5.3	2.6	
	KY	5.3	2.1	
Texture	BY^{A}	5.9	3.4	0.001
	P^{A}	4.1	2.5	
	KY^{B}	10.0	1.4	
Sweetness	BY^{A}	6.7	2.9	0.046
	P^{B}	5.8	3.2	
	KY^{A}	9.0	2.3	
Aftertaste	BY	7.3	1.9	0.082
	P	5.5	2.4	
	KY	5.0	2.7	
Overall Acceptability (Liking)	BY	5.8	2.8	0.253
	P	6.6	3.1	
	KY	7.8	1.9	

[11] Alphabetical subscript denotes posthoc significant difference at P < 0.05 according to Tukey's HSD test. Significant P values are highlighted in grey.

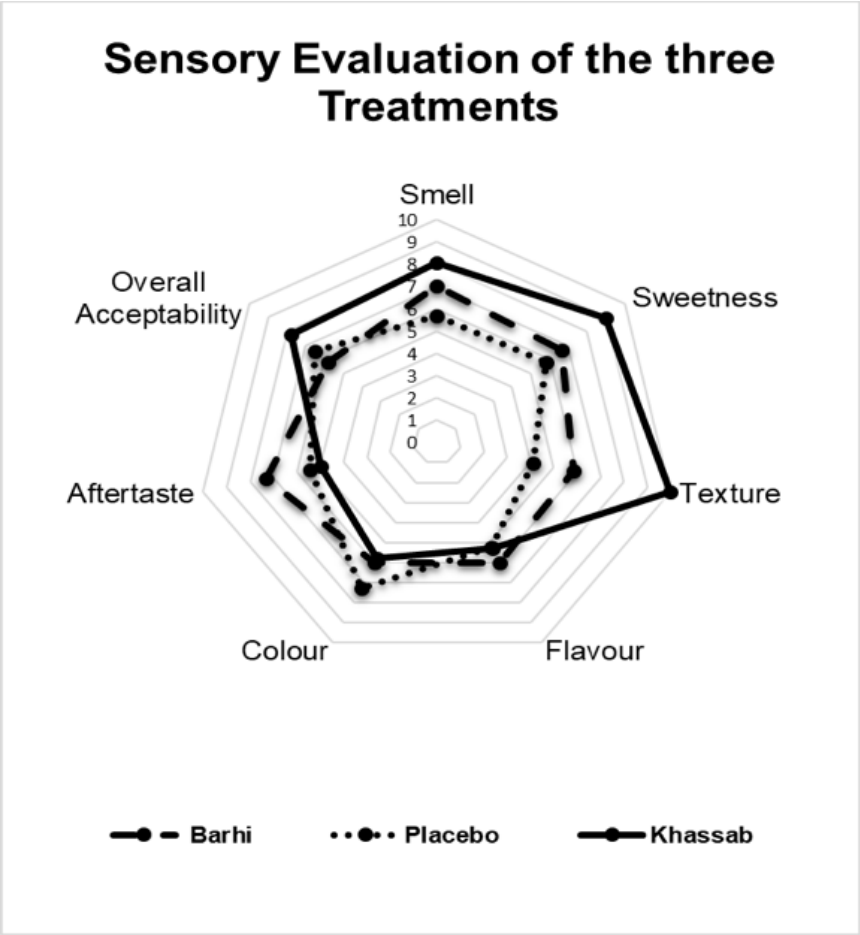


Figure 22. The sensory profile of the seven attributes, smell, sweetness, texture, flavour colour, aftertaste and overall acceptability of the three treatments.

'Texture' went from 'smooth' as low to 'gritty' as high, 'flavour' went from 'mild' as low to 'intense' as high, 'colour' went from 'pale' as low to 'dark' as high, while sweetness, smell, aftertaste, and overall acceptability went from 'very little' to 'very much'.

2.7.5 GI and GL

Mean glycaemic indices of the date and yoghurt mixed treatments were calculated as 38 for Khassab and 51 for Barhi, while the mean for the glycaemic load was 18 for Khassab and 24 for Barhi (Table 16 and Figure 23). Furthermore, the glycaemic

responses of all study treatments: Barhi, Khassab and the reference drink of 50 g of glucose are shown in Figure 23.

Table 16. G**lycaemic index and glycaemic load** of date-containing treatments.

Food	***Barhi***	***Khassab***
Participants n	9	9
Available CHO (g)	44	44
Experimental portion (g)	270.3	274.6
GI (mean ± SEM)	51 ± 12	38 ± 7
Category[12]	Low	Low
GL (mean ± SEM)	24 ± 6	18 ± 3
Category[13]	High	Medium

* The number of participants tested, weights of carbohydrates consumed, weights of date and yoghurt meals consumed and mean ± standard errors of glycaemic index and glycaemic load.

12 Glycaemic indices categories: high (>70), medium (56–69), and low (<55). Glycaemic loads categories: high (>20), medium (11–19), and low (<10) SYDNEY, T. U. O. 2019. GI testing research [Online]. Available: http://www.glycemicindex.com/ [Accessed 28/11/2019 2019].

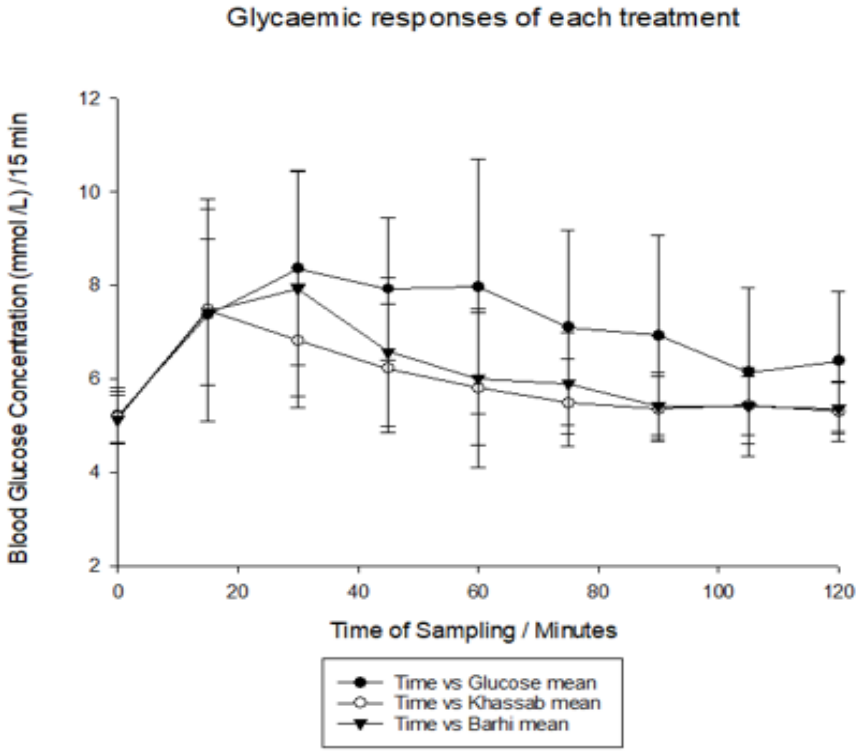


Figure 23. Blood glucose concentrations of all study treatments

*Barhi, Khassab and the reference drink of 50 g of glucose. Values are the mean for nine participants with their SEM represented by the vertical bars.

A paired T-test on GI data did not show a significant difference between the two date-containing treatments (P= 0.760). Glycaemic response curves were elicited by 50 g available carbohydrate portions of reference glucose, and 46 g of available carbohydrate of Barhi and Khassab mixed with 150 g of 0% fat yoghurt, including the yoghurt sugar content. It is important to mention that the placebo was not included as a fourth treatment in the GI experiment for different reasons. Firstly, to make it more comfortable for the participants in terms of the number of visits and more importantly, the number of finger pricks. Secondly, as the total sugar content of the yoghurt containing treatments used was 46 g/per serving, including the sugar content of the low-fat yoghurt media which was 8.5 g/100 g, was close to the 50 g of glucose utilised in the reference drink, thus the placebo treatment made of yoghurt, fibre and sugars was not included as an arm in the GI and GL study.

2.7.6 Power calculation

Due to the lack of research investigating the effects of date fruit consumption on mood and cognitive function, it was a challenge to find the most relevant paper to be the basis for the proposed trial power calculation. Since the initially anticipated psychoactive component was the TPC of date fruit, it was decided to power this study according to one of the most relevant studies in this domain. Several studies which investigated the acute effects of the consumption of different polyphenolic compounds on mood and cognitive function in humans and utilised different doses of polyphenols are included in Table 4. However, regardless of the variation in the doses and the type of the phenolic compound, Watson et al. (2015) investigated the acute effects of the phenolic content of blackcurrant extracts on mood and cognitive performance in healthy young volunteers; this was deemed most appropriate to be used for the power calculation as a similarly young, healthy population was recruited in this thesis, and it is a more recent study in comparison to the others.

It reported an increase in the percentage of the accuracy of the rapid visual information process (RVIP) measurement of 64.53±17.59 at baseline and a difference of -0.23, -1.56, -1.95, -1.72, -1.72, -1.56, and -2.03 at the first up to the seventh repetition of the RVIP task respectively. This study made use of the required sample size to detect a difference of 9.41, the average between all seven means of the many repetitions (number of post-dose tests) with a power of 95%, 90%, 85% or 80% and an alpha of 5%. The expected difference between groups is 9.41. Assumed Standard Deviation of RVIP accuracy = 12.15 to allow for maximum variation. Inputting these numbers in Minitab version 17 revealed the results shown in Table 17.

Table 17. Results from the Minitab power calculation

Difference	*Sample size*	*Power*	*Actual Power*
9.41	*42*	*0.95*	*0.950337*
9.41	*34*	*0.90*	*0.906482*
9.41	*28*	*0.85*	*0.852986*
9.41	*24*	*0.80*	*0.803693*

It was decided to use a power of 90% and a sample size of 34. To compensate for possible dropouts, a sample size of 36 was used, thus, a total of 36 healthy participants aged between 18 and 35 were recruited through advertisement via posters and flyers. All participants were required to undergo a screening visit.

2.8 Discussion

2.8.1 Sensory evaluation panel

The purpose of the sensory analysis was to determine to what extent organoleptic differences in the treatments were detectable by participants. This was to ensure treatments were not too easily distinguishable from one another and thus qualify their credible use in a nutritional intervention trial. To enable this, doses of active ingredients in the treatments had to remain under tight control giving rise to a considerable challenge during product development. This is reflected in the presence of some significant differences between the date-containing treatments, particularly concerning the Barhi treatment.

A statistically significant difference was detected in only one attribute tested, texture. However, it was not a concern for the study, as this difference was observed between the placebo and Khassab treatments. It is important to emphasise that the sensory testing was a direct comparison, so the treatments were presented together, making it much easier to detect differences than when the treatments were presented

separately, one week apart, and covered in foil. Due to this, even though sensory differences did exist, it was unlikely that the participants would be able to identify and distinguish the placebo treatment from the two date treatments, therefor such differences would not cause a bias. Since it is often challenging to make treatments indistinguishable in food and nutrition studies, the focus should be to ensure that they are unrecognisable. Some minor sensory differences are acceptable if this can be achieved since they will not cause a bias (Welch et al., 2011).

2.8.2 TPC quantification

Regarding finding the phenolic content of the five tested date cultivars, as mentioned earlier in 2.5.1, these cultivars are from different maturation stages and different regions of KSA. The ripening stage plays an important role in the phenolic content of dates as proven by Eid et al. (2013), in which three different cultivars (Ajwah, Barhi, and Khalas) at different stages of ripening (*Kimri, Khalal, Rutab, and Tamer*) were tested for their phenolic content. These same cultivars were included in our analysis, with some variation in the maturation stage. However, the highest TPC in all the three date cultivars was detected in the *Khalal* stage. Therefore, our quantification of the TPC agreed with Eid et al. (2013). Most importantly, our chosen maturation stage was deemed appropriate, as it confirmed that the Khalal stage has the highest phenolic content among other stages, as demonstrated by Eid et al. (2013).

Additionally, Allaith (2008) evaluated the TPC of ten of the Bahraini date cultivars and found a considerable variation in the level of phenolics between cultivars and between stages among the same cultivars. Two of the same cultivars of the five utilised in this study (Barhi, Khalas and Hallwa) were included in Allaith's (2008) study. The TPC of the Hallwa date cultivar was reported by Allatiah (2008) to be 90 mg of GAE/100 g of FW in the *Khalal* stage. Moreover, the TPC of the Barhi date cultivar was 99.8 mg of GAE/100 g of FW in the *Khalal* stage. These findings emphasised that both *Khalal* and *Tamar* stages have the highest TPC among all stages. However, the most important question to be answered is: are these levels of phenolic content sufficient to impact cognitive function? According to the literature, doses which have shown a statistically

significant impact on cognitive function when studied acutely ranged from 150 mg in Haskell et al. (2017) to 520 mg in Scholey et al. (2012), therefore, it is difficult to predict but worth investigating.

The TPC of Barhi and Khassab was 159± 18 and 369±12 mg of GAE/100 g of FW, respectively, were to some extent in line with the reported average contents of phenolics which ranged from 193.7 to 280 mg/100 g for fresh dates, as reported in Al-Farsi and Lee (2008).

It is also important to mention the two methods for quantifying the TPC of dates: Folin-Ciocalteu and HPLC. For example, the TPC for Barhi obtained by Folin-Ciocalteu was 293.5± 13.5 of GAE/100 g of FW, while it was 159.17± 18.18 of GAE/100 g of FW when obtained by HPLC. The variations observed in the TPC could be due to the use of two different methods and can be explained as the Folin-Ciocalteu (or spectrophotometric method) lacks specificity which may cause overestimates in the total phenolic compounds. Escarpa and González (2001) compared different approaches to quantifying the content of total phenols in food samples, concluding that in some cases, where there is an interference with non-phenolic materials like sugars or partially dissolvable protein, this overestimated the polyphenolic content in comparison to the chromatographic method (HPLC) (Escarpa and González, 2001).

2.8.3 GI and GL determination

Regarding the GI and GL results, the data provides the GI and the GL values of two local Saudi date cultivars commonly consumed within the Kingdom of Saudi Arabia. The results underline that Khassab and Barhi in a freeze-dried form mixed with 0% fat yoghurt may promote a low to medium postprandial glucose response compared to that expected based on their natural sugar content. According to Sydney (2019), the categorisation for the glycaemic indices are as follows: high (>70), medium (56–69), and low (<55), and for the glycaemic loads: high (>20), medium (11–19), and low (<10). However, despite their low GI, the GLs assessed for these freeze-dried date yoghurt treatments were quite high but in line with a study by Miller et al. (2003) who assessed the GI and the GL of date yoghurt mixed meals. Although there was no

significant difference in GI between Barhi and Khassab treatments, there was a difference in the pattern of glucose uptake between the date-containing treatments and glucose, which means the blood glucose changed differently over time after the consumption of date-containing treatments compared to glucose. Therefore, blood glucose levels were considered a factor when analysing the cognitive performance data in the human intervention.

2.9 Conclusion

This chapter aimed to create novel date-containing treatments and a placebo to be used in the human nutritional trial to investigate the acute effects of consuming such treatments on mood and cognitive functioning in healthy young volunteers. Among the hundreds of date varieties cultivated in the KSA, five popular varieties, which are representative of different geographical regions, were nominated and screened for their phenolic content. The two date varieties with the highest content were selected and freeze-dried to produce a standardisable form of dates. The average consumption of date fruit in a sitting per capita in the Gulf countries which equates to 48 and 34.5 g of Barhi and Khassab, respectively, was used to define the experimental dose of dates for the treatments. Fat-free yoghurt (150 g per portion) was chosen to be the carrier of the freeze-dried dates and for the placebo. Iterative trial and observation were applied in the very early stages of the recipe development to help achieve both palatable and homogeneous treatments, which were similar enough to not easily be recognised by the participants. Although there was a statistically significant difference between Khassab and placebo in the sensory attribute texture, the treatment recipes can be considered successful in producing relatively similar treatments as discussed in section 2.8.1. The success of the blinding element was further confirmed by the satisfactory results of the sensory evaluation panel. The final treatments contained the freeze-dried powder dates in an equivalent amount to the average fresh fruit intake within KSA (115 g/day); which worked out to be 48 g of freeze-dried Barhi and 35.5 g of freeze-dried Khassab. The treatments contained different total phenolic contents, with 184 mg GAE/115 g FW for Barhi and 424 mg GAE/115 g FW for the Khassab treatment as determined using HPLC method. The placebo was matched for sugar

content but contained a very low phenolic content (16 ± 6 mg/150 g FW). Moreover, HPLC quantification of the TPC also revealed different TPCs for the same date varieties after mixing with yoghurt. The TPC for Barhi with yoghurt was 139 mg GAE/115 g FW, while that for Khassab with yoghurt was 238 mg GAE/115 g FW. This difference was considered when discussing the results of the human intervention.

To help provide a better understanding of the postprandial glucose response of the created and developed date-containing treatments, a trial to determine GI and GL was conducted on a small cohort.

Moreover, based on data gathered from a similar study which investigated the acute effects of the consumption of a different phenolic constituent (anthocyanin), the power calculation to determine the sample size for the human intervention was completed. A HACCP plan was made to provide the third party with guidance when making the treatments for the human trial (see Appendix KK).

Finally, the next chapter will discuss the double-blind, placebo-controlled, crossover study of thirty-six healthy young participants (18–35 years) to assess cognitive function using computerised tests for attention, working and episodic memory.

Chapter 3. An investigation of the acute effects of mood and cognitive function following the administration of two different varieties of Saudi date fruit on healthy young volunteers: A double-blind, placebo-controlled, crossover design

3.1 Introduction

The main focus of the current study, which is positive outcomes on mood and cognitive functions using the treatment of date extract, can be observed in animal research as detailed in section 1.12. The majority of the literature uses several tools to measure cognitive function and demonstrates inconsistent outcomes. While the phenolic content of dates has been considered as the main cause for interpretation and attribution of the mitigation of symptoms of the induced neurodegenerative diseases in experimental animals, the deficiency of data on the preparation of the date treatments used for supplementation is concerning. It was noted that a lack of information about the date variety, ripening stage and most crucially, the TPC quantification was apparent in a sizeable portion of the literature. This gives rise to complications in determining the precise effects on the alleged neuroprotective actions from phytochemical compositions due to the influences of differing cultivars, locations and stages of fruit picking on phenolic content (Baliga et al., 2011). Consequently, the beneficial association between the phenolic content consumption and the neuroprotective actions cannot be entirely conclusive, however, a separate example in the literature of a well-designed trial has presented a definitive benefit of anthocyanins (Toufektsian et al., 2008). It is important to draw some attention to the fact that such benefits of phenolics still only apply to the long-term neuroprotective effects and not specifically relevant to short-term effects due to the long lifetime of cells. It is evident that there is a scarcity of acute studies investigating the effect of dates on animals' cognition.

Reflecting on the phenolic content of date fruit, a review of approximately eighty studies (Al-Farsi and Lee, 2008) concluded that date fruit is a good antioxidant source

with notable carotenoid and phenolic contents compared to other fruits and vegetables regarded as functional foods (Table 4). The quantification of the phenolic compounds of the five most popular and most consumed date varieties in the KSA was conducted using different methodologies as detailed in chapter 2, section: 2.7.2.1. The TPC obtained using Follin- Ciocalteu method of Ajwah, Khalas, Barhi, Sukarri and Khassab ranged between 46.26 ± 10.26 to 293.51 ± 3.52 GAE mg/100 of fresh weight, while the TPC of the two chosen cultivars for this study were those with the highest phenolic content. Information about the TPC of Barhi and Khassab is available below in Table 18.

Table 18. Total phenolic content (TPC) of the chosen date cultivars (Barhi & Khassab) for the human intervention

Date cultivar	***First batch TPC (GAE mg/100 g) of FW***	***Second batch TPC (GAE mg/100 g) of FW***
Barhi	294 ± 4	159 ± 18
Khassab	233 ± 10	369 ± 12

*TPC was quantified by Folin-Ciocalteu for the first batch (to aid the selection of the date varieties with the highest TPC) and by HPLC for the second batch for the chosen and imported date varieties.

Currently, no peer-reviewed human interventions examining the acute or chronic effects of date fruit on mood and cognitive function have been published. However, an intervention study demonstrated outcomes of a 6-week intervention of consumption of Ajwah date fruits on cognitive processing, indicating a significant enhancement of reaction time of the Stroop, 1-back and 2-back working memory tests and the tension subscale of the profile of mood scale (POMS) (Abdullah et al., 2019). However, the study contained no control or placebo group. Welch et al. (2011) recommended a single or double-blind, placebo-controlled design when planning and completing nutritional intervention analyses to assess functional foods, and this was not adhered to. Therefore, there is a possibility of both researcher and participant bias due to the constraints of the design and methods utilised, thus challenging the reliability of the results obtained.

Existing literature was utilised to format the rationale of the current study, to assist the piloting of treatment design as a substitute route due to a deficiency in date research. Justification of each stage of the development plan for treatments and placebo creation was outlined in chapter 2, however, they have also been outlined within this section to aid consideration of the anticipated effects.

An important intervention study is the randomised, controlled, double-blinded, balanced, crossover trial conducted by Scholey et al. (2010) which observed the effect of drinks containing 520 mg or 994 mg of cocoa flavanols compared to a matching control on young, healthy adults. They aimed to test the efficacy of cocoa flavanols on the alleged cognitive improvements but obtained both differing and conflicting outcomes. In comparison to the placebo, the consumption of both active drinks resulted in increases in working memory and/or psychomotor performance (as evaluated by the serial threes subtractions task). Only the 994 mg drink, in comparison to the placebo, provided observations for enhancements in sustained attention (comprehended from the reduced reaction times in the rapid information processing task). However, this dose also gave rise to more errors in the serial sevens subtractions task. Conversely, supplementation with 520 mg increased self-reported mental fatigue (Scholey et al., 2010). Moreover, Field et al. (2011) reported that the acute consumption of dark chocolate (containing 720 mg cocoa flavanols and 38 mg caffeine) versus white chocolate elicited enhancements in visual contrast sensitivity (ability to read numbers which increasingly become more alike in luminance to the background) and reduced the time taken to distinguish motion direction after 90 minutes. This outcome establishes a refinement in visual-spatial memory tasks after the consumption of dark chocolate (Field et al., 2011).

A further example of intervention studies is those which have evaluated the consumption of berries on cognitive functions in a young, healthy cohort. Four published interventions stated differing outcomes. Acute supplementation with grape juice on implicit memory or mood provided evidence of no significant effects, (Hendrickson and Mattes, 2008), whereas the acute administration of 368–968 mg of polyphenols acquired from an anthocyanin-enriched blackcurrant drink (DelCyan™)

significantly enhanced rapid visual information processing task accuracy in young, healthy adults (Watson et al., 2015). Furthermore, a blackcurrant-based treatment comprising 500 mg/serving was investigated in a double-blind and placebo-controlled crossover design and had a positive statistically significant effect on the speed of responses during the choice reaction time task in young, healthy volunteers, post-supplementation of the blackcurrant treatment (Watson et al., 2019). Also, a comparable dose of 578.82 mg/30.1 g sourced from blueberry extracts statistically significantly positively affected a variety of tasks in the global cognitive function post-consumption of the control compared to the enriched treatment group (Dodd et al., 2019). All of the aforementioned studies are summarised in Table 4.

Furthermore, the time to reach maximum concentration C_{max} of a single dose of 150 mg of anthocyanins from berries in humans has been reported to be 1.5 h with a range from 0.75 to 4 h in plasma (Manach et al., 2005), while Qadir (2017) reported the C_{max} concentration of chlorogenic acid from 70 g of lettuce to be 4.5 h with a range from 3 to 6 h. Therefore, the cognitive tests with the maximum concentration of the date phenolics were considered when designing the study.

To conclude, the information discussed above provides a starting point for this research, which includes three measures: the precise and defined form of dates with defined maturation stage and phytochemical composition, objective computer-based measures for cognitive performance, and meticulous method designs, which integrate paradigms that have displayed sensitivity in comparable nutritional interventions for animal models and human trials.

The aim of the current study was therefore focused on the impact of two standardised dates on various cognitive functioning indices and mood. The form of dates used was a freeze-dried form of the date cultivars commercially available in the KSA. Both date cultivars were matched for their quantities of sugars, colour and flavour but contained differing phenolic profiles and quantities. Due to the phenolic acids being hypothesised as the major active compounds in dates, the cognitive assessments were conducted with the phenolic acid levels at maximum concentration C_{max}; between 45- and 135-minute post-supplementation. As reviewed, biofluids were not included in the

collection or analysis, and consequently were not used to consider the bioavailability of the phenolic content.

3.2 Study design

The study investigated the acute effects on human cognitive function and mood after the administration of a single dose of two different date fruit yoghurts against a control matched for sugar, flavour, fibre, taste and appearance but with different quantities of polyphenols and differing phenolic profiles. The study followed a double-blind, counterbalanced, placebo-controlled, repeated measures design to investigate the effects of two standardised date extracts on cognitive functioning and mood. The date extracts were freeze-dried powder of two different date varieties: Barhi and Khassab in the Bisir maturation stage. The dose of each date variety was equivalent to the KSA average fresh fruit intake, 115 g/day. This was obtained with 48 g of Barhi and 34.5 g of Khassab in freeze-dried powder with 184 mg GAE/115 g FW and 424 mg GAE/115 g FW respectively as determined using the HPLC method. Detailed information regarding the quantification of the TPC and the execution of the study treatments can be found in chapter 2, section 2.7.2.

3.3 Methods

3.3.1 Ethical approval

The study was approved by the Faculty of Medical Sciences (FMS) Ethical committee at Newcastle University. The application number was 1408/118/2017 and registered on ClinicalTrial.gov (NCT03350100) (see Appendix L and Appendix M).

3.3.2 Participants

A total of 36 healthy participants aged between 18 and 35 were recruited through advertisement via poster and flyer from Newcastle University and each received a £20 voucher as a gesture for their participation. The number of participants required was estimated according to a power calculation based on alertness, as explained in detail

in chapter 2, section 2.7.6. According to the power calculation, 34 participants were needed to achieve a power of 95%, however, recruitment was stopped after a total of 36 participants were successfully recruited to compensate for possible dropout.

Due to missing data, full data sets were only obtained for 35 of the participants. Before enrolment commenced, all participants attended a 120-min screening-training session. The demographic data are provided in Table 19.

Table 19. Descriptive statistics of the participant characteristics

(n= 35; 23 females and 12 males).

Measured Characteristics	Min	Max	Mean	SD
Age	18	35	24	4.5
Height (cm)	145	191	168	11
Weight (kg)	43.1	98.3	67.5	14.5
BMI	18.2	31.0	24.2	3.5

3.3.2.1 Screening and training

The screening ensured that any contraindications to the study were identified, and the training aimed to remove any practice effects by completing four repetitions of the study tasks. During the screening-training session, the participants signed a consent form and reported themselves to be healthy, not pregnant, not consuming any over the counter medication or supplementations, had no food allergies or sensitivity to the treatment constituents, were non-tobacco users and had a body mass index below $35/m^2$ but no lower than $18/m^2$.

Screening for any contraindications to the study was completed with the use of an exclusion questionnaire and a case report form (see Appendix N, in particular, pages S3 and S4 for the questionnaire).

3.3.3 Study treatments

Participants received the three treatments of yoghurt mixtures in an order dictated by random allocation to a counterbalancing (Williams Latin Square) order, with at least a one-week washout between the visits (see Figure 24 for an illustration).

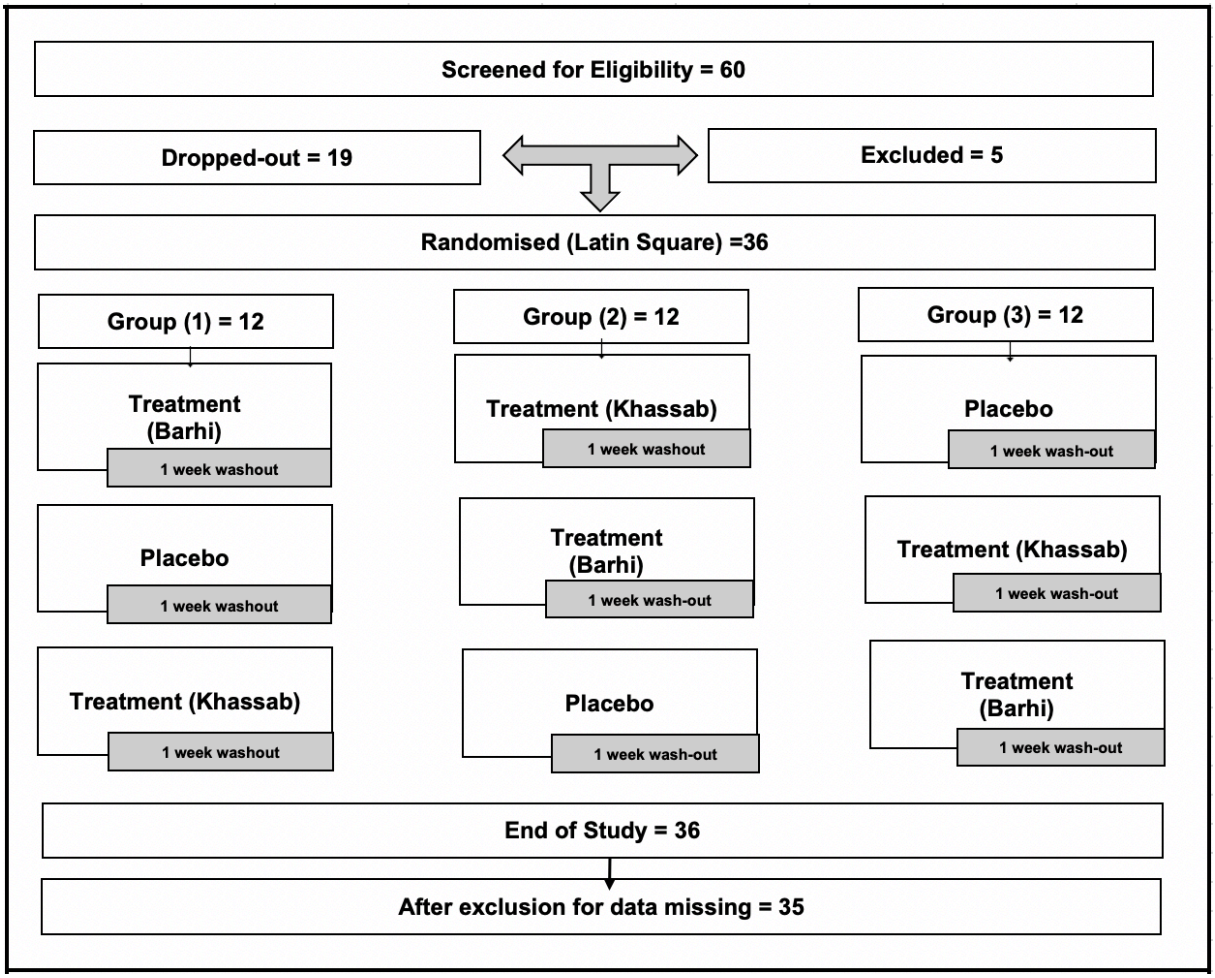


Figure 24. Overview of the study process and management of participants

The date extracts were assessed for their phytochemical constituents as described in chapter 2, sections 2.7.2 and 2.7.3 (see Table 20 for treatment details). Yoghurt was used as the vehicle for the freeze-dried powders to ensure participant blinding, with a yoghurt control containing a low phenolic content of 16.30 mg also produced. The naturally occurring sugars in both of the date varieties were quantified using the Barhi variety with the highest sugar level as a reference, and both Khassab and placebo

were supplemented with the appropriate amounts of sugars (Table 2) to ensure caloric equality. In each case, all treatments were matched for sugars and contained 16.8 g of glucose and 15.8 g of fructose. The date freeze-dried powder extracts were stored frozen in the walk-in freezer at the NU-Food facility at Newcastle University until the day of use.

Table 20. The treatment ingredients

Ingredients	***Barhi treatment***	***Khassab treatment***	***Placebo***
Total phenolic content (TPC) as determined using HPLC	184 (mg/115 g FW) GAE	424 (mg/115 g FW) GAE	16 (mg/150 g) GAE
Date powder	48 g	34.5 g	0 g
0% fat yoghurt	150 g	150 g	150 g
Naturally available glucose	16.78 g	11.85 g	0 g
Added glucose for matching	0	4.94 g	16.78 g
Naturally available Fructose	15.84 g	9.36 g	0 g
Added fructose for matching	0	6.48 g	15.84 g

Treatments were coded and prepared fresh each morning by a third party who had no further part in the running of the study. The treatments were served in a plastic bowl, covered with foil and labelled with the participant number to ensure blinding. No member of the investigation team was aware of the treatment coding until a blind-data review was completed, therefore until after the completion of the statistical analysis. Food safety during treatment execution was ensured throughout the investigation according to the HACCP plan available in Appendix KK.

The sensory evaluation panel conducted in chapter 2 indicated a successful blinding procedure which aimed to cause uncertainty regarding which of the mixtures were date-containing treatments and which one contained the placebo. However, a further

question was added to the written debrief of the current study, which was given to the participants after the completion of the trial, which was as follows:

“Which of the three treatments you had was the date-containing yoghurt?”This question aimed to monitor the success of the blinding procedure.

3.3.4 Cognitive function test CogTrack

An arrangement of nine online cognitive tests in the CogTrack system (Wesnes Cognition) was used to assess cognition. This is a software application which was purposely designed for the flexible delivery of randomly generated parallel versions of standard and novel cognitive assessment tasks. These tasks have previously been shown to be sensitive to a range of nutritional interventions and are reliable, sensitive and valid for detecting cognitive changes caused by an energy drink (Wesnes et al., 2017b) and polyphenols in blackcurrant (Watson et al., 2019). CogTrack is a platform which allows the delivery of standardised cognitive tests assessing aspects of attention, information processing, as well as working and episodic memory. For exploratory analysis purposes, these tasks were selected to assess attention performance and cognitive flexibility.

The nine tasks require approximately 18 minutes to perform, and the stimuli for all tasks are presented on the screen. The instructions for each task are presented on the computer screen and remain there until the volunteer initiates the test by pressing the right arrow on the keyboard. The in-task responses are also made using the computer keyboard. The tasks are described below in the order in which they were administered.

3.3.4.1 Immediate word recall (Wesnes et al., 2017b)

One word every 2 seconds is presented on the screen, with a total list of fifteen words, for the volunteer to remember. The volunteer is then provided with one minute to recall all words by typing, in any order, using the computer keyboard, with the number of words which were correctly recalled and those which were not in the list (errors), recorded.

3.3.4.2 Pattern separation (Wesnes et al., 2017b)

The volunteer is instructed that the pictures in this first stage will be reshown later mixed with very similar ones. The volunteer is then presented with a series of twenty pictures on the computer screen, one every 3 seconds of everyday scenes and objects.

3.3.4.3 Simple reaction time (Wesnes et al., 2017b)

The volunteer is informed that only one stimulus, a 4.5 cm by 3.5 cm image of a right-facing arrow containing the word 'YES' will be presented and remain on the screen until a response is made. Fifty stimuli are presented with an inter-stimulus interval which varies randomly between 1–3.5 s. The volunteer is instructed to press the right arrow key on the keyboard as quickly as possible every time a stimulus is presented in the centre of the screen. Throughout the test, the volunteer is required to keep the right index finger resting lightly on the right arrow key and the speed of each response is recorded.

3.3.4.4 Digit vigilance (Wesnes et al., 2017b)

A target digit between 1-9 is randomly selected and constantly displayed on the right-hand side of the screen. The volunteer is required to keep the right index finger resting on the right arrow keyboard key throughout the task and instructed to press the right arrow key as quickly as possible every time a digit matches the target digit as the digits are presented one at a time in the centre of the screen at a rate of 150 per minute. A total of 450 digits are presented, with fifteen target digits in each block of 150 digits. The digits and the target digit are 2 cm wide by 3 cm high. Correct detections, the speed of the detections and responses made in error (false alarms) are recorded.

3.3.4.5 Choice reaction time (Wesnes et al., 2017b)

The two possible stimuli in this task are either the right-facing arrow used in the Simple reaction time or an equivalently sized left-facing version of the arrow with the word 'NO' in the middle. The volunteer is required to maintain the left and right index fingers resting lightly on the appropriate keyboard keys and to respond as quickly and

accurately as possible. On each of fifty successive trials, one of the two stimuli is selected randomly (but with equal probability) and presented in the centre of the screen, remaining there until a response is made. The interval between successive trials varies randomly between 1–3.5 seconds, and the accuracy and speed of each response by the volunteer are recorded.

3.3.4.6 Spatial working memory (Wesnes et al., 2017b)

A 3×3 array of light bulbs is presented on the screen for 10 sec. Four of the nine light bulbs are lit and the volunteer has to remember the position of the lit bulbs. There are then thirty-six subsequent presentations of the 3×3 array, each time with only a single bulb lit. Over the thirty-six presentations, each of the nine bulbs is lit on four occasions, the order being randomised, following presentation rules that the two same 'lit positions' should not be presented consecutively and no more than four target or distractor stimuli should be presented consecutively. For each presentation, the volunteer is required to decide whether or not the lit bulb was one of those lit in the original presentation, pressing the right keyboard arrow if it was and the left if it was not. The images remain on the screen until a response is made, the volunteer again being required to maintain the index fingers resting on the left and right keyboard arrows and to respond as quickly and accurately as possible. The accuracy and speed of each response are recorded.

3.3.4.7 Numeric working memory (Wesnes et al., 2017b)

A series of five different digits is presented on the screen at the rate of one every 1.2 seconds and the volunteer is instructed to hold these digits in memory. This is followed by a series of thirty probe digits, each of which remains on the screen until a response is made. In the series of thirty probe digits, each of the digits 0–9 is presented three times in a randomised order following the presentation rules that the same digit should not be presented consecutively and no more than four target or four distractor stimuli should be presented consecutively. The volunteer again maintains the index fingers resting on the left and right keyboard arrows throughout the task. For each stimulus, the volunteer has to indicate whether or not it was in the original series, pressing the

right keyboard arrow if it was and the left if it was not, as quickly and accurately as possible. The accuracy and speed of each response are recorded.

3.3.4.8 Delayed word recall (Wesnes et al., 2017b)

The number of words correctly recalled and the number of words recalled that were not in the original list (errors) presented earlier are recorded as the volunteer is provided one minute to type as many of the words, in any order, using the computer keyboard.

3.3.4.9 Word recognition (Wesnes et al., 2017b)

The fifteen original words plus fifteen distractor words are presented one at a time in a randomised order, and each word remains on the screen until a response is made. The volunteer again maintains the index fingers resting on the left and right keyboard arrows, and for each word is required to indicate whether or not it was from the original list of words by pressing the right keyboard arrow if it was and the left if it was not as quickly as possible. The accuracy and speed of each response are recorded.

3.3.4.10 Pattern separation (Wesnes et al., 2017b)

In this second stage the original pictures, plus the twenty very similar distractor pictures are presented one at a time in a counterbalanced order. Each picture remains on the screen until a response is made. The volunteer again maintains the index fingers resting on the appropriate keyboard arrows throughout the task, and for each picture the volunteer has to indicate whether or not it was the precise picture shown earlier, pressing the right keyboard arrow if it was and the left if it was not as quickly and accurately as possible. Half of the original pictures are presented before the very similar distractor versions and half afterwards. The accuracy and speed of each response are recorded.

3.3.4.11 Core measures from the CogTrack tasks

The selected nine tests are comparable to the outcome measures used in the standard battery of cognitive tests in the CDR system, including immediate/delayed word recall,

word recognition, picture recognition, simple reaction time, digit vigilance, choice reaction time, numeric working memory and spatial working memory.

Moreover, the eight indices of core measures on CogTrack combine the same task measures as used in the CDR system to form the factor scores named: the power of attention, continuity of attention, quality of memory and speed of memory etc. Composite scores, or cognitive indices, were repeatedly used in an assortment of interventions assessing cognitive functioning such as Kennedy et al. (2000), Scholey and Kennedy (2004), and Wesnes et al. (2003).

The calculation of each index is described below, however, a full description of the equation of each index can be found in Appendix EE. Furthermore, a diagram to show the composites of each index can be seen in Figure 25.

Attentional Intensity Index: The three-speed scored attention tasks, digit vigilance, simple and choice reaction time were summed to form a score, named the attentional intensity index and the accuracy scores were combined.

Sustained attention index: The accuracy scores of digit vigilance, simple and choice reaction time tasks were combined into a score named the sustained attention index.

Attentional fluctuation index: Derived by calculating the combined scores for the three tasks of digit vigilance, choice reaction time and simple reaction time.

Cognitive reaction time index: Derived by calculating the combined scores of the two tasks of choice reaction time and simple reaction time.

Working memory capacity index: The accuracy scores from the spatial working memory and numeric working memory tasks were combined to form the working memory capacity index.

Memory retrieval speed index: The speed scores from the numeric working memory, spatial working memory, word recognition and pattern separation tasks loaded together on a single factor and combined to create the speed of retrieval index.

Episodic memory capacity index: Derived by calculating the combined percentage accuracy scores (adjusted for the proportion of novel and new stimuli where appropriate) from all secondary memory tests: word recognition, picture recognition, immediate word recall and delayed word recall (with adjustment to the total percentage, corrected for errors and intrusions on the latter two tasks).

Quality of memory index: Derived by calculating the combined percentage accuracy scores (adjusted for the proportion of novel and new stimuli where appropriate) of all working memory tests and secondary memory tests: spatial working memory, numeric working memory, word recognition, picture recognition, immediate word recall and delayed word recall (with adjustment to the total percentage corrected for errors and intrusions on the latter two tasks).

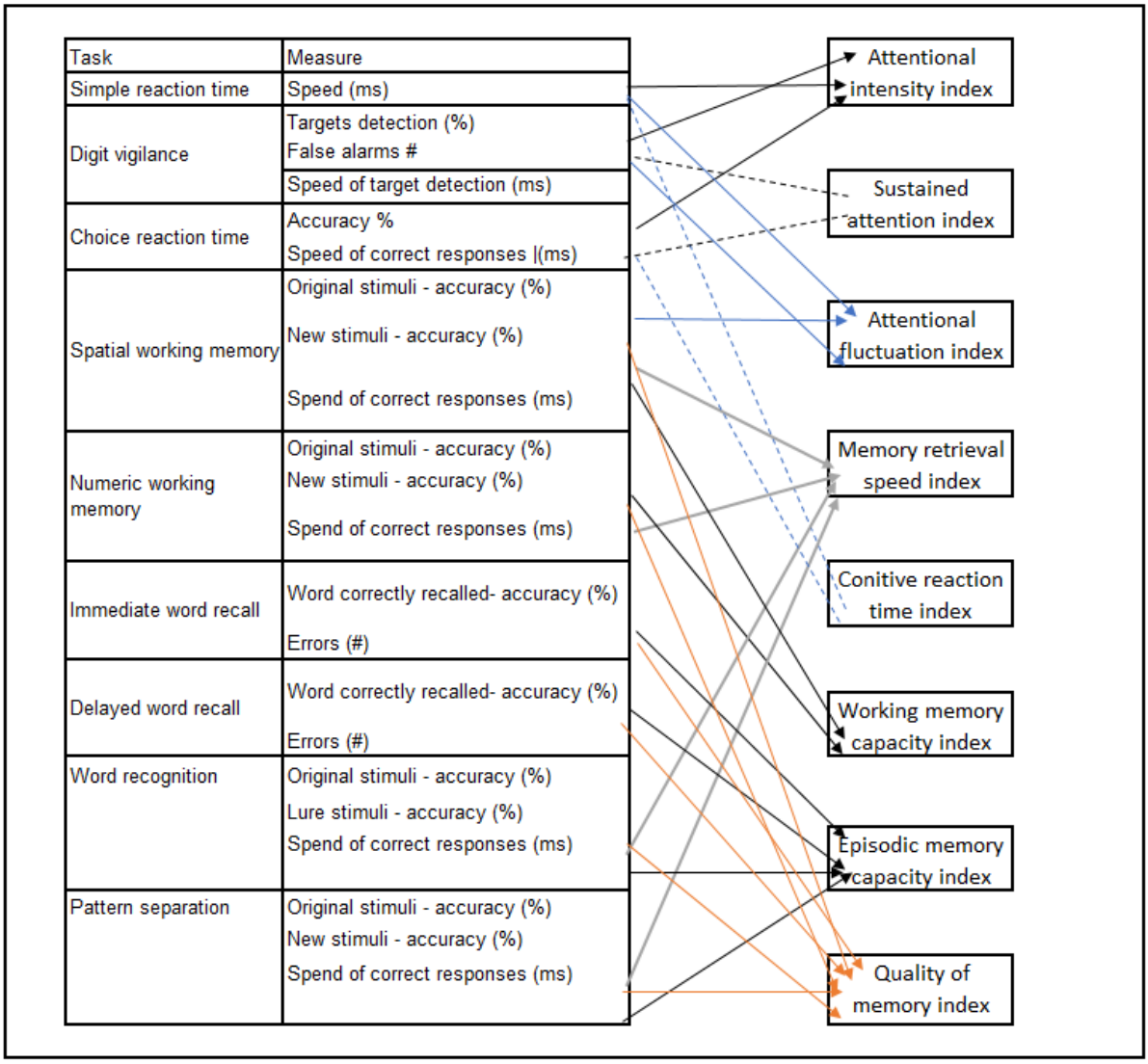


Figure 25. Graphic representation of the core measures for the CogTrack battery

Showing (from left to right) the running order of tasks, individual task outcome measures and the composition of the eight indices derived by factor analysis. Arrows indicate that a task outcome measure contributes to the given index: Attentional intensity, Sustained attention, Attentional fluctuation, Memory retrieval speed, Cognitive reaction, Working memory, Episodic memory and Quality of Memory (adapted from (Kennedy et al., 2000).

3.3.5 Mood scale: Bond-Lader VASs of mood and alertness

Bond-Lader visual analogue mood scales (Bond and Lader, 1974), as used in several nutritional intervention studies (Kennedy et al., 2006, Haskell et al., 2008, Haskell et al., 2010, Wesnes et al., 2017b) were employed. The scales comprise a total of sixteen

100 mm lines anchored at either end by antonyms (e.g. alert-drowsy, calm-excited) on which participants mark their current subjective position. To facilitate the data collection, the sixteen Bond-Lader visual analogue scales were converted into an electronic version by an expert member of staff from the Faculty of Natural and Environmental Sciences at the University of Newcastle. Scores from the sixteen Bond-Lader visual analogue scales were combined, as recommended by the authors, to form three mood factors: 'alert', 'calm' and 'content' (Bond & Lader, 1974). The reliability and validity of these visual analogue scales have been demonstrated (Ahearn, 1997). The calculation of each of the three factors is described in Appendix FF.

3.3.6 Profile of mood scale (POMS) (McNair et al., 1992)

The profile of mood scale questionnaire (POMS) is a well established (McNair, 1992), factor-analytically derived measure of psychological distress for which high levels of reliability and validity have been documented. POMS has been used in several studies such as Wesnes et al. (2017b) and (Kennedy et al., 2010) and norms have been published for a variety of patient and non-patient groups. The POMS questionnaires consist of sixty-five adjectives rated on a 0–4 scale that can be consolidated into 'depression-dejection', 'tension-anxiety', 'anger-hostility', 'confusion-bewilderment', 'vigour-activity' and 'fatigue-inertia' sub-scales. The latter two sub-scales can be interpreted as measures of fatigue and have been validated as separate factors in several studies. The purpose of using this mood scale was to assess the overall mood status of the participants before the treatment administration.

3.3.7 Blood glucose (BG)

Blood glucose was measured from a finger prick-puncture with the use of a portable BG monitor system (ACCUCHEK One call-EZ, Roche Ltd, Basel, Switzerland) at baseline, 45, 90- and 135-minutes post-supplementation. The ACCUCHEK reader has a reported coefficient of variance (CV) of less than 5%.

3.4 Procedure

Before the screening day commenced, the participants were asked to sign the informed consent form. See Figure 26 for an illustration of the study timeline.

The volunteers were instructed to limit their consumption of fruits and avoid alcohol for 24 hours before their arrival, as well as fasting and not consuming caffeine since 21:00 the previous evening. On each of the three study visits, the participants were required to attend the laboratory at 09:00, with each visit separated by seven days.

A baseline assessment of CogTrack, BG, Bond-Lader and the POMS scales was completed. The volunteers were then given the allocated treatment yoghurt and a cup of water, all of the treatment had to be consumed within fifteen minutes. BG samples and the Bond-Lader were taken after each performance of the CogTrack system and were repeated at 45, 90- and 135-min post-consumption of the treatment.

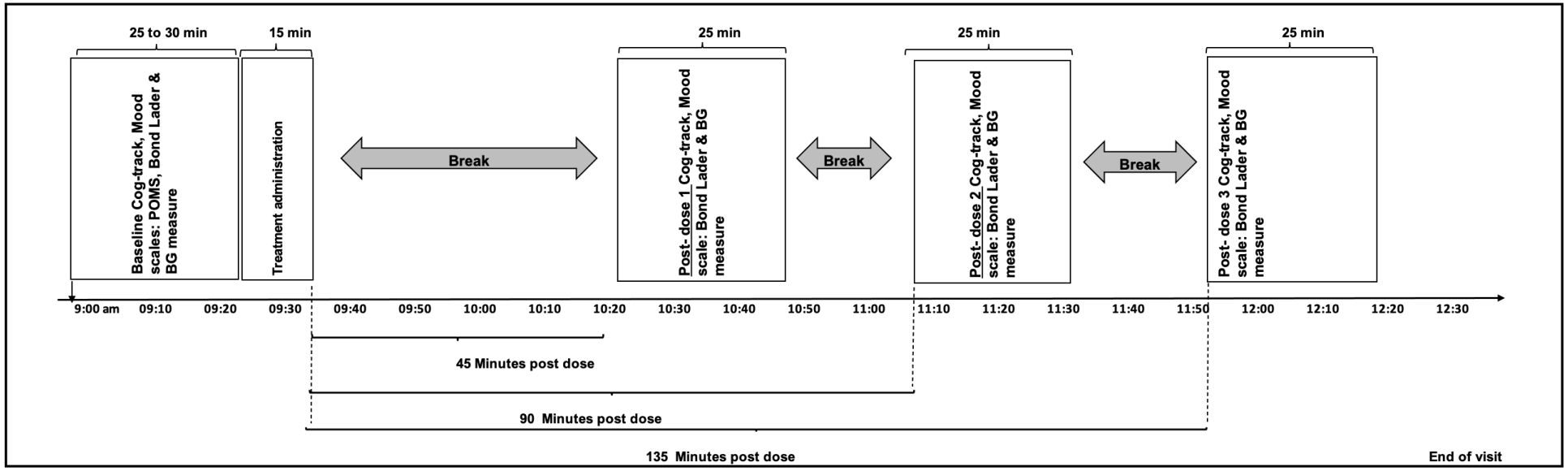


Figure 26. Study session timeline

3.5 Dietary restrictions and nonintervention foods

The volunteers were instructed to limit their consumption to a maximum of one portion of certain fruits such as oranges, apples, grapes, peaches, grapefruit juice, cherries, blueberries, pomegranate juice, raspberries, cranberries, black elderberries, blackcurrants, plums, blackberries, strawberries, apricots the day before each visit as they are rich in phenolic content. Additionally, they were asked to avoid alcohol 24 h before arrival, as well as have fasted and not consumed caffeine since 21:00 the previous evening. As the participants had been fasting since 21:00, they were provided with a standardised lunch to break their fast once the study day had been completed.

After the completion of the three visits, the participants were given a £20 voucher to thank them for their participation and a written debrief. In the debrief, there was a question regarding the treatment recognition as follows: “which of the three treatments you had was a date-containing yoghurt? You may choose more than one answer” (see Appendix P).

3.6 Statistical analyses

Most of the utilised cognitive tasks were orthogonal or statistically independent, and the usual approach in this field is not to adjust for multiple statistical comparisons. However, due to the novelty of this research, it was a challenge to predict which cognitive domains were sensitive to the phenolic content of dates and thus there were difficulties in pre-defining one outcome as the primary outcome. Also, it was difficult to find a very definitive instruction from the literature regarding which indices may be correlated or orthogonal. Therefore, an adjustment for multiple comparisons was used. The purpose of applying a correction for multiplicity during the data analysis was not meant to discard or undermine any detected effects but rather to express caution in the interpretation of the results for such original treatments. The use of adjustment for multiple comparisons was well explained and justified in (Chen et al., 2017). Consequently, two statistical approaches that represented two different schools of thought, less cautious and cautious, were applied for the cognitive and mood

outcomes. In the less cautious approach, linear mixed model analyses (MML) were conducted on the change from baseline data for all outcomes (CogTrak: individual tasks and indices, and Bond-Lader mood scales outcomes). Study treatments (three levels), time of testing (three levels), and the interaction between study treatment and time of testing were fitted as fixed factors. The analysis was conducted using the SPSS statistical package version 24 for windows. For measures which had significant main effects of treatment and/or interactions, between-condition pairwise comparisons were performed using simple mixed model repeated measure (MMRM) analyses of variance (ANOVAs) with multiple comparisons.

While in the more cautious approach, the mixed model linear (MML) was conducted on the same outcomes but with Bonferroni correction performed to identify the degree of any significant differences between the study treatment conditions and to protect from Type I errors arising from the multiple ANOVAs. The alpha level of 0.05 was divided by the number of variables measured as outcomes for cognitive function, therefore, the alpha level was divided by thirty for the thirty individual cognitive tasks, divided by eight for the eight measures for the CogTrack indices, and divided by three for the three measures for the Bond-Lader mood scales.

Furthermore, the baseline scores over the three study conditions for POMS mood scale outcomes were compared using MMRM ANOVAs to ensure that there were no systematic variations over the study conditions.

For the blind monitoring of the study treatments, Chi-square was calculated for the true and false answers in the survey provided to the participants in the written debrief (see section 3.3.2).

3.6.1 Supplementary analysis

Since all treatments were standardised for the sugar content, the data as changed from baseline were analysed again irrespective of treatment using multivariate analysis of variance (MANOVA). This approach aimed to detect the sugar effect on mood and cognitive performance. Moreover, to explore the change in blood glucose

in relation to cognitive and mood composites scores and the individual tasks scores, an analysis of the correlation matrix was performed. Linear mixed model analyses (MML) were conducted on the change from baseline data for any outcomes that showed a significant correlation. The interaction between study treatment and blood glucose levels and time of testing was fitted as a fixed factor.

Finally, Chi-square was used to examine the proportion of the positive to the negative outcomes to determine the general direction of each treatment effect. Therefore, the change from baseline for each treatment was pooled together for the Chi-square test. Summing the data from each treatment may cause result reinforcement which cannot be seen in an ANOVA. The Chi-square of positive and negative outcomes was calculated for each measure across the three treatments individually only when clear variations among the treatments were observed.

For the baseline of each of the three visits, the POMS, and the MMRM ANOVA's methodology was undertaken by using the actual scores obtained at baseline for each study visit. None of the POMS outcomes measures approached significance (all $p>0.05$), and thus the MML was conducted as planned.

3.7 Results

3.7.1 Missing data

There were some missing data which occurred during the trial: Subject three, Barhi, Baseline, tasks missing: numeric working memory reaction time (NWMNRT) and thus numeric working memory reaction time Median (NWMNRTM). Since the missing data occurred in the baseline scores, this participant was excluded from the data analysis of all measures: BG, POMS and Bond-Lader. Therefore, the number of participants decreased from 36 to 35 but the exclusion of this participant did not affect the sample size, as this study was powered to 95% by recruiting 34 participants.

3.7.2 Baseline

For the baseline of each visit, the CogTrack measures, Bond-Lader mood scales' factors and the MMRM ANOVA's methodology were undertaken by using the actual scores obtained at the baseline. None of the measures approached significance (all $p>0.05$), thus, the MML was conducted as planned.

3.7.3 CogTrack individual task outcomes

3.7.3.1 A less cautious approach

Immediate word recall task

There was a significant main treatment effect on the percentage of correct words recalled measure (F=3.79, P=0.02). The pairwise comparison revealed that the increase in the percentage of correct words recalled was significant for Barhi (P=0.041) and Khassab (P=0.048).

Digit Vigilance task

There was a significant main treatment effect on the average speed (msec) of the digit vigilance task (F=8.60, P=0.001) and the false alarms (F=3.08, P=0.047). The pairwise comparison revealed that the increase in the average speed was significant for Barhi (P=0.003), while the decrease was not significant for Khassab (P=0.87) and there was a significant difference between date treatments (P=0.001). The decrease in the false alarms was significant for Barhi (P=0.038) but not for Khassab (P=0.58), and no difference between date treatments was detected (P=0.33).

Delayed words recall

There was a significant main treatment effect on the percentage of the correct words in the delayed word recall task (F=4.72, P=0.01). The pairwise comparison revealed that the attenuation of the decline was significant for both Barhi (P=0.027) and Khassab (P 0.017), with no difference between date treatments (P=0.98).

Word recognition

There was a significant main treatment effect on the percentage of the original stimuli accuracy of the word recognition task (F=7.33, P=0.001). The pairwise comparison revealed that the increase was significant for Barhi (P=0.003) and Khassab (P=0.003), with no difference between date treatments (P=0.99).

However, for the remaining five CogTrack tasks, there was no statistically significant main treatment effect, repeated post-treatment test sessions, or an interaction between the two on most measures. The overall changes from pre-dose values for all CogTrack task measures are presented in table 21.

3.7.3.2 A more cautious approach

As explained in the statistical analysis section 3.6, a cautious approach was also used to increase the certainty about the previously reported tasks that had reached a significant level. Thus, the alpha level for the CogTrack tasks was corrected by Bonferroni correction by dividing the alpha level of 0.05 by 30 (number of outcomes) = 0.0017. Therefore, the results were readjusted with only the average speed (msec) of the digit vigilance task (F=8.60, P=0.001) remaining significant, while none of the CogTrack tasks reached significance (all p>0.005).

3.7.4 Indices of CogTrack, Bond-Lader and BG

3.7.4.1 A less cautious approach

Sustained attention index

There was a significant main treatment effect (F=3.644, P=0.029). The pairwise comparison revealed that the improvement was significant for Barhi (P=0.003) but not for Khassab (P= 0.99), and there was a significant difference between date treatments (P=0.004).

Episodic memory capacity index

There was a significant main treatment effect (F=3.134, P=0.047). The pairwise comparison revealed that the attenuation of the decline was significant for Khassab (P=0.012) but not for Barhi (P= 0.08), with no difference between date treatments detected (P=0.77).

Alertness index

There was a significant main treatment effect (F=6.033, P=0.003). The pairwise comparison revealed that the increase in alertness level was significant for Khassab (P=0.005) but not for Barhi (P= 0.63) and there was a significant difference between date treatments (P=0.001).

Calmness index

There was a significant main treatment effect on the calmness index (F=4.868, P=0.009). The pairwise comparison revealed that the reduction for Khassab was significant (P=0.001) but the enhancing effect for Barhi was not (P=0.43), and there was a significant difference between date treatments (P=0.02).

However, for the remaining six CogTrack indices, there was no statistically significant main treatment effect, of repeated post-treatment test sessions, or an interaction between the two on most indices. The overall changes from pre-dose values for all CogTrack, Bond-Lader and blood glucose measures are presented in Table 22.

3.7.4.2 A more cautious approach

The same cautious approach used in 3.7.3.2 was used for the CogTrack indices to increase the certainty about the two indices. Thus, the alpha level for the CogTrack indices was corrected by Bonferroni correction by dividing the alpha level of 0.05 by eight (number of indices) = 0.00625. Also, the alpha level for the Bond-Lader factors was corrected by Bonferroni correction by dividing the alpha level of 0.05 by three (number of factors) = 0.0166. Therefore, the results were readjusted with none of the CogTrack indices reaching significance (all p>0.0045). However, both mood indices,

alertness and calmness, reached significance (all p<0.0166) and remained significant after the Bonferroni correction.

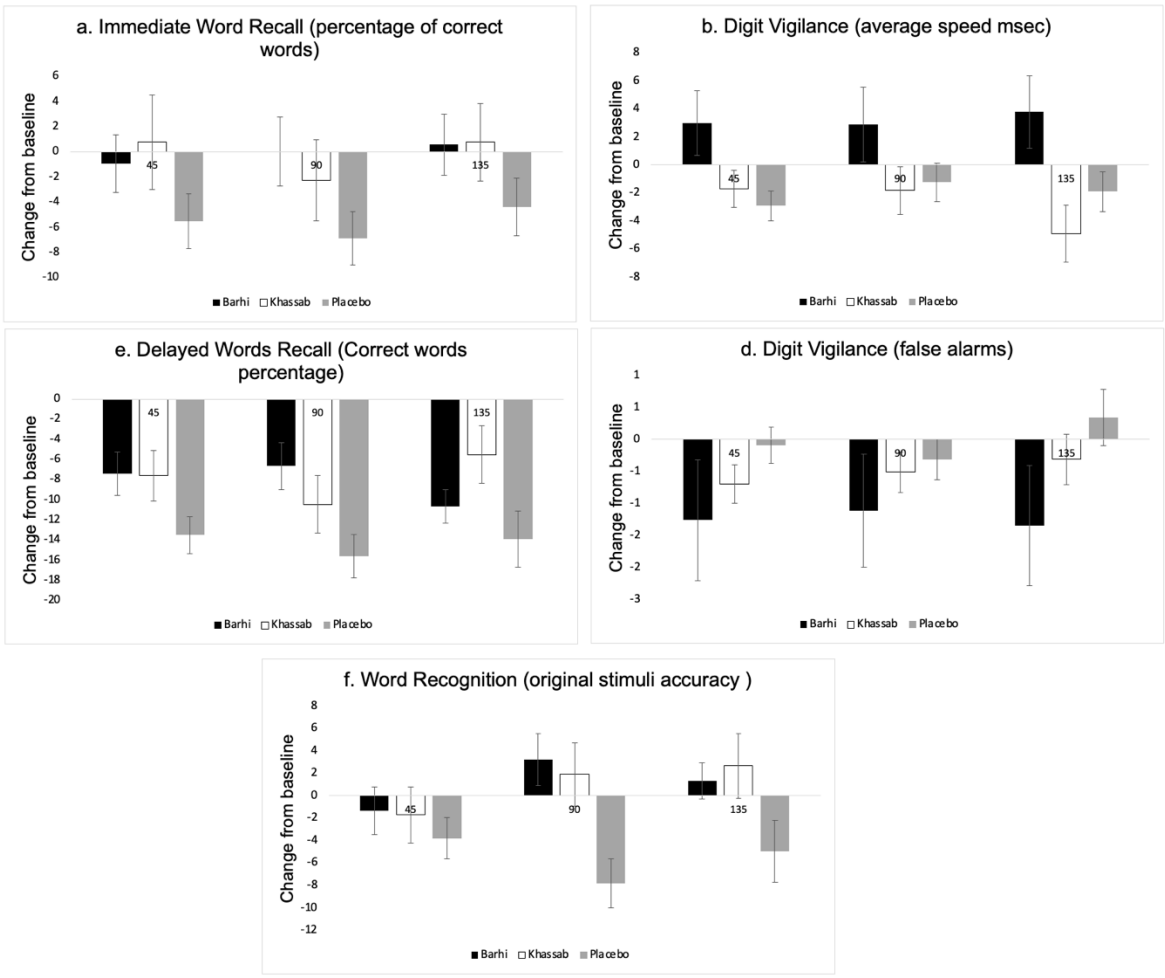


Figure 27. Main effects of change from baseline scores for the CogTrack task outcomes

(a) Percentage of immediate word recall task, (b) average speed for digit vigilance task, (c) percentage of targets detected for digit vigilance task, (d) false alarms for digit vigilance, (e) percentage of delayed word recall task, and (f) original stimuli accuracy of the word recognition task. Scores are presented as means and standard errors. Descending scores reflect impairments compared to baseline levels, and ascending scores reflect improvements, except for average speed and false alarms where descending and ascending scores indicate the opposite.

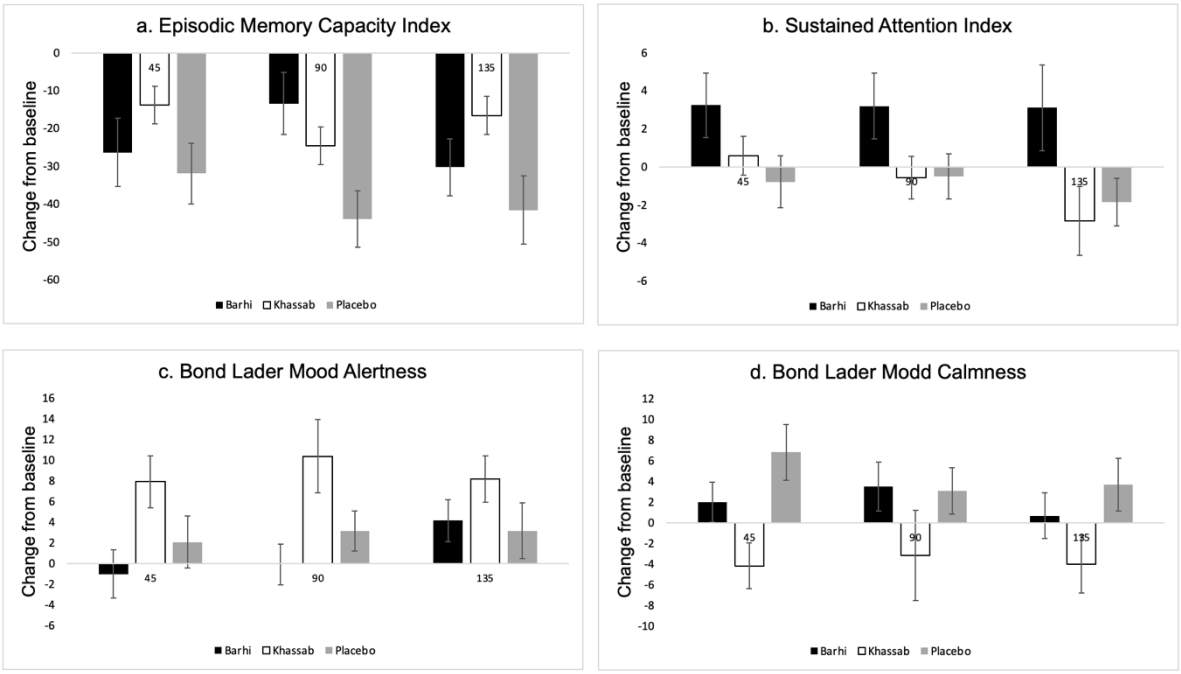


Figure 28. Main effects of change from baseline scores for the core measures.

(a) Episodic memory capacity index, (b) sustained attention index, (c) alertness mood index, and (d) calmness mood index. Scores are presented as means and standard errors. Descending scores reflect impairments compared to baseline levels; ascending scores reflect improvements.

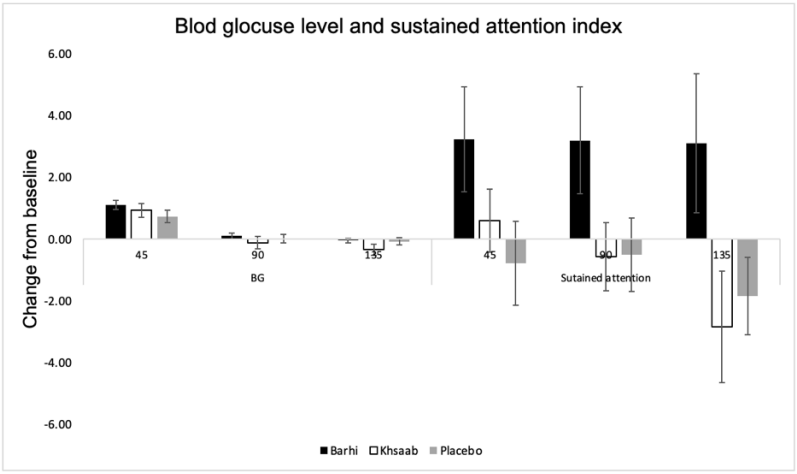


Figure 29. Main effects of change from baseline scores for blood glucose levels and sustained attention index.

Showing different patterns of treatments * time intervals interaction.

Table 21. The mean and standard error for pre-dose baseline and change from baseline and *ANOVA* for the individual tasks of CogTrack following the administration of two different varieties of date fruit (Barhi, Khassab) and a placebo mixed with 0% fat yoghurt (n=35; 23 females and 12 males)

Task	*Measure*	*Treatment*	*Baseline*	*Post-dose change*			*Effect of treatment*		*Effect of treatment*time intervals*	
			0 Min	*45 min*	*90 min*	*135 min*	*F*	*P*	*F*	*P*
Immediate Word Recall	*Correct words %*	*Barhi*	*44.44 ± 19.78*	*-0.95 ± 13.82*	*-0.00±16.41*	*0.57 ± 14.58*	*3.79*	*0.02*	*0.14*	*0.97*
		Khassab	*44.26 ± 14.79*	*0.76 ± 22.56*	*-2.2±19.40*	*0.76 ± 18.49*				
		Placebo	*47.9 ± 21.07*	*-5.52 ± 12.98*	*-6.85±12.67*	*-4.38 ± 13.07*				
	Number of incorrect words (errors)	*Barhi*	*0.58 ± 0.77*	*0.20 ± 0.68*	*0.06±1.00*	*-0.03 ± 0.79*	*0.47*	*0.62*	*0.16*	*0.96*
		Khassab	*0.42 ± 0.69*	*0.14 ± 1.06*	*0.14±1.03*	*0.03 ± 0.89*				
		Placebo	*0.56 ± 0.81*	*0.00 ± 0.73*	*0.09±0.89*	*-0.11 ± 0.83*				
Simple Reaction Time	*Average Speed msec*	*Barhi*	*354.37 ± 116.88*	*1.85 ± 80.94*	*-6.81±95.69*	*12.02 ± 104.88*	*0.85*	*0.43*	*0.08*	*0.99*
		Khassab	*348.6 ± 65.31*	*8.26 ± 40.87*	*11.58±45.52*	*13.02 ± 49.20*				
		Placebo	*376.38 ± 164.13*	*-6.72 ± 108.57*	*-16.24±144.11*	*-0.17 ± 178.00*				
Digit Vigilance	*Average speed (msec)*	*Barhi*	*467.4 ± 66.68*	*3.00 ± 13.82*	*2.89±15.95*	*3.79 ± 15.61*	*8.60*	*0.00*	*0.48*	*0.75*
		Khassab	*463.0 ± 46.97*	*-1.70 ± 7.90*	*-1.81±10.22*	*-4.90 ± 12.06*				
		Placebo	*465.30 ± 49.69*	*-2.91 ± 6.47*	*-1.23±8.13*	*-1.90 ± 8.52*				
	Targets Detected %	*Barhi*	*90.51 ± 15.49*	*0.15 ± 65.28*	*-4.00±62.31*	*0.90 ± 65.34*	*2.94*	*0.05*	*0.61*	*0.66*
		Khassab	*92.65 ± 8.65*	*12.25 ± 30.46*	*3.01±20.91*	*15.74 ± 33.67*				
		Placebo	*92.51 ± 9.96*	*17.43 ± 24.31*	*16.49±36.89*	*6.27 ± 39.55*				
	False Alarms	*Barhi*	*3.67 ± 5.94*	*-1.26 ± 5.65*	*-1.11±5.28*	*-1.34 ± 5.65*	*3.03*	*0.05*	*0.15*	*0.97*
		Khassab	*3.14 ± 3.96*	*-0.69 ± 1.81*	*-0.51±1.92*	*-0.31 ± 2.31*				
		Placebo	*1.97 ± 1.93*	*-0.09 ± 1.66*	*-0.31±1.92*	*0.34 ± 2.62*				
Choice Reaction Time	*Accuracy %*	*Barhi*	*95.89 ± 3.13*	*0.34 ± 3.71*	*0.51±3.57*	*-0.29 ± 3.46*	*0.18*	*0.84*	*0.24*	*0.92*
		Khassab	*95.78 ± 3.57*	*0.69 ± 2.82*	*-0.23±2.94*	*-0.74 ± 4.23*				
		Placebo	*95.17 ± 3.98*	*0.57 ± 4.24*	*-0.23±4.10*	*-0.51 ± 4.53*				
	Average Speed (msec)	*Barhi*	*471.70 ± 70.29*	*11.41 ± 79.14*	*-0.94±57.98*	*0.22 ± 66.10*	*1.24*	*0.29*	*0.03*	*1.00*
		Khassab	*462.1 ± 67.48*	*19.30 ± 98.51*	*3.30±75.44*	*10.92 ± 73.38*				
		Placebo	*472.25 ± 78.40*	*4.66±65.49*	*-9.15±51.96*	*-8.63 ± 65.45*				
Spatial Working Memory	*Original Stimuli Accuracy %*	*Barhi*	*94.10 ± 11.85*	*-2.14 ± 9.08*	*-1.07±6.51*	*-2.32 ± 7.43*	*1.77*	*0.17*	*0.09*	*0.99*
		Khassab	*94.79 ± 5.88*	*-1.96 ± 11.11*	*-1.96±12.57*	*-2.14 ± 9.93*				
		Placebo	*93.0 ± 8.81*	*-1.78 ± 12.63*	*0.36±11.13*	*0.89 ± 9.72*				
	New Stimuli Accuracy %	*Barhi*	*94.17 ± 10.59*	*-2.43 ± 13.63*	*1.00±5.92*	*-0.86 ± 7.12*	*2.31*	*0.10*	*0.68*	*0.61*
		Khassab	*96.25 ± 5.53*	*-0.57 ± 9.98*	*-2.29±9.87*	*-1.43 ± 7.03*				
		Placebo	*94.58 ± 8.57*	*1.14 ± 9.48*	*1.29±8.94*	*1.29 ± 9.87*				
	Original Stimuli Average Speed (msec)	*Barhi*	*709.16 ± 196.21*	*-27.70 ± 206.87*	*-40.21±140.03*	*-33.95 ± 161.24*	*0.80*	*0.45*	*0.20*	*0.94*
		Khassab	*706.57 ± 208.00*	*-66.12 ± 168.36*	*-62.49±169.96*	*-52.38 ± 179.65*				

Task	Measure	Treatment	Baseline		Post-dose change		Effect of treatment		Effect of treatment*time intervals	
			0 Min	45 min	90 min	135 min	F	P	F	P
		Placebo	653.06 ± 201.80	-15.15 ± 214.60	-29.17±199.77	-52.79 ± 170.82				
	New Stimuli Average Speed (msec)	Barhi	798.42 ± 264.91	-64.41 ± 148.99	-39.34±112.78	-8.62 ± 166.20	0.21	0.81	1.28	0.28
		Khassab	738.64 ± 209.49	-44.83 ± 156.31	-31.96±121.21	-33.27 ± 177.39				
		Placebo	691.41 ± 160.18	2.76 ± 142.26	-28.82±146.32	-51.19 ± 123.60				
	Average Speed	Barhi	760.55 ± 212.52	-48.16 ± 153.38	-39.04±104.19	-18.72 ± 143.57	0.44	0.65	0.68	0.61
		Khassab	724.69 ± 203.42	-53.88 ± 150.65	-45.18±127.71	-42.23 ± 168.54				
		Placebo	674.61 ± 169.76	-5.46 ± 156.83	-28.39±154.67	-52.26 ± 127.27				
Numeric Working Memory	Original Stimuli Accuracy %	Barhi	93.89 ± 6.83	-0.19 ± 9.76	-1.14±12.67	-2.85 ± 10.51	0.30	0.74	0.47	0.80
		Khassab	93.15 ± 7.39	-1.33 ± 8.21	-1.14±10.53	0.38 ± 9.69				
		Placebo	93.33 ± 8.43	-1.14 ± 10.73	-2.47±10.10	-1.71 ± 8.75				
	New Stimuli Accuracy %	Barhi	93.89 ± 16.82	0.19 ± 6.36	0.19±6.76	0.00 ± 6.26	3.79	0.02	0.41	0.80
		Khassab	97.41±3.99	-2.66 ± 7.08	-1.90 ± 7.15	-2.66 ± 7.61				
		Placebo	96.85±4.64	-0.95 ± 6.69	-3.04 ± 7.29	-1.71 ± 8.13				
	Original Stimuli Average Speed (msec)	Barhi	657.58 ± 192.76	2.91 ± 95.38	-30.23 ± 1 34.79	-21.48 ± 1 58.57	1.36	0.26	0.43	0.79
		Khassab	602.56±113.21	13.85 ± 74.70	22.84 ± 217.29	8.98 ± 80.11				
		Placebo	604.00±130.37	-14.17 ± 103.20	-20.61 ± 80.94	9.51 ± 239.63				
	New Stimuli Accuracy (msec)	Barhi	701.77±191.28	4.44 ± 154.12	-25.13 ± 174.15	52.64 ± 246.56	0.49	0.61	0.83	0.51
		Khassab	685.33±157.65	-19.64 ± 149.65	-31.52 ± 114.18	16.40 ± 188.89				
		Placebo	655.22±123.95	8.56 ± 118.47	1.27 ± 131.67	-13.46 ± 148.97				
	Average Speed (msec)	Barhi	688.81±197.79	3.39 ± 105.98	-27.34 ± 140.19	19.06 ± 195.53	0.04	0.96	0.23	0.92
		Khassab	644.91±122.71	-3.22 ± 101.60	-6.90 ± 130.33	11.85 ± 124.16				
		Placebo	629.76±119.30	-1.88 ± 100.00	-9.89 ± 88.38	-2.36 ± 166.00				
Delayed Word Recall	Correct words %	Barhi	37.96±18.91	-7.42 ± 14.07	-6.66 ± 13.52	-10.66 ± 14.11	4.27	0.01	0.62	0.65
		Khassab	34.81±17.39	-7.61 ± 22.41	-10.47 ± 21.46	-5.52 ± 20.09				
		Placebo	40.56±19.93	-13.52 ± 15.46	-15.61 ± 14.45	-13.90 ± 15.70				
	Number of incorrect words (errors)	Barhi	0.75±1.05	0.14 ± 1.12	0.03 ± 1.01	0.03 ± 1.22	0.04	0.96	0.50	0.74
		Khassab	0.64±0.80	0.09 ± 1.22	0.14 ± 1.30	0.03 ± 1.15				
		Placebo	0.72±0.91	-0.06 ± 1.08	0.09 ± 1.09	0.31 ± 1.27				
Word Recognition	Original Stimuli Accuracy %	Barhi	79.45±13.08	-1.33 ± 12.81	3.23 ± 13.94	1.33 ± 9.80	7.33	0.00	1.02	0.40
		Khassab	75.37±13.97	-1.71 ± 14.93	1.90 ± 17.07	2.66 ± 17.20				
		Placebo	77.04±12.79	-3.80 ± 11.11	-7.80 ± 13.08	-4.95 ± 16.67				
	New Stimuli Accuracy %	Barhi	88.70±13.78	-5.33 ± 15.15	-3.42 ± 11.92	-8.95 ± 13.70	0.04	0.97	0.62	0.65
		Khassab	91.67±9.48	-4.00 ± 9.17	-6.09 ± 12.87	-6.66 ± 12.20				
		Placebo	91.30±12.73	-4.76 ± 9.91	-6.85 ± 11.02	-6.47 ± 13.95				
	Original Stimuli Average Speed (msec)	Barhi	945.43±438.88	13.38 ± 728.14	-69.99 ± 489.41	-135.58 ± 452.11	2.09	0.13	0.64	0.64
		Khassab	795.66±221.99	15.58 ± 350.58	66.69 ± 353.96	106.93 ± 134.61				
		Placebo	782.16±215.76	24.31 ± 257.80	-25.07 ± 305.31	-31.18 ± 236.72				
	New Stimuli Average Speed (msec)	Barhi	936.04±250.98	-11.72 ± 194.67	-36.58 ± 170.05	-77.78 ± 183.73	1.23	0.29	1.48	0.21
		Khassab	892.54±342.09	-44.01 ± 288.84	-40.30 ± 310.30	114.09 ±687.62				

Task	Measure	Treatment	Baseline	Post-dose change			Effect of treatment		Effect of treatment*time intervals	
			0 Min	45 min	90 min	135 min	F	P	F	P
Pattern Separation	Average Speed (msec)	Placebo	812.33±232.84	67.74 ± 284.72	-4.01 ± 249.52	14.27 ± 275.90	1.74	0.18	1.66	0.16
		Barhi	944.20±296.42	5.53 ± 390.28	-51.68 ± 263.53	-113.44 ± 266.88				
		Khassab	848.57±250.69	-35.90 ± 202.14	3.85 ± 257.62	106.09 ± 629.24				
	Original Stimuli Accuracy %	Placebo	792.50±193.52	51.43 ± 239.54	-13.35 ± 182.20	-11.74 ± 184.78	0.36	0.70	0.28	0.89
		Barhi	81.53±14.78	-2.57 ± 14.47	-2.43 ± 16.42	-5.71 ± 13.94				
		Khassab	76.94±15.37	1.14 ± 14.95	-3.57 ± 16.20	-3.00 ± 16.27				
	New Stimuli Accuracy %	Placebo	74.72±17.65	-1.00 ± 12.17	-2.29 ± 16.42	-4.86 ± 14.01	1.19	0.31	0.19	0.94
		Barhi	79.03±13.03	-6.43 ± 11.73	-3.57 ± 11.85	-6.86 ± 13.23				
		Khassab	77.36±13.86	-0.86 ± 13.90	-2.14 ± 15.20	-4.43 ± 16.12				
	Original Stimuli Average Speed (msec)	Placebo	79.44±15.01	-3.71 ± 14.77	-3.43 ± 17.48	-5.71 ± 17.62	0.18	0.83	0.27	0.90
		Barhi	1048.83±314.25	59.25 ± 240.41	53.16 ± 345.56	-0.86 ± 295.10				
		Khassab	1081.11±309.14	69.27±790.00	-21.11±464.27	-8.69 ± 460.93				
	New Stimuli Average Speed (msec)	Placebo	918.49±220.26	26.15±182.89	69.15±378.72	50.12 ± 429.89	1.73	0.18	0.87	0.48
		Barhi	1065.06±184.07	138.67±678.08	89.80±424.86	-39.25 ± 173.08				
		Khassab	1125.42±619.11	60.61±447.42	-49.79±223.30	-77.71 ± 278.57				
	Average Speed (msec)	Placebo	974.17±230.06	39.96±263.89	119.24±514.37	104.53 ± 704.96	0.64	0.53	0.67	0.61
		Barhi	1057.44±227.22	88.19±378.03	66.09±346.53	-21.50 ± 210.00				
		Khassab	1098.22±408.02	70.30±609.29	-34.35±264.69	-28.62 ± 252.67				
		Placebo	947.89±214.41	25.77±201.67	77.50 ± 393.34	77.92 ± 567.43				

Table 22. The mean and standard error for pre-dose baseline and change from baseline and *ANOVA* for CogTrack, Bond-Lader mood and BG measures following the administration of two different varieties of date fruit (Barhi, Khassab) and a placebo mixed with 0% fat yoghurt **(**n= 35; 23 females and 12 males)

Measures	*Treatments*	*Baseline*	*Post-dose change*			*Effect of treatment*		*Effect of treatment* time intervals*	
		0 Min	***45 Min***	***90 Min***	***135 Min***	***F***	***P***	***F***	***P***
Attentional Intensity Index	Barhi	1230.41 ± 22.47	2.16 ± 12.15	8.22 ± 13.31	13.86 ± 15.85	0.74	0.48	0.83	0.51
	Khassab	1223.19 ± 23.06	15.12 ± 15.12	11.28 ± 10.37	29.66 ± 13.67				
	Placebo	1234.52 ± 23.85	28.62 ± 11.50	14.77 ± 9.23	11.69 ± 13.32				
Sustained Attention Index	Barhi	87.71 ± 2.22	3.24 ± 1.70	3.20 ± 1.73	3.10 ± 2.25	3.64	0.03	0.40	0.81
	Khassab	89.69 ± 1.07	0.59 ± 1.02	-0.57 ± 1.11	-2.84 ± 1.80				
	Placebo	89.47 ± 1.36	-0.79 ± 1.36	-0.50 ± 1.19	-1.85 ± 1.25				
Attentional Fluctuation Index	Barhi	73.47 ± 4.58	3.71 ± 6.27	-6.82 ± 4.98	1.09 ± 5.82	1.76	0.18	0.65	0.62
	Khassab	67.91 ± 4.42	7.89 ± 5.70	2.76 ± 5.55	6.21 ± 5.13				
	Placebo	76.79 ± 5.72	-3.32 ± 5.68	-2.88 ± 4.92	-3.63 ± 5.12				
Memory Retrieval Speed Index	Barhi	3029.33 ± 74.17	-55.68 ± 42.60	115.41 ± 52.93	-125.92 ± 54.59	2.46	0.09	0.86	0.49
	Khassab	2918.43 ± 75.22	69.81 ± 155.97	-19.73 ± 63.79	110.62 ± 142.43				
	Placebo	2779.50 ± 72.06	20.89 ± 35.93	28.65 ± 56.35	-30.81 ± 62.12				
Cognitive Reaction Time	Barhi	138.26 ± 7.09	6.18 ± 37.97	6.27 ± 41.45	9.67 ± 34.11	1.16	0.32	0.94	0.44
	Khassab	124.67 ± 7.27	12.86 ± 29.79	7.63 ± 36.93	10.75 ± 36.74				
	Placebo	130.52 ± 8.44	7.06 ± 38.75	6.42 ± 42.33	8.82 ± 38.15				
Working Memory Capacity index	Barhi	188.18 ± 1.50	-4.38 ± 3.62	0.12 ± 1.79	-3.18 ± 2.02	1.37	0.26	0.81	0.52
	Khassab	189.14 ± 1.75	-5.20 ± 3.95	-6.16 ± 4.56	-6.24 ± 3.07				
	Placebo	184.24 ± 3.14	0.01 ± 3.51	-1.40 ± 2.91	0.47 ± 3.09				

Measures	*Treatments*	*Baseline*	*Post-dose change*			*Effect of treatment*		*Effect of treatment* time intervals*	
		0 Min	***45 Min***	***90 Min***	***135 Min***	***F***	***P***	***F***	***P***
Episodic Memory Capacity Index	Barhi	204.71 ± 12.04	-26.33 ± 8.98	-13.43 ± 8.18	-30.29 ± 7.54	3.134	0.05	1.83	0.13
	Khassab	194.00 ± 9.79	-13.81 ± 11.43	-24.57 ± 9.20	-16.57 ± 9.74				
	Placebo	203.38 ± 11.13	-31.95 ± 7.97	-44.00 ± 7.49	-41.62 ± 9.01				
Quality of Memory Index	Barhi	200.27 ± 7.48	-16.16 ± 6.48	-8.41 ± 5.98	-21.75 ± 5.44	0.16	0.85	1.94	0.11
	Khassab	198.09 ± 6.68	-10.95 ± 7.99	-22.11 ± 6.96	-15.90 ± 6.59				
	Placebo	194.90 ± 8.50	-14.61 ± 5.95	-23.15 ± 6.28	-18.87 ± 5.50				
Bond-Lader Mood (Alertness)	Barhi	61.22 ± 2.49	-1.05 ± 2.34	-0.10 ± 1.98	4.14 ± 2.05	6.03	0.00	0.93	0.45
	Khassab	55.46 ± 2.27	7.91 ± 2.53	10.38 ± 3.56	8.19 ± 2.25				
	Placebo	59.42 ± 2.79	2.04 ± 2.52	3.14 ± 1.94	3.15 ± 2.72				
Bond-Lader Mood (Contentment)	Barhi	64.02 ± 3.09	1.97 ± 1.85	3.20 ± 1.60	3.66 ± 1.43	0.11	0.90	0.46	0.76
	Khassab	65.01 ± 2.56	2.05 ± 2.18	2.91 ± 4.45	2.06 ± 2.18				
	Placebo	64.23 ± 2.54	2.76 ± 1.58	4.69 ± 1.46	2.78 ± 1.76				
Bond-Lader Mood (Calmness)	Barhi	58.22 ± 2.47	2.00 ± 1.95	3.49 ± 2.36	0.67 ± 2.22	4.87	0.01	0.62	0.65
	Khassab	62.17 ± 2.61	-4.20 ± 2.22	-3.17 ± 4.37	-4.04 ± 2.80				
	Placebo	57.89 ± 2.09	6.81 ± 2.68	3.07 ± 2.23	3.69 ± 2.55				
Blood glucose concentration	Barhi	4.75 ± 0.09	1.11 ± 0.15	0.11 ± 0.10	-0.05 ± 0.08	1.40	0.25	0.55	0.70
	Khassab	4.88 ± 0.07	0.94 ± 0.22	-0.12 ± 0.20	-0.34 ± 0.18				
	Placebo	4.81 ± 0.08	0.73 ± 0.20	0.02 ± 0.14	-0.07 ± 0.12				

3.7.5 POMS

For the six POMS mood scale factor scores (named tension, depression, anger, vigour, fatigue and confusion, plus an overall score named total mood disturbance) the same MMRM ANOVA methodology was undertaken using the actual scores obtained at the baseline of each study visit only.

A MMRM ANOVA with a Greenhouse-Geisser correction determined that the mean tension score differed significantly between the three visits ($F (1.970, 66.964) = 15.001$, $P<0.001$). Additionally, for vigour, it showed that the score significantly differed between the three visits ($F (1.908, 64.877) = 4.158$, $P<0.022$). However, none of the remaining POMS mood scores approached significance (all $p > 0.05$). Pairwise comparisons revealed that the difference in the tension score was detected between visit three and both visits one and two ($P=0.001$ and 0.046). The same difference in the vigour score was detected between visit three and visits one and two ($P = 0.015$ and 0.047). Participants were less tense with less vigour in visit three than in visits one and two.

3.7.6 Treatment guesses (confirmation of blinding)

The calculated Chi-square of the true and false answers for the survey question “Which of the three treatments you had was the date-containing yoghurt?” see Appendix P.

The Chi-square test for the participants who guessed the date-containing treatments correctly differed from those who did not, $X^2 (1, n\ 36) = .605$ ($p = 0.\ 437$) $p < 0.05$ which means that the number of expected true or false answers was not significantly different from the observed answers. This indicates that the participants were not able to identify the date-containing treatments significantly better than by chance, as explained in Table 23.

Table 23. Values and percentages for observed correct and false answers vs expected correct and false answers

Observed Correct answers	***Observed false answers***	***Expected correct answers***	***Expected false answers***
27	9	24	12
75 %	25 %	67 %	33 %

3.7.7 Supplementary analysis results

3.7.7.1 Sugar effect

The MANOVA for the eight CogTrack indices and the three mood indices showed that there was no statistically significant main effect of repeated post-treatment test sessions F (22, 590) = 0.965 p > 0.05 irrespective of treatments. The overall changes from pre-dose values for all CogTrack and Bond-Lader measures are presented in the SPSS output table available in Appendix Q. Furthermore, the Pearson Correlation examined the relationship between blood glucose levels and mood and cognitive indices scores and individual tasks. The relationship was not significant for any indices except for the sustained attentional index, with a positive correlation between blood glucose level and sustained attentional index *r* (313) = [0.18], (P= 0.001) (the SPSS output table is available in Appendix NN). However, there was a positive correlation between blood glucose level and the average speed of digit vigilance *r* (313) = [0.11], (P=0.037) for the individual tasks of cognitive function. The MML revealed that there was no statistically significant effect of the interaction between study treatment and blood glucose levels and repeated post-treatment test sessions for sustained attention index F (31, 129) = 1.1, P = 0.38, and for the average speed of digit vigilance task F (34, 125) = 0.74, P = 0.85. However, there was a statistically significant effect of the interaction between blood glucose levels and repeated post-treatment test sessions for sustained attention index F (39, 129) = 5.57, P=0.001.

3.7.7.2 Effects' direction

The calculated Chi-square of the positive and negative outcomes for the CogTrack and Bond-Lader measures of all the date-containing treatments summed together is

$X^2_{(1)} = 0.007$ ($p = 0.9332$), showing no statistically significant difference between the dates (Barhi + Khassab) and placebo.

3.8 Discussion and conclusion

The current study was the first to demonstrate improvements in self-reported alertness after the consumption of Khassab.

3.8.1 Cautious approach and effect size

As mentioned earlier in section 3.6, the novelty of this research made the prediction of the primary outcome challenging. Furthermore, the inconsistency observed in the literature regarding phenolic doses and effects and the diversity of phenolic types and varying domains of cognition utilised in previous research also challenged any simulation-based predictions of primary outcomes. When pre-identification of a single primary outcome is difficult in a study with multiple measures, then the appropriate solution would be to correct the p-value for multiplicity such as applying the Bonferoni correction (Ranstam, 2016). Therefore, two statistical approaches, less and more cautious, were performed when analysing the data of both individual tasks and indices of cognitive tests. Results with the less cautious approach showed that there were some significant effects in two indices of cognitive functions, four outcomes of the individual cognitive tasks, and two of the Bond-Lader mood indices. For cognitive and mood indices, improvement in sustained attention index was found after consumption of Barhi, while the consumption of Khassab was associated with improvements in episodic memory capacity index and alertness index. For outcomes of the individual tasks, both Barhi and Khassab exerted some small enhancing effects on immediate word recall, delayed word recall and word recognition. In the false alarm of the digit vigilance task, only Barhi was found to improve the participant's performance. Regardless of the two analyses performed on the cognitive functions data as individual tasks or as indices, the results were to some extent confirmative. The measures of the digit vigilance task and the choice reaction time are the components of the sustained attention index. Further, the measures of the immediate word recall task, the delayed word recall task, the word recognition and the pattern separation task are the components of the episodic memory capacity index (Figure 25). In the digit vigilance

task, the significant decrease in the false alarms after the consumption of Barhi may have contributed to the significant increase in the sustained attention index. At the same time, the significant increase in the percentage of immediate word recall after both date treatments, the significant attenuation of the decline in the delayed word recall of both date treatments, and the significant increase in the original stimuli accuracy may have also contributed to the significant attenuation of the decline in the episodic memory capacity.

Despite the argument about the nature of the cognitive tests as orthogonal, using a cautious approach may be deemed appropriate in this study. The p-value is an informative indicator regarding whether an effect exists or not, while Cohen's d is an appropriate effect size for comparing two means. Cohen's d or the standardised means difference is one of the most common methods to measure effect size in psychology trials. Three categories are used to classify the effect size, d=0.20 is interpreted as small effects, d=0.50 as medium effects, and d =0.80 as large effects. Therefore, Cohen's d was used to explore the effect size of all outcomes that reached the significance level without the Bonferroni correction.

For the individual task outcomes, the calculated effect size for the immediate word recall task was medium for Khassab (*d*=0.55). The effect size for delayed word recall medium for Barhi was 0.59 and Khassab was 0.50. The effect size for the word recognition task was medium for both Barhi (*d*= 0.71) and Khassab (*d*= 0.60). The effect size for the false alarms digit vigilance was medium for Barhi (*d* =0.42).

For the index scores, the calculated Cohen's d for the effect size for the sustained attention was medium *(d*=0.58) for the enhancing effect for Barhi, while the size effect of the decline in the attenuation in episodic memory capacity for Barhi was very small (*d*=0.03) and medium for Khassab (*d*=0.79). The effect size of the increase in alertness levels and the decrease in calmness levels for Khassab was medium (*d*=0.61 and *d*=0.75, respectively).

Although all size effects calculated above have shown small and moderate improvements, they provide a promising rationale for future research on Barhi and Khassab.

3.8.2 Phenolic content potential in enhancing cognitive functions

In terms of the self-ratings of mood indices, Khassab produced a significant increase in 'alertness' compared to Barhi and placebo. In a previous study which used the same questionnaire to study the effect of different doses of the phenolic content (520.89 mg) of chlorogenic acid in a special coffee blend, the same increase in alertness was reported in a healthy elderly population (Cropley et al., 2012). Moreover, Watson et al. (2015) found some trends toward an increase in alertness after the consumption of blackcurrant juice containing an average anthocyanin content of 525 ± 5 mg/ 60 kg. In contrast, Haskell-Ramsay et al. (2017) found an increase in self-rating 'calmness' following the consumption of 150.4 mg of anthocyanin in purple grape juice by young healthy volunteers.

Although the significant improvement observed in the sustained attention index following the consumption of Barhi compared to Khassab and placebo did not reach the corrected alpha level, the Cohen's d effect confirmed the effect of Barhi to be a medium effect size. This enhancing effect of Barhi on sustained attention has a clear and consistent pattern through the three-time intervals, as illustrated in Figure 29, and it can be attributed to the phenolic content of Barhi of 184.04 mg GAE/115 g. Therefore, the significant correlation seen between the sustained attention index and the glucose blood levels detected in the supplementary analysis does not imply an association between the enhancing effect and the sugar content. Further, the inconsistent pattern in the participants' blood glucose levels at the three-time intervals does not correspond to their performance in the sustained attention index, which may explain the absence of a significant interaction of time intervals and treatment and blood glucose levels detected by MML.

Again, although it is not possible to make a direct comparison between the effect of different doses or compounds of phenolics in different studies, other studies also reported enhanced attention domains in young, healthy volunteers. Anthocyanin in the grape juice was associated with the attention reaction time composite score of healthy young volunteers generated from the COMPASS cognitive battery (Haskell et al., 2017). Regarding the cognitive battery used in many of the published studies investigating the acute effect of polyphenols on mood and cognitive performance, such as Watson et al. (2015) and Field et al. (2011), the utilisation of a high intensity

paradigm to manipulate the baseline scores has been observed. This paradigm consists of several (3-7) repetitions of the attention tasks and should be considered when designing a further study in the same field.

The enhancing effect of oral glucose ingestion has been established in the literature. In a comprehensive review of the 'glucose memory facilitation effect' by Smith et al. (2011), it has been found that among other cognitive function indices, verbal episodic memory tends to be sensitive to glucose ingestion. In a meta-analysis by Riby (2004) of the effects of glucose on cognitive function, it was concluded that in young, healthy volunteers, tests of verbal episodic, visuospatial episodic and working memory showed highly significant overall benefits of glucose. In contrast, the other task groups did not have significant effects, including attention, executive, motor, implicit memory, and semantic retrieval. Furthermore, the optimum dose for improving memory was 25 g, with more minor benefits distinguished at the higher doses studied; this was most commonly at 50 g. In contrast, in our study, no significant improvements were seen in the participants' performance in similar tasks, although the total sugar content of treatments was 45.37 g, including the lactose from the treatment media, of which, 16.78 g was glucose. The absence of the glucose effect can be attributed to the difference in the glucose content of the study treatments and the reported optimal dose of glucose in the literature. Alternatively, it may indicate that the cognitive tests were not sufficiently sensitive to capture the changes.

3.8.3 Date-containing treatments

This study summarises the performance of the date-containing treatments with both the cautious and less cautious approaches. Both date-containing treatments enhanced delayed word recall tasks and word recognition tasks for the individual tasks. However, only Khassab showed a positive effect on the immediate word recall task, while only Barhi had a positive effect on the false alarm task. None of the previous outcomes remained significant with the cautious approach. Regarding the indices, only Khassab had enhancing effects on memory capacity and alertness indices, while only Barhi enhanced the sustained attention index. Only Khassab's effect on the alertness index remained significant after applying the cautious approach. The findings of this study demonstrate that the overall performance of the date-containing treatments was not

better than the placebo, and in some cases, only one individual date treatment had a superior effect over the other, regardless of the use of the cautious statistical approach. This variation may indicate that the effect of the individual date treatments is most likely a result of type 1 error, supporting the choice of the cautious approach.

The justification for conducting this research trial, as discussed in the literature review in section 3.1, was founded on animal research (Essa et al., 2015; Susban et al.,2014; Susban et al., 2015), where a transgenic mouse model of Alzheimer's disease given a diet comprising either 2% or 4% of dates for 14 weeks showed a significant dose-dependent escalation of cognitive functioning, such as spatial memory and motor functions. While the quantity of date fruit applied in the current study was founded on the daily ingestion of fresh date fruit in KSA and UAE, a comparison between this and the quantity employed in the animal research literature was attempted. The body surface area (BSA) can be employed in translating drug doses among species (Reagan-Shaw et al., 2007). However, due to the lack of information in the aforementioned studies, the BSA equation was unable to be implemented, as, without the chemical composition and calorie content of the mouse diet, this dose cannot be converted from animal to human. In the three animal research studies, the same date diet maker was cited (Research Diet Inc., NJ, USA), and thus their database was examined to locate the essential data. However, this was inconclusive and consequently, the company was approached by email. The company replied that the diet product ID in the paper is wrong and they encouraged me to contact the corresponding author for the correct information (see Appendix MM for the original email). Unfortunately, the author did not respond to multiple emails, so no information was included in these studies regarding the mice's food intake per day or the weight of the daily doses.

Furthermore, there is a lack of information in the only published human trial which examines the effects of dates on mood (Abdullah et al., 2019), which was published after the completion of this trial. This intervention focused on a dose of seven dates of the Ajwah variety ingested daily for six weeks, with a claimed outcome being a significant effect of Ajwah dates in heightening mood. The deficiency of information concerning the ripening stage and phenolic content of Ajwah dates further increases

the complication of drawing comparisons in the current study dose and specified literature.

Date fruit has been postulated as a rich source of phenolic content and in the current study, 115 g of fresh date fruit generated 48 g of Barhi freeze-dried powder and 35 g of Khassab freeze-dried powder, with a TPC of 184 and 424 mg of GAE/115 g GAE, respectively (see Table 20), which is equivalent to 1.60 and 3.69 mg of GAE/g FW of Barhi and Khassab, respectively. However, this is a relatively small dose in contrast to the different quantities from sources of polyphenols applied in alternative studies. Detailed information regarding different doses of polyphenols and cognitive functioning was illustrated in chapter 1, section 1.6.

Increasing the date fruit quantity in the treatments to increase the phenolic content is not feasible due to the high sugar content of the date fruit, and subsequent concerns for the participants' health. This is due to sugar constituting 80% of the date fruit (Al-Farsi and Lee, 2008). The total sugar content of the yoghurt containing treatments used in the current study was 45.37 g/250 g of total product, including the sugar content of the low-fat yoghurt media which was 8.5 g/100 g. Both of these are particularly elevated compared to many other fruit yoghurts available, for example, a 120 g serving of strawberry Nestle ski smooth yoghurt contains 15.8 g of total sugar, there is 7.3 g of sugar in Danone light & free blueberry yoghurt, and 12.4 g of total sugar per 175 g serving of Muller light strawberry yoghurt (Diabetes.org.uk, 2020). Moreover, FAGE 0% fat natural yoghurt contains 3 g/100 g of total carbohydrate (FAGE), which is much lower than the Yeo Valley yoghurt used in the current study. Due to these differences, Yeo Valley was contacted to request further information on the processes employed to assess the sugar content of their products, which advised as follows:

"*Yoghurt is exempt from declaring its ingredients on packaging, as long as the yoghurt only contains milk products and cultures. If, however, the yoghurt contains any other ingredients, for example, sugar, thickeners or fruit, the manufacturer would have to declare this on the packaging under 'added ingredients'. Our Yeo Valley Natural yoghurts only contain milk products and cultures. Natural yoghurt only contains sugars that occur naturally in milk (lactose), we do not add any sugar to these products.*"

Presuming that the nutritional information of 8.5 g/100 g of yoghurt is precise, this could advocate the use of an alternative yoghurt with a lesser quantity of sugar if conducting a comparable study, which could permit an increment in the dose of date fruit applied.

Separately, as discussed in chapter 2, section 2.6.10, the use of low-fat yoghurt is of concern regarding the binding effects of dairy proteins and the phenolic content of the date fruit powder. A significant decrease in the TPC of strawberries when mixed with low-fat yoghurt has been observed (Oliveira et al., 2015), however, they did not explore the addition immediately after combining, and therefore, potentially is not relevant to the current study whereby the treatments were composed immediately before consumption. The HPLC analysis of the treatments with yoghurt showed a reduction in the Barhi and yoghurt TPC from 159 ± 18 mg/100 g of GAE to 121 ± 2 mg/100 g of GAE, and for Khassab, from 368 ± 12 mg/100 g of GAE to 207 ± 9 mg/100 g of GAE after addition to the yoghurt.

3.8.4 Power and sample size

Since Cohen's d can be calculated retrospectively to compare the sample size of the current study to a previous study in the literature (Lovakov and Agadullina, 2021), the mean and SD for the Barhi post doses (45, 90 and 135) were averaged to permit the application of Cohen's *d*. Decisively, it is imperative to consider the data (mean and SD) which was utilised in calculating the study power of the human intervention and the possibility that it may not be entirely accurate due to two reasons. Firstly, the mean and SD used were obtained from the alertness index of the Bond-Lader mood scale, whereas the primary outcome of the current study was the composite of cognitive tasks in eight indices, and therefore the use of data from a cognitive measure may have been more applicable and appropriate.

A retrospective calculation was conducted in line with this using data from one cognitive measure, the speed of attention task in a comparable study (Watson et al., 2019). This research employed a parallel polyphenol source in addition to similar doses, population and design. Remarkably, this provided the same results as those previously calculated, with 36 participants essential to achieve a power of 95%.

However, this data was collected from stressed and fatigued participants which may indicate incomparability.

As explained in section 2.7.6, the current study was powered based on the study by Watson et al. (2015). The dose of blackcurrant treatment was elicited from body weight, which worked out on average as 525 ± 5 mg/ 60 kg. This dose is notably higher than the one utilised in the current study for both treatments comprising dates, with the highest phenolic content being sourced from the Khassb treatment with 368.11 ± 11.75 mg/100 g of GAE of FW. An additional retrospective calculation was undertaken based on this information to consider both the power and sample sizes. With the Khassab treatment potentially possessing a 75% efficacy in comparison to the blackcurrant treatment, the mean was divided by 3, indicating a sample size of 99 participants to obtain % power (see Appendix S). While this sample size appears impractical, it elucidates that the employed power calculation was not suitable and requires meticulous contemplation for comparable future research. However, since the power calculation of a new study is based on previous research, then the Watson et al. (2015) study was also underpowered.

To aid in measuring if there was an overall trend of the date treatments in contrast to the placebo, further analysis of Chi-square was completed to assess if there was any preponderance of positive or negative outcomes of the measures. However, as mentioned in section 3.7.7.2, this did not show any overall effect, so this approach failed to provide a well-defined basis for subsequent research.

3.9 Conclusion

To conclude, the current study results indicated enhancing effects of the administration of Khassab on alertness. Furthermore, the overall outcomes from Bonferroni corrected tests have shown some promising potential of the phenolic content of both Khassab and Barhi when mixed with low-fat yoghurt to enhance some domains of cognitive performance within a “young and healthy” adult cohort. Despite the cautious approach and the Chi-square test of the pooled date-containing treatments together may indicate otherwise, results from individual cognitive tasks and the composites scores

of the CogTrack have indicated some potential, especially for Barhi, to enhance cognitive performance. Therefore, future research on dates' efficacy in enhancing cognitive performance would require substantial changes in experimental design, such as a lower 'baseline' performance (6.1), a higher (more concentrated) dose (3.8.3) or a longer-term treatment (1.13). However, the current study employed a robust design and method regardless of the issues raised during the creation of the date-containing treatments and the placebo, and during the attempts to replicate any of the amounts of phenolic content reported in the literature to have a positive effect on mood and cognitive functions.

Chapter 4. A product development plan: designing three treatments (active, positive control and placebo) for the investigation of the acute effects of roasted date seeds drink consumption on mood and cognitive performance in healthy young volunteers

4.1 Introduction

As presented in chapter 3, only small or inconsistent enhancing effects were observed on some mood factors and only some potential to enhance cognitive performance in the domains tested after the consumption of Barhi or Khassab dates with a TPC of 184 mg of GAE/48 g of FW for Barhi and 424 mg of GAE/34 g of FW of Khassab when utilised as mixtures of date freeze-dried powder and yoghurt. Various reasons for this have been extensively discussed within chapter 3 but have resulted in numerous further questions such as how to pursue conducting a second human trial that fits within the scope and the hypothesis of this thesis, or whether the date fruit flesh should even be investigated for the second trial.

A range of ideas was deliberated throughout the brainstorming phase, including increasing the date doses, altering the vehicle previously used (yoghurt), increase the duration of study to cover the chronic effects, as well as changing to a different date fruit cultivar.

Regarding the proposed chronic study design, it was considered to administer frozen yoghurt to the participants instead of the previously used fresh mixture of date and yoghurt; this would also have followed a parallel design. Despite maintaining the double-blind placebo control study design, the logistics, however, in providing the necessary quantity of the “chilled” study treatments were complicated. Separately, it was also proposed that a differing date variety with a longer shelf-life (Khalas) could be tested against a dissimilar fruit which contained a low phenolic content (such as bananas) within a double-blind, placebo-controlled, and crossover design. Alterations could be made to the participant information sheet to advise the study aims to investigate the effect of fruit consumption on mood and cognitive performance rather than focusing on dates to overcome any issues of bias.

However, utilising the date fruit flesh within the second trial protocol was still challenging, for example:

- The influence of the high sugar content of the date fruit on participants' blood sugars.
- The short shelf-life of the dates within the *Khalal* stage could affect the phenolic content.
- The logistics of:

1. Importing the large quantities required from KSA.
2. Supplying the participant with the needed quantities of the study treatments.
    - The complications encountered when creating recipes for study treatments and the placebo, while ensuring the masking of the sensory characteristics.

The aforementioned ideas and both the advantages and disadvantages for each are summarised in Table 24 and Figure 30.

Table 24. Summary of proposed ideas.

Proposed idea*	***Advantages***	***Disadvantages***
Date fruit frozen yoghurt against a placebo using a double-blind, placebo-controlled, parallel-group study design	Extending the shelf-life of the dates and yoghurt	Food manufacturing issues Logistical issues
Dates against bananas using a double-blind, placebo-controlled, crossover study design	Fruits provided to participants with no processing or preparation required	The Khalas cultivar is in the fully mature stage and has the highest phenolic content (70.80 mg/100 g GAE FW see section 2.7.2.1) that is still considered low, so it could be problematic as the date dose/day will be large Time-consuming study design
Increasing the dose of either Khassab or Barhi against placebo using a double-blind, placebo-controlled, parallel-group study design	Using the same materials, protocol and recipes for both date-containing treatments and placebo	Repeating the trial which showed a complete absence of an effect Increasing the dose will increase the sugar content

* Aiming at a study of the 'chronic effects' of date fruit on mood and cognitive function, with their advantages and disadvantages.

Furthermore, for a clear illustration of the critical analyses of the proposed protocols and the progression of the ideas, please see Figure 30.

4.1.1 Date seed as a "like coffee beverage"

The inaugural proposal for the PhD project was centred around establishing the consequences on mood and cognitive function after consumption of date fruit with various phenolic contents using an array of studies with differing date cultivars (fresh or dry) or study continuity (either acute or chronic). However, as outlined in Figure 30, it was concluded that persisting with date flesh was unsatisfactory for the completion of the thesis. It, therefore, became crucial to develop an alternative treatment which accommodates the same scope and hypothesis and is compatible with previous projects. The overall scope of this thesis is to consider the acute effect on mood and cognitive performance after the administration of dates, and it has been hypothesised that the phenolic content of the dates may be the cause for any effect observed.

Date fruit has been endorsed as a functional food due to its rich phenolic content, with a range of literature describing the phenolic content as high. However, a comparison of the phenolic content of dates and other fruits is available in Table 4 and indicates otherwise. The TPC of a variety of date cultivars within the *Khalal* stage was within a range of 134 to 280 mg GAE/100 g FW (Al-Farsi and Lee, 2008), and those within the *Tamer* stage as between 572 to 661 mg GAE/100 g FW (Wu et al., 2004).

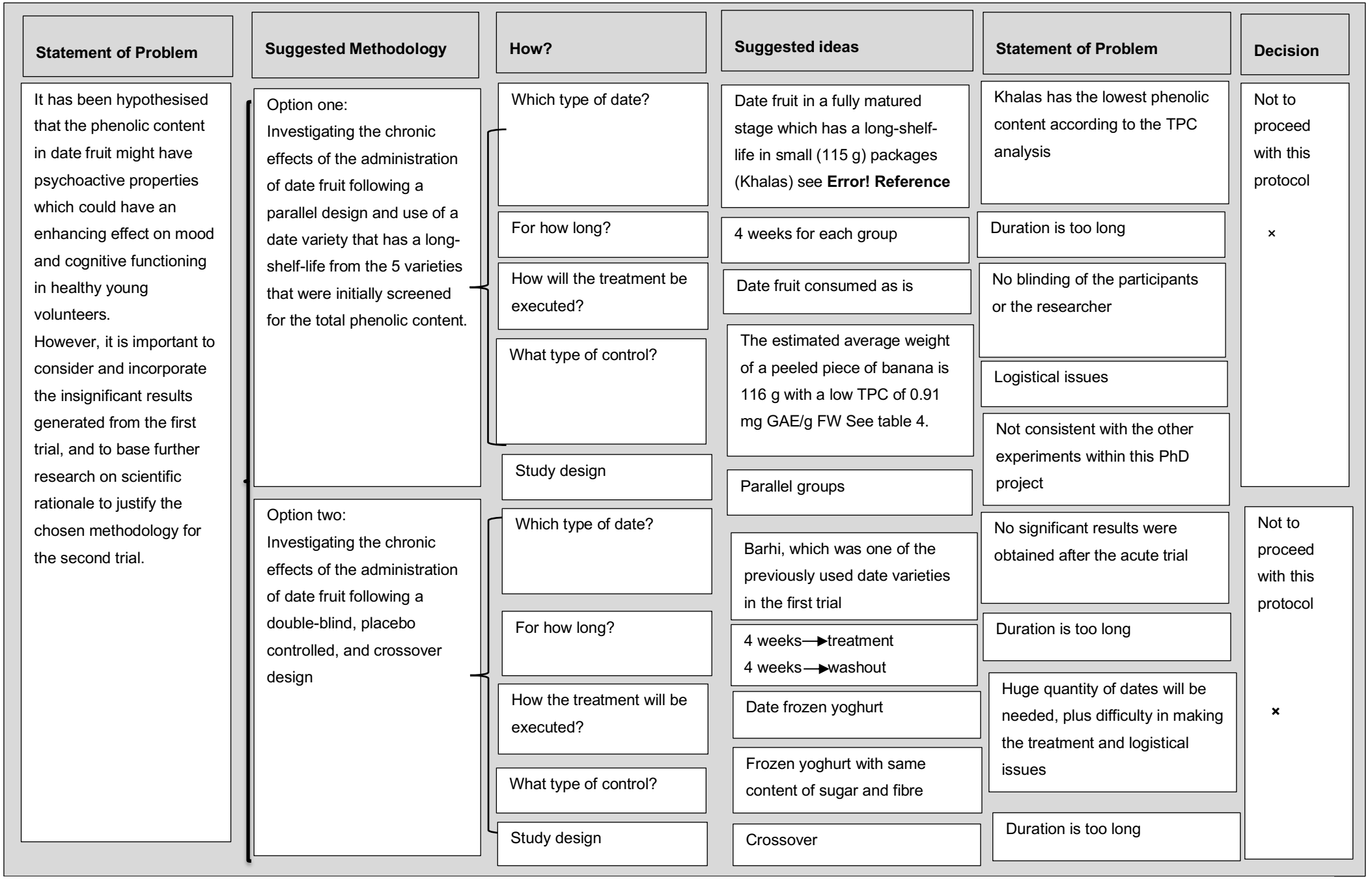


Figure 30. The critical analysis adopted in developing the proticol for the second trial.

Statement of Problem	Suggested Methodology	How?	Suggested ideas	Statement of Problem	Suggested ideas
Cont.	Option three: Investigating the acute effects of the administration of a drink “like a coffee” made with date seeds, following a double-blind, placebo controlled, and crossover design.	Will the drink be made in the Nu-Food pilot kitchen or purchased?	Date seeds drink will be purchased from a manufacturer	Different cultivar seeds (Majdool)	Test different seeds for TPC
		What is the DSD dose/drink?	45 g - suggested as this has been tested elsewhere for its palatability	No available information regarding the TPC and phenolic profile of date seed drink	HPLC to quantify phenolic
		How will the treatment be executed?	A filter coffee machine will be used	Formulating the positive control to have 75 mg of caffeine	Preserving the serving temperature of the drink
		What type of controls?	A positive control containing an effective dose of caffeine, plus a placebo with 0 caffeine and 0 phenolic	Formulating the date seeds drink recipe to have an effective dose of phenolic content	Measuring the TPC of the positive control and confirming the caffeine content
		Study design	Double blind placebo-controlled with a cross-over design	Formulating the placebo with 0 psychoactive properties.	Sensory evaluating the treatments
Decision	To proceed with this protocol				

However, analysis of the five different varieties completed within the study on the TPC demonstrated dissimilar ranges to those listed above. During study analysis, date cultivars within the *Khalal* stage ranged between 232.64 ± 10.20 mg/100 g FW to 293.51 ± 13.52 mg/100 g FW of GAE, while those within the *Tamer* stage ranged between 46.26 ± 1.45 mg/100 g FW to 82.08 ± 10.26 mg/100 g FW of GAE. Further information can be found in chapter 2, section 2.7.2

Despite these variances, designing a treatment containing date fruit to the reported phenolic doses (773 mg or 520 mg or 994 mg of flavanol) (Field et al., 2011, Scholey et al., 2010), which are anticipated to be efficient in delivering an acute enhancing effect on cognitive functions was problematic due to the high sugar content. The use of different units for the phenolic content was previously explained in 2.5.5. Being mindful of this, the most suitable option was to shift away from the use of the date fruit flesh to utilising the seeds.

While date seed was previously perceived as being unproductive and worthless other than for use within animal feed (Chao and Krueger, 2007), date seeds have now been shown as providing a superior total phytochemical content than the flesh, which has likewise been observed in some other fruits and seeds. Abundant in bioactive compounds, the date palm seeds (*P. dactylifera*) present as substantial contenders as functional food additives and nutraceuticals. Encouraging conclusions concerning neurodegenerative disease protection (Kalantaripour et al., 2012a, Majid et al., 2008a) and cerebroprotective effects (Kalantaripour et al., 2012c) were recognised within animal studies reviewing date fruit seed consumption. These were credited to the antioxidant and anti-inflammatory traits of large quantities of phenolic compounds (Majid et al., 2008b). The high phenolic contents range from 3102 to 4430 mg /100 g DW of GAE (Al-Farsi and Lee, 2008) and the total flavonoids of 3670 mg/100 g of Rutin Equivalent (Majid et al., 2008a) result in a rich antioxidant status of the date seed. It is important to note that for date seeds, the dry weight is almost the same as the fresh weight due to very low water content.

Additionally, date seed has considered high in fibres (676–742 g/kg) (Majid et al., 2008b) depending on the variety, as well as considerable minerals, vitamins, lipids and proteins which contribute to their promoted nutritional quality. Furthermore, the

date seed is sugar-free (Al-Farsi and Lee, 2008) but unprocessed date seeds are not edible.

Preservation of health *in vivo* within animal trials was associated with the date seed phenolic content (Habib and Ibrahim, 2011); (Thouri et al., 2017). Although there is a prominent lack of verification of these effects in humans, the evidence within animal health postulates a concept of opportunity. Separately to this, the studies assessed the health benefits of date seeds and employed non-aqueous solvents to quantify the TPC of the utilised animals' diets (Majid et al., 2008b) (Habib et al., 2014b), so it cannot be established whether these quantities would be representative of the roasted date seed drink for human consumption which will be brewed with hot water. Therefore, it was essential to determine the phenolic content of the date seed drink, as well as regular coffee before concluding the favourable effects being acknowledged as reliable or representable.

Devising a system of consumption that was characteristic of human cognition was essential, and this is why a roasted date seed drink was selected to achieve this. Although a novel product within the western world, roasted date seed drinks are very popular and regularly consumed within Arab countries. Similar to coffee, the seeds are roasted before being ground to a fine powder and then combined with hot water. Algarni (2020) considered the preparation of cappuccinos and lattes from roasted date seeds utilising different ratios of date seeds to regular coffee (10:90, 20:80, 30:70, 40:60, 50:50 and 60:40), concluding that a cappuccino consisting of 50% roasted date seed and 50 % of regular coffee provided a statistically significant level of acceptance during sensory evaluation in comparison to other ratios and the control of pure regular coffee (Algarni, 2020).

During a pilot study by Cropley et al. (2012), decaffeinated coffee with a high chlorogenic acid content (521 mg CGA/cup) was tested against a placebo, decaffeinated coffee with regular chlorogenic acid (244.26 mg of CGA/cup) and regular coffee (223.8 mg of CGA and 167.4 mg of caffeine/cup). The high chlorogenic acid decaffeinated coffee exhibited an improvement in some mood and cognitive measures, although to a lesser extent than regular decaffeinated coffee. These effects reached a statistically significant level in alertness and contentment indices of the Bond-Lader mood scales (same mood scale mentioned earlier) and showed trends

toward a significant level in the tendency to impair delayed recall in the visual-verbal test. Although these findings showed a robust positive effect of the regular coffee which contains caffeine, they also suggest that a decaf coffee with high CGA may be capable of some acute enhancing effects and are worthy of further research (Cropley et al., 2012). The date seed drink is a feasible and practical supply of phenolic compounds. High phenolic contents ranging from 3102 to 4430 mg /100 g FW of GAE were reported in Al-Farsi and Lee (2008) and a range of 1722 to 3444 mg/100 g FW of GAE was reported in Habib and Ibrahim (2011). By comparison, the TPC of three different cultivars of roasted date seeds (Ajwah, Aseel and Hallawi) was quantified in a study by Ahmed et al. (2016) and ranged between 843.54 to 1204.7 mg/100 g FW of GAE.

In contemplation of all the aforementioned information, and due to this new “coffee alternative” product becoming both commercially produced and attainable, as well as more widely accepted by the consumer, a coffee-based study showed potential. However, presently, there are no human trials which have been conducted to explore the effect of the consumption of date seeds drink on human cognitive function and mood. Therefore, the second intervention study in this thesis examined the acute effect of the date seeds drink on mood and cognitive function in healthy, young volunteers. However, prior to this, the lack of information regarding the effect of cultivar type and the effect of date seed processing, such as roasting time or/and temperature, needed to be considered, thus raising multiple questions about how to prepare the desired (in terms of TPC and flavour) “coffee-like” beverage from the roasted date seeds: How many grams of DSD should be used? What will the TPC yield of this amount be? Is there any difference in the TPC among different date seed cultivars? Which date seed cultivar is utilised in the chosen commercial DSD and what is its TPC? Will the quantification of the TPC in the DSD differ from the TPC in the final DSD treatment? And finally, what to utilise to mask the flavour, colour and smell in all of the three treatments: DSD, RC and the placebo?

4.2 Aim

This study aimed to establish a recipe for a roasted date seed “like a coffee” beverage with both quantified and justified levels of phytochemicals, which had similar sensory characteristics to a positive “normal coffee’’ control and a placebo.

For this, several attempts were made to create and modify the recipes, testing the homogeneity and confirming an acceptable level of participants’ uncertainty to discriminate one treatment from the others.

4.3 The objectives

- To conduct an HPLC analysis for product development of the phytochemical constituents of both phenolic and caffeine content.
- Formulate a standardised dose and brewing strategy for the roasted date seed drink based on the HPLC results.
- Conduct a sensory test to standardise the roasted date seed drink formulation against a placebo and positive control through the assessment of product differences concerning specific product attributes.
- Implement a HACCP plan for the preparation of the treatment.
- Determine the sample size (Power calculation) for the second human intervention study utilising reported positive effects of the postulated psychoactive ingredients in the literature.
- To incorporate all obtained results in finalising the treatment recipes for the protocol of the second human intervention in this thesis.

4.4 The study design development plan

Due to the multiple methodologies used within this chapter, and to facilitate the transition between the steps of this development plan, all steps are summarised in a flow chart in Figure 31.

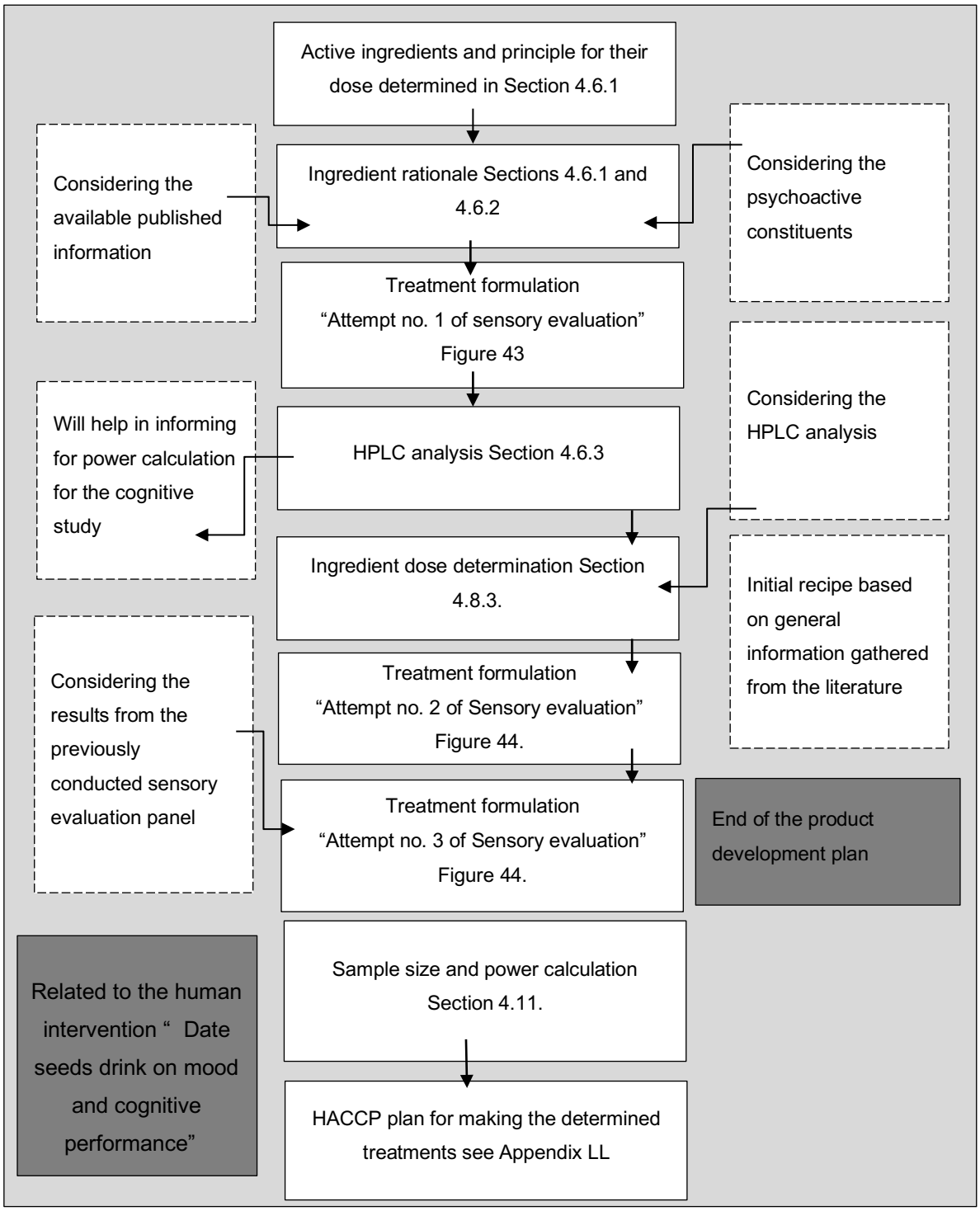


Figure 31. Study design guideline and timeline development

4.5 The rationale for the product development plan and recipe creation

As discussed above, this is the first study to investigate the acute effects of a coffee made from roasted date seed powder, therefore it was vital to replicate methods and designs used in well-documented but different nutritional interventions within the same field. Consequently, a positive control of regular coffee was added as a third arm with the DSD and the placebo. The amount of the regular coffee was a replicate of a regular coffee used in several published studies and more information on this is available in section 4.8.2. The gradual increase of the quantity of DSD from 10 g to 45 g and the justification for such change is illustrated in section 4.8.3.1.

The recipes were developed based on the quantification of the psychoactive properties (phenolics) obtained by TPC and then confirmed and identified by HPLC in parallel with results from the sensory evaluation panels to ensure the flavour masking process. A reflection of each cycle also helped in navigating the recipe creation, which is explained in Section 4.7. Therefore, the development plan for the recipe treatment creation included the following steps:

- Step 1: Determining the active ingredients and their principles for the doses
- Step 2: Quantifying the phenolic and caffeine content of all treatments
- Step 3: Discussing the proposed psychoactive ingredients doses, and justification and reflection on the HPLC results
- Step 4: Determining the treatment ingredients of both proposed and finalised recipes
- Step 5: Creating the recipes through the stages of the development plan
- Step 6: Conducting sensory evaluation panels

The rationale and the aim of each phase of the recipe creation will be illustrated in detail in the following subheadings.

4.6 Materials and methods

4.6.1 Step 1 Determining the active ingredients and their principles for the doses

To fulfil the experimental aim and design for the placebo-controlled trial, it was decided to evaluate the date seeds drink against a negative placebo which contained no psychoactive properties (phenolic). A third treatment was added as a positive control (regular coffee). The caffeine within coffee has an established enhancing effect on both mood and cognitive function, so this was used to test the robustness of the study design when reproducing a similar effect of caffeine as reported in the literature.

In summary, the chosen study treatments were:

- Date seeds drink (DSD)
- Placebo (P)
- Regular coffee (RG)

Although the phenolic content was postulated as the psychoactive ingredient within the date seeds drink, all the treatment beverages are also rich in phenolic content. Furthermore, caffeine is also considered a psychoactive ingredient and is available in regular coffee. Therefore, the initial intention was to carefully formulate the study treatments to ensure that all psychoactive ingredients in both the positive and negative controls were in lower proportions than in the experimental treatment (date seeds drink). As illustrated in Figure 32, the creation of the recipe was informed by the results of the HPLC analysis.

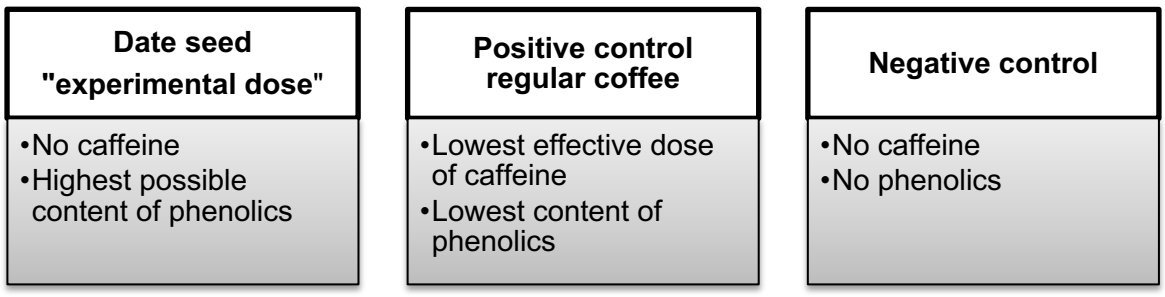


Figure 32. Overview of the treatments and their initially intended desired characteristics

Note that all treatments should have a sufficient level of similarity in terms of sensory characteristics.

4.6.2 Proposed ingredients: have been used in different doses for certain purposes during recipe development

The proposed ingredients to be utilised within the treatments were as follows:

- Roasted date seed drink – Coffee Date, Hagor, Israel
- Roasted and ground regular coffee (a unique blend of Arabica and Robusta) intensity 5/10 - Lavazza Qualita Rossa, Turin, Italy
- Roasted and ground decaffeinated coffee (100 % Arabica) intensity 3/10 - Lavazza Café Decaffeinato, Turin, Italy
- Brown liquid food colouring – The Vanilla Valley, Cardiff, UK
- Natural coffee flavouring liquid – Sensient Flavours, Milton Keynes, UK

All beverages used in this study were roasted and ground filter coffee/drinks, refer to Figures 42, 43 and 44 for the ingredients. The justification for the use of particular ingredients is outlined in further sections as not all ingredients were utilised in the final treatments.

4.6.3 Step 2 HPLC analysis for phenolic acids, flavonoids and caffeine composition

The HPLC analysis was conducted according to the method described in chapter 2, section 2.5.14.1.

The HPLC analysis was run in three different cycles and each cycle had a different extraction method. Before the sample extraction methods are explained, the specific purpose of each cycle was as follows:

4.6.3.1 Cycle 1

The purpose of this initial analysis was to explore if there were any significant differences in terms of the TPC quantification of several different roasted date seeds, roasted Barhi seeds, roasted Khassab seeds, roasted Ajwah seeds, roasted Sukkari

seeds, roasted Khalas seeds, and Majdool seeds. The first five seeds were from the same dates which were nominated for the creation of the date-containing treatment in chapter 2 due to their popularity in KSA. The Medjool was also evaluated as it is the same cultivar seeds of the proposed commercial roasted date seed powder which would be used when making the date seeds drink. Each sample was analysed in triplicate.

4.6.3.2 Cycle 2

The purpose of this cycle was to quantify the TPC and the caffeine content of the proposed roasted date seed powder, positive control of regular coffee, and any other ingredients that may contain phenolic or caffeine content. This quantification navigated the recipe creation plan and facilitated making decisions regarding whether to include or exclude any ingredients, providing results on what may interfere with the postulated psychoactive compounds in the study arms. Three types of beverages were analysed: date seeds drink, regular coffee and decaffeinated coffee. Each sample was analysed once with no replicates.

4.6.3.3 Cycle 3

The purpose of the final analysis was to quantify the TPC in the date seeds drink and both the TPC and caffeine in the regular coffee, in the same ratio of coffee to water which was to be used within the finalised recipes for the treatments. Each sample was analysed in triplicate.

4.6.4 Sample extraction

Sample preparation before extraction

The seeds of the Barhi and Khassab dates were collected from the dates imported for the previous investigation that had been dried and stored in a cold dry place. This process was relatively straightforward and it was easier to remove the seeds from the less mature stages like *Khalal* and *Rutab* compared to the fully matured stage like Tamar when the dates were stickier. Furthermore, seeds were also collected from dates in a *Kimir* stage (information about date maturation stages is provided in Figure

3). Meanwhile, Ajwah and Medjool dates were purchased from the Newcastle Halal superstore (Newcastle, UK); these were in the *Tamer* stage.

The seeds were soaked in water for 5 minutes and then washed to remove any adhering flesh. The dried whole pits were ladled and roasted according to Rahman et al. (2007) at 220ºC for 15–20 min, before being cooled to an ambient temperature. Each of the roasted seed cultivars was then converted into a fine powder in two steps due to the firmness of the seeds. Firstly, all seeds were milled and crushed into small pieces. Secondly, they were transferred into a heavy-duty coffee grinder machine; Fresh Grind®, model 080335R from Hamilton Beach, to create a very fine powder. The ground material was stored in airtight, food-grade, labelled coffee bags until they were used for the extraction. Some steps of preparation before extraction are illustrated in Figure 33.

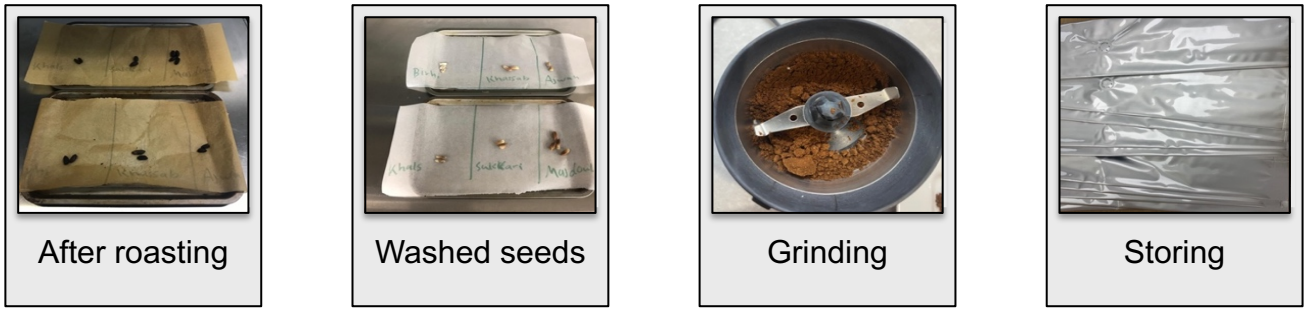


Figure 33. Photos taken in the Newcastle University pilot kitchen of date seed preparations prior to the HPLC analysis.

4.6.4.1 Sample extraction for cycle 1 samples

A 100 mg quantity of each sample was weighed into a 15 ml polypropylene centrifuge tube, and 5 ml of methanol: water (80:20, v/v) was added according to the modified method of Qadir et al. (2017). The mixture was agitated for 30 min with a shaker and centrifuged for 10 mins at 4000 rpm, then 300 µL of supernatant was transferred into an HPLC vial before analysis on the same day.

4.6.4.2 Sample extraction for cycle 2 samples

The same extraction method as used in cycle 1 was utilised to extract:

- Date seeds drink powder (DSP)
- Decaffeinated coffee powder (DCP)

(see section 4.6.2 for source information)

4.6.4.3 Sample extraction for cycle 3 samples

Since each coffee was made per the final treatment recipe, each sample was made with a specific coffee to water ratio:

- DSD (experimental treatment): a 160 mg amount of each sample was weighed into a 10 mL polypropylene centrifuge tube, and 5 mL of freshly boiled water was added while it was still hot. The mixture was centrifuged for 10 minutes at 4000 rpm and 300 µL of supernatant was transferred into an HPLC vial.
- RC (positive control): a 105 mg amount of each sample was weighed into a 10 mL polypropylene centrifuge tube, and 5 mL of boiled water was added. The mixture was centrifuged for 10 minutes at 4000 rpm and 300 µL of supernatant was transferred into an HPLC vial.

It is important to mention that these two samples, and all of their replicates, were made fresh as they were inserted into the tray of the HPLC machine. The batch file was prepared and the location for the coffee samples was designated and left empty when the cycle was run. Then, according to the batch file, and with the aid of monitoring the HPLC screen showing the percentages and ratio of solvents added at a specific time, each coffee sample was inserted into the HPLC tray within 5 min before the end of the analysis of the previous sample to ensure that every coffee sample was injected into the column only a few minutes after being prepared. This was completed to simulate the actual treatments in terms of the temperature and freshness. The temperature when the coffee was freshly made was 65ºC, however, a drop of 5–9ºC was deemed acceptable and was expected due to the time taken to centrifuge the samples and transferring them to the HPLC vials.

4.6.5 Chemicals

All standards and solvents are listed in Table 10 and * Compounds were selected based on Alarcón-Flores et al. (2016) and Llorach et al. (2008) and used to compare and identify the phenolics in date fruits. Serial dilutions of each standard were made to make standard curves.

Table 11 within section 2.5.13.1 of Chapter 1, except for the caffeine standard (see Table 25).

Table 25. Standards for caffeine

Standards	***CAS No***	***Sourced***
Caffeine	C0750-500G	(Sigma-Aldrich®)

4.6.6 Quantification of flavonoids and phenolic acids

As described in chapter 2, section 2.5.14.2, the only addition was the caffeine quantification at 270 nm and a retention time of 19.69 minutes, as shown in Figure 34.

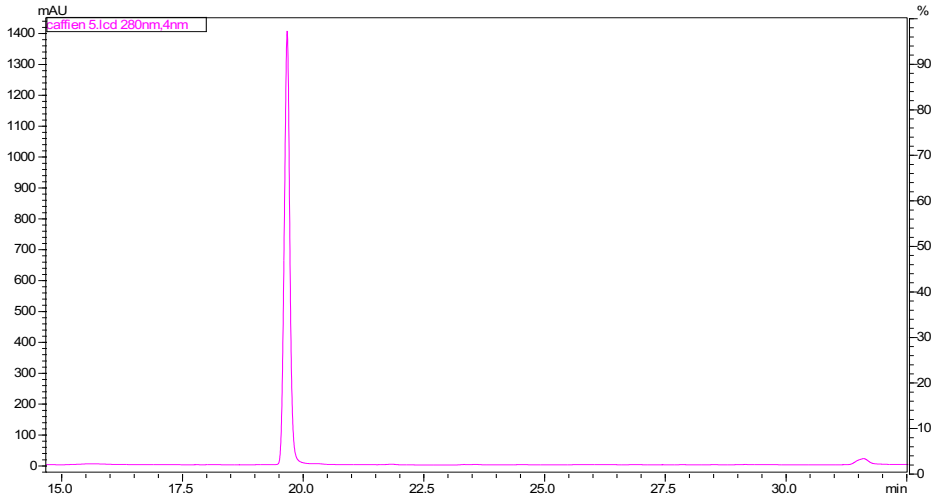


Figure 34. Caffeine chromatogram @ 270 nm

4.6.7 Results

4.6.7.1 Cycle 1

One-way ANOVA showed a significant difference in the TPC among the seeds of the six cultivars (F (5,11)=92.22, p = 0.001; see Appendix T). A Tukey post hoc test for multiple comparisons revealed that the TPC of the Barhi seeds were significantly higher than the Khalas (P = 0.001), Ajwah (P = 0.001), Sukkari (P = 0.001), and Majdool seeds (P = 0.001), with the Medjool seeds having the lowest TPC. The phenolic profile of the different date seeds can be found in Table 26 and Figure 35.

Table 26. The phenolic profile[13] of the six different date seeds.

Seed name	***GAE (mg/100 g) of DW***	***CAE (mg/100 g) of DW***	***EPE (mg/100 g) of DW***	***TPC (mg/100 g) of DW***
Roasted Barhi	131 ± 5	84 ± 4	5984 ± 338	6200 ± 338
Roasted Khassab	182 ± 32	ND	2622 ± 502	2804 ± 497
Roasted Ajwah	111 ± 6	ND	4215 ± 37	4326 ± 30
Roasted Majdool	122 ± 4	ND	2276 ± 4	2398 ± 7
Roasted Sukkari	108 ± 21	ND	4109 ± 108	4216 ± 91
Roasted Khalas	233 ± 2	ND	3274 ± 11	3507 ± 10

[13] Gallic acid equivalent (GAE), chlorogenic acid equivalent (CAE), epicatechin equivalent (EPE), total phenolic content (TPC) and caffeine content of the six different roasted date seeds in dry matter. Values are the mean ± SD of replicates.

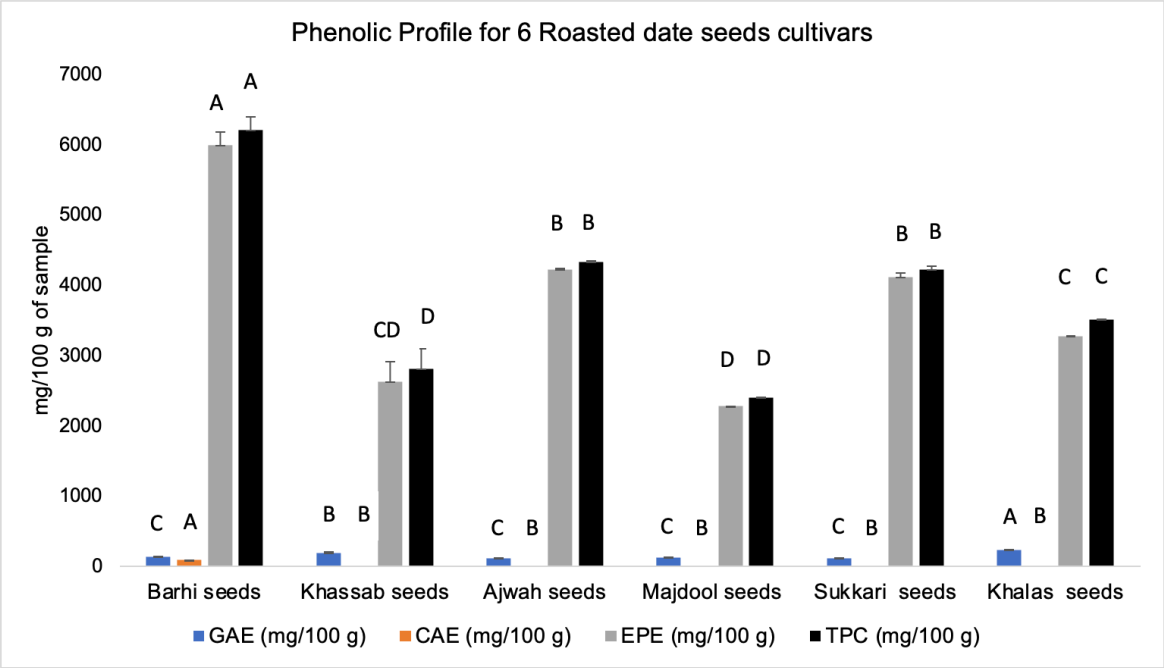


Figure 35. The phenolic profile of the six different date seeds

*Gallic acid equivalent (GAE), chlorogenic acid equivalent (CAE), epicatechin equivalent (EPE), total phenolic content (TPC) and caffeine content of six different roasted date seeds in dry matter. Each bar represents the mean ± SE calculated from triplicates and the bars denoted with different letters indicate a significant difference at $P<0.05$ according to Tukey's HSD test.

4.6.7.2 Cycle 2

The phenolic and caffeine contents of the decaf coffee powder and date seeds drink powder are shown in Table 27 and the chromatogram of the DCP in Figure 36. A comparison of the TPC of three different amounts of DSP and the proposed amount of DCP is available in Table 28.

Table 27. The phenolic profile and caffeine content[14] of decaf coffee and date seeds drink

COFFEE TYPE	*GAE (MG/100 G)*	*CAE (MG/100 G)*	*EPE (MG/100 G)*	*TPC (MG/100 G)*	*CC (MG/100 G)*
DSP	*71.72*	*0.00*	*563.80*	*635.53*	*0.00*
DCP	*27.31*	*2510.83*	*799.61*	*3337.75*	*27.11*

[14] Gallic acid equivalent (GAE), chlorogenic acid equivalent (CAE), epicatechin equivalent (EPE), total phenolic content (TPC) (as sum of the phenolics measured by HPLC) and caffeine content (CC) of roasted date seeds drink powder DSP and decaf coffee DCP in dry matter.

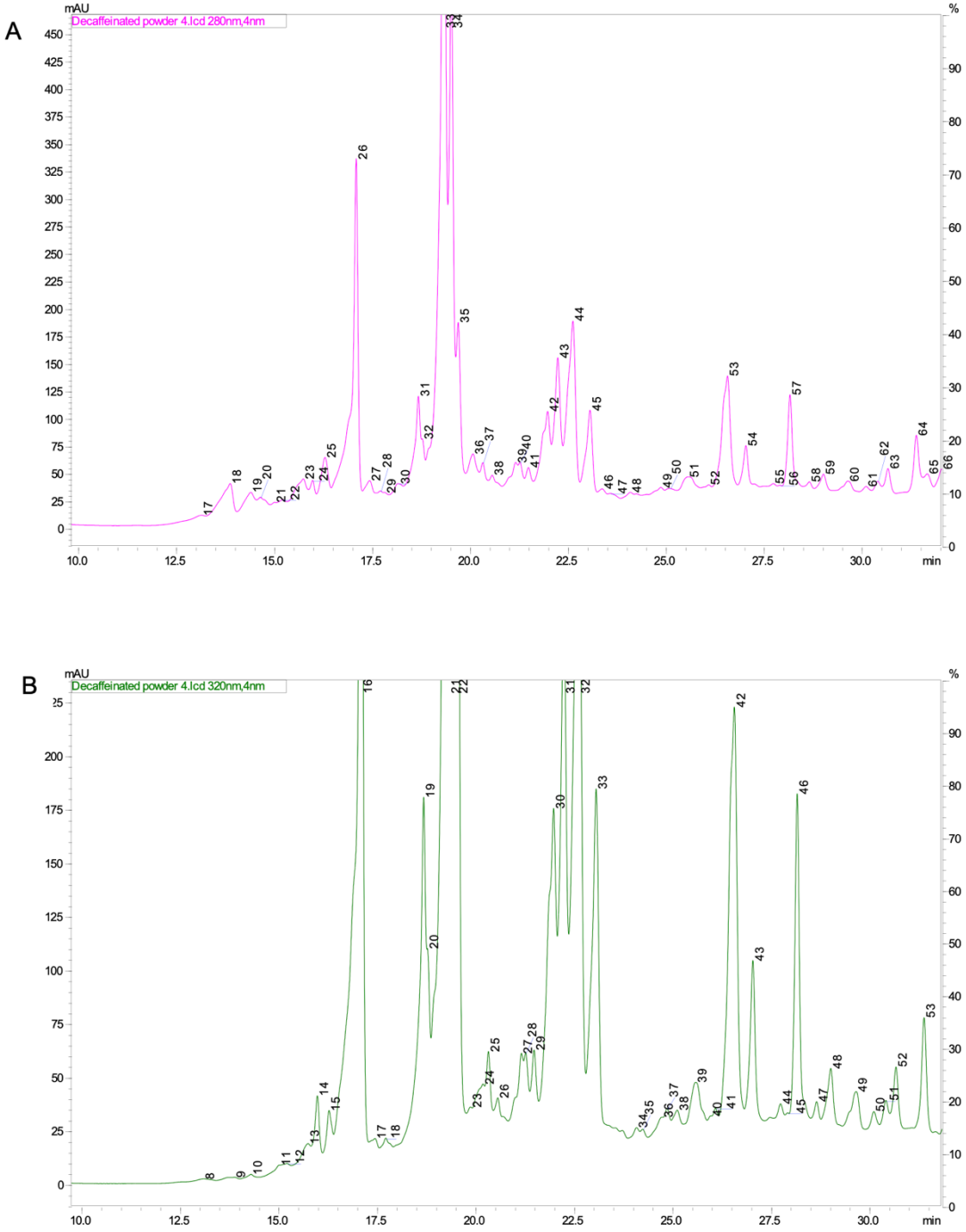


Figure 36. HPLC chromatogram of phenolic compounds in decaf coffee @ 270 and 320 nm

*The first chromatogram **A** is for decaf coffee at 280 nm: peak 26 is gallic acid (GA), peaks 17 to 25 are gallic acid equivalents (GAE), peak 33 is epicatechin (EP) and peaks 27 to 45 are epicatechin equivalent (EPE), peak 35 is caffeine (CAF). The second chromatogram **B** is for decaf coffee at the 320 nm: peak 16 is chlorogenic acid (CA), peaks 11 to 36 are chlorogenic acid equivalents (CAE).

Table 28. The phenolic profile and caffeine content[15] of different doses of date seeds drink and decaf coffee

Coffee dose	*GAE (mg)*	*CAE (mg)*	*EPE (mg)*	*TPC (mg)*	*CC (mg)*
DSP/ 10 g dose	7.2	0.00	568	64	0.00
DSP/ 30 g dose	21.5	0.00	169	191	0.00
DSP/ 45 g dose	32.3	0.00	254	286	0.00
DCP/ 3 g dose	0.8	75.32	24	100	0.81

4.6.7.3 Cycle 3

One-way ANOVA showed a significant difference in the TPC and CC between the date seeds drink and regular coffee ($F(1,4) = 1678.99$ $p < 0.001$). There was a statistically significant difference in two of the phenolic compound equivalents, CAE, EPE and the caffeine content between DSD and RC ($P < 0.001$), with exception of GAE. Figure 37 and Table 29 illustrate the detected differences, while Table 30 shows the TPC and the CC of the final amount of each coffee type for each treatment. The chromatogram of both regular coffee and date seeds drink are available in Figure 38 and Figure 39 respectively.

[15] Gallic acid equivalent (GAE), chlorogenic acid equivalent (CAE), epicatechin equivalent (EPE), total phenolic content (TPC) (as sum of the phenolics measured by HPLC) and caffeine content (CC) of the three different doses of roasted date seeds drink DSP (10, 30 and 45 g) and the 3 g of the decaf coffee DCP used as a flavour in dry matter.

Table 29. The phenolic profile and caffeine content[16] of roasted date seeds and regular coffee beans

Beverage type	*DSD*	*RC*
GAE (mg/100 g)	*47 ± 4*	*52 ± 9*
CAE (mg/100 g)	*0.00 ± 0.00*	*6255 ± 258*
EPE (mg/100 g)	*198 ± 20*	*2076 ± 54*
TPC (mg/100 g)	*244 ± 15*	*8383 ± 303*
CC (mg/100 g)	*0.00 ± 0.00*	*1152 ± 47*

Table 30. Total phenolic content (TPC) and caffeine content (CC) [17] of the finalised study treatment doses of date seeds drink and regular coffee

DSD (experimental dose)		**RCD (positive control)**	
TPC mg/ cup of 280 ml	***Caffeine mg/ cup of 280 ml***	***TPC mg/ cup of 280 ml***	***Caffeine mg/ cup of 280 ml***
110 ± 9	*0.00 ± 0.00*	*503 ± 18*	*69 ± 7*

[16] Gallic acid equivalent (GAE), chlorogenic acid equivalent (CAE), epicatechin equivalent (EPE), total phenolic content (TPC) (as sum of the phenolics measured by HPLC) and caffeine content (CC) of roasted date seeds (DSD) and regular coffee beans (RC) in dry matter. Values are the mean ± SD of three replicates.

[17] Contents were calculated from the values in Table 29 of the DSD (45 g in 280 ml of hot water) and of the regular coffee powder (RCD) (6 g of in 280 ml of hot water) of the final recipes in dry matter. Values are the mean ± SD of triplicates.

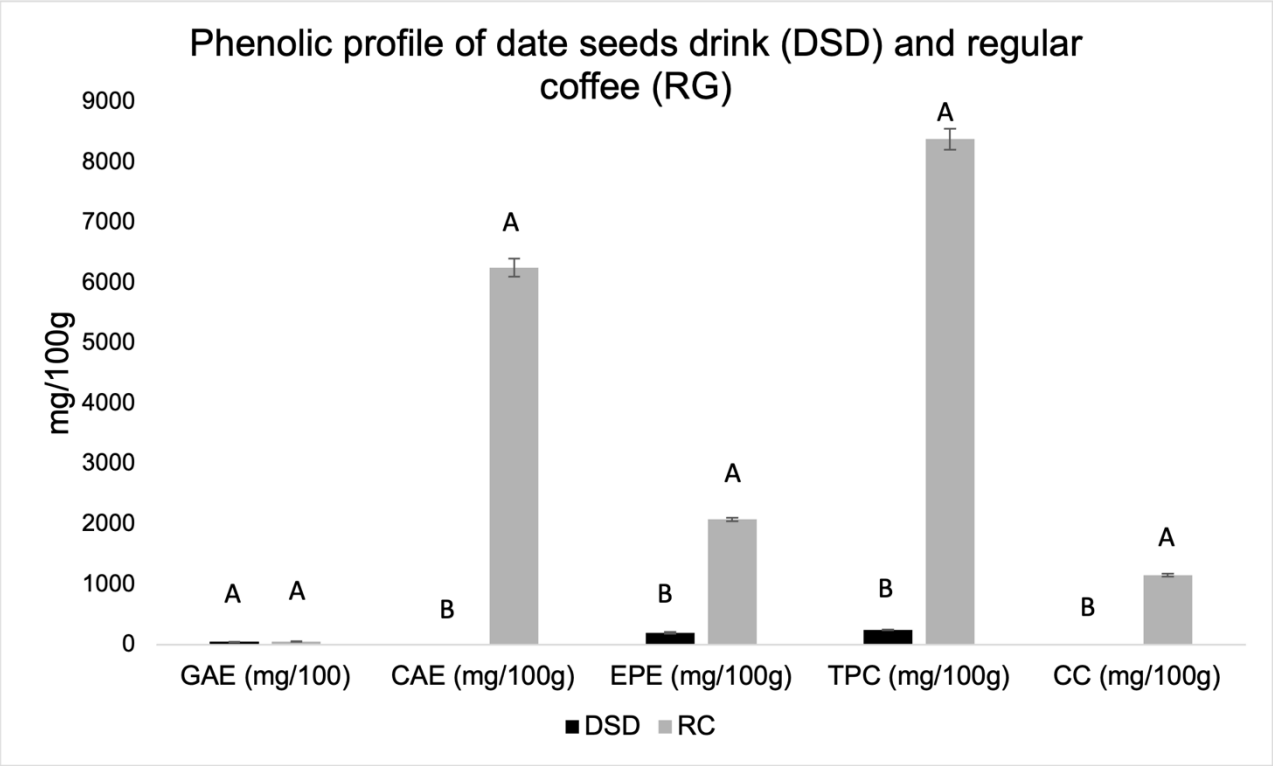


Figure 37. Phenolic profile and caffeine content of the final study treatment doses of date seeds drink and regular coffee.

*Gallic acid equivalent (GAE), chlorogenic acid equivalent (CAE), epicatechin equivalent (EPE), total phenolic content (TPC) (sum of phenolics measured by HPLC) and caffeine content (CC) of roasted date seeds (DSD) and regular coffee beans (RC). Each bar represents the mean ± SE calculated from triplicates in dry matter. The bars with different letters indicate a significant difference at $P < 0.05$ between drinks according to Tukey's HSD test.

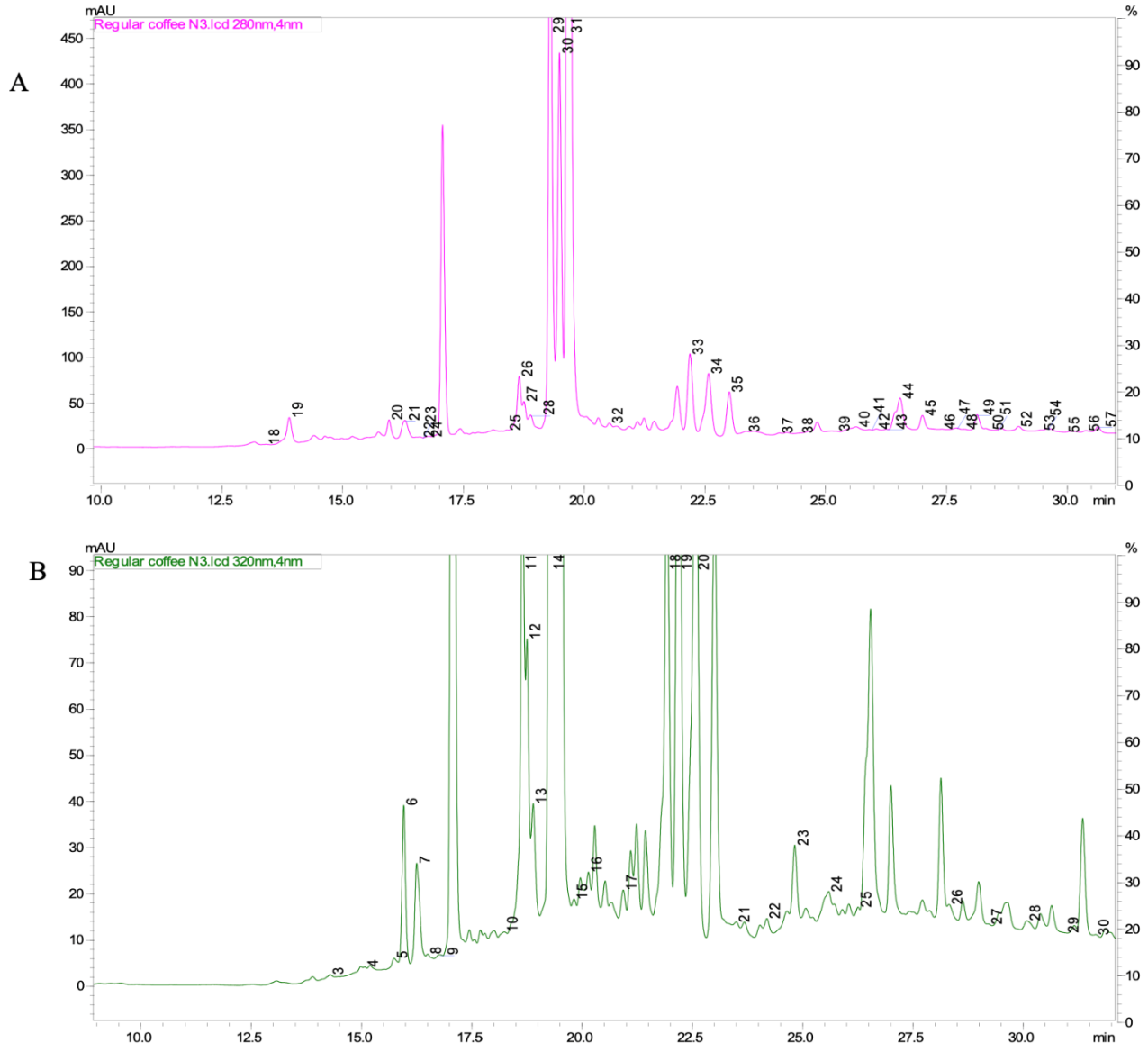


Figure 38. HPLC chromatogram of phenolic compounds in regular coffee @ 280 and 320 nm.

*The first chromatogram **A** is for regular coffee at 280 nm: peak 24 is gallic acid (GA), peaks 18 to 23 are gallic acid equivalents (GAE), peak 29 is epicatechin (EP) and peaks 25 to 35 are epicatechin equivalent (EPE), peak 31 is caffeine (CAF). The second chromatogram **B** is for regular coffee at the 320 nm: peak 14 is chlorogenic acid (CA), peaks 6 to 23 are chlorogenic acid equivalents (CAE).

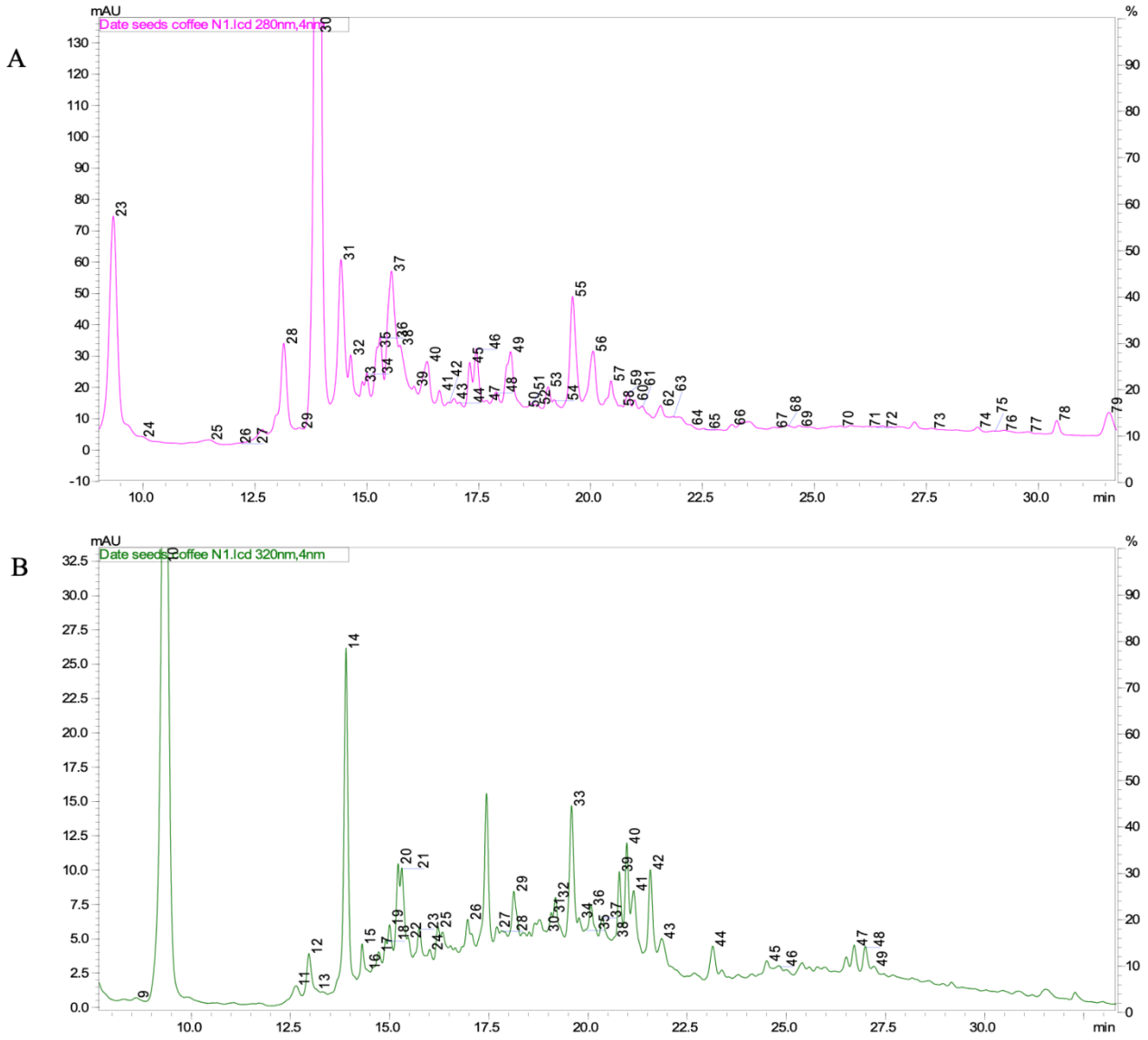


Figure 39. HPLC chromatogram of phenolic compounds in date seeds drink @ 280 and 320 nm

*The first chromatogram **A** is for date seeds drink at 280 nm: peak 23 is gallic acid (GA), peaks 24 to 27 are gallic acid equivalents (GAE), peak 30 is epicatechin (EP) and peaks 28 to 60 are epicatechin equivalent (EPE), The second chromatogram **B** is for date seeds drink at 320 nm: peak 10 is chlorogenic acid (CA), peaks 11 to 28 are chlorogenic acid equivalents (CAE).

4.7 Reflection on the phenolic and caffeine contents quantified by HPLC on the development plan of trial treatments

4.7.1 Cycle 1

This analysis showed that there was difference among the date seed cultivars in their phenolic content and that the Medjool was not the best candidate to make the date seeds drink but as the available date seed drink products on the market were very limited, the decision was made to proceed with this powder made of roasted Mejdool seeds. This was partially because making our own roasted seeds might be considered as a novel food. Furthermore, it is important that products are properly labelled, safe for consumers and have a pre-market authorisation according the European Commission. However, most importantly, these initial HPLC results showed that catechins were the predominant phenolic compounds within the date seeds drink. Although only preliminary results were conducted at this stage, it was sufficient to navigate the direction of the inclusion of the psychoactive ingredients and calculating the sample size in the final human intervention.

4.7.2 Cycle 2

This analysis showed that decaf coffee has a high TPC and a low CC (Table 27 and Table 28); it was later omitted from the final treatment recipes. Although the analysed samples in this cycle were in powder form and not as drinks, it was presumed that the phenolic compounds are 100% extractable in hot water as they would be prepared and served to the participants. Regardless of the confirmed low CC within decaf coffee and the flavouring enhancing effect, 3 g of decaf coffee was added to the first recipe

4.7.3 Cycle 3

This analysis quantified the phenolic and caffeine contents of the study treatments prepared and extracted to simulate the final treatment recipes.

4.8 The proposed psychoactive ingredients doses, justification and reflection of the HPLC results

4.8.1 Catechins

Since the predominant phenolic compounds in DSD, according to our HPLC analysis, were catechins (see Table 26 and Table 29), and due to the reported enhancing effect of catechins on mood, cognitive function and/or cerebral blood flow in the literature, it was hypothesised that catechins and their derivatives in DSD would acutely enhance mood and improve cognitive functions. The intended double-blind, placebo-controlled, crossover study therefore was initially designed to investigate the effects of a single 300 mg dose of epigallocatechin gallate (EGCG) on cognitive function and mood measured post-administration. The chosen dose of catechins was based on a study by Scholey et al. (2012) where 300 mg of EGCG showed an increase in self-rated calmness and reduced self-rated stress which was associated with a significant overall increase in alpha, beta and theta wave activity when measured by electroencephalogram (EEG). However, cognitive functions were not assessed in Scholey et al. (2012). Additionally, acute enhancements were reported after the consumption of two different doses (270 mg and 135 mg) of EGCG in CBF as physiological effects (Wightman et al., 2012). Although CBF is a predictor for mood and cognitive enhancements, these were not associated with any significant modulation of mood or cognitive function within the Wightman et al. (2012) study. Dietz et al. (2017) investigated the effect of 280 mg of EGCG of 4 g of matcha in the form of bars on healthy young volunteers and found small effects in basic attention abilities and psychomotor speed response. Moreover, it is important to mention that there is a large number of studies which have looked at the EGCG content within tea or matcha as a psychoactive ingredient besides other psychoactive ingredients like theanine and caffeine, however, these were deliberately neglected as persistent questions remain: whether the observed effect can be attributed solely to the catechin compounds? And more importantly, whether or not catechins are the main components in tea and matcha?

The results from cycle 3 of HPLC to quantify the total catechins in the selected commercial date seeds drink confirmed that the amount of catechins present in the

coffee was too low to achieve an effective dose of 300 mg of catechins. An impracticable amount of DSD would be required to be consumed to reach this dose, and this raised concerns about making a treatment with a very low-level palatability. The gradual increase of the date seeds dose can be seen further in the treatment ingredients determination in section 4.6.2.

4.8.2 Caffeine

Due to the fact that DSD was to be tested for the first time in a human intervention, it was decided to include regular coffee as a positive control for two reasons. First, to test the robustness of the methodology by demonstrating the similar confirmed effects of caffeine. Second, to aid the provision of an alternative route for the calculating the study power and sample size.

To formulate the caffeine dose in the ‘’positive control’’, it was important to collect and gather data from well-designed studies, namely, those investigating the effects of caffeine on mood and cognitive functioning in attention tasks, and to use this as guidance for the scientific rationale justifying the use of the selected caffeine dose.

A review by Ruxton (2008) of the impact of caffeine on mood, cognitive function, performance and hydration was used to extract the information needed regarding the effect of caffeine on mood, cognitive function and the used dose. This review only included studies which assessed the acute effect of caffeine on healthy adults using randomised, double-blind, placebo-controlled methodologies. Studies using combinations of caffeine and other substances (e.g. glucose, herbs and drugs) were excluded. However, as there was some missing or unclear information in the review, all papers were revisited and any missing information was gathered as shown in Figure 40.

The reviewed studies presented evidence demonstrating that caffeine influences mood e.g. increasing alertness (Yeomans et al., 2002) and cognition e.g. benefits in memory and attention (Smit and Rogers, 2000). It may be reasonable to suggest that these caffeine effects are not dose dependent, as, within each study, a range of doses was utilised. These studies considered a range of cognitive measures, such as memory, accuracy, vigilance and speed, as well as self-reported mood and perceived

fatigue. The caffeine dose varied depending upon the study, with most using a single bolus of caffeine ranging from 37.5 to 450 mg. While the duration of most studies may appear short, it is worth bearing in mind that plasma caffeine levels peaked at 60–90 minutes post-ingestion, thus, any cognitive effects would be expected to occur in the short-term.

Nine out of fourteen studies showed that a single caffeine dose ranging between 50 to 100 mg significantly affected mood and cognition like enhancing alertness and increased the number of hits and decreased reaction times in a vigilance task (Robelin and Rogers, 1998, Hindmarch et al., 1998, Hindmarch et al., 2000, Quinlan et al., 2000, Smit and Rogers, 2000, Brice and Smith, 2002, Smith et al., 2005, Haskell et al., 2005, Hewlett and Smith, 2006, Childs and de Wit, 2006).

All reported doses from the fourteen reviewed and included studies were extracted and presented in Figure 40.

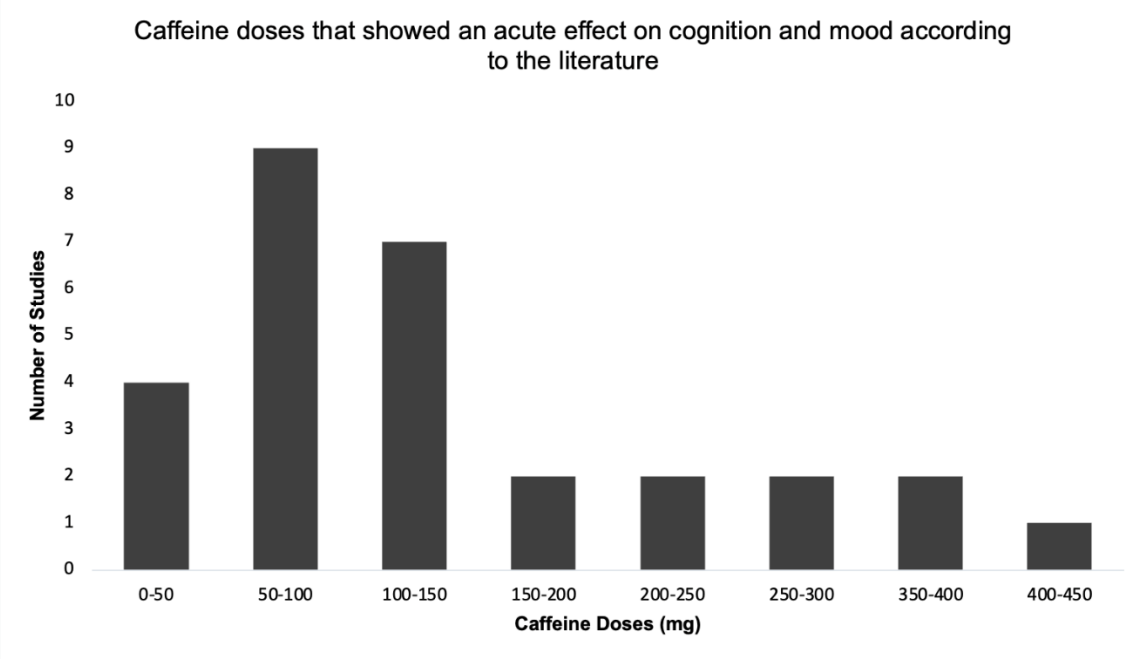


Figure 40. Caffeine doses which showed an acute effect on cognitive performance in the literature

*Data extracted from Ruxton 2008 and McLellan et al. (2016).

Ruxton (2008) concluded that the optimal intake of caffeine on mood, cognitive function performance was 38 to 400 mg per day, equivalent to 1 to 8 cups of tea or 0.3 to 4 brewed cups of coffee per day. Accordingly, the decision was made to apply

a regular coffee dose that provided an average of 50-100 mg of caffeine, therefore, a 75 mg caffeine dose was selected for the positive control treatment. According to the literture review, this dose has been utilised in three studies (Quinlan et al., 2000, Hindmarch et al., 2000, Haskell et al., 2005), one of which had a parallel sample size and power arithmetic (Haskell et al., 2005).

Normally, a dose of 75 mg is obtained from 6 g of ground coffee, which was almost equivalent to the canonical coffee weight (Clifford and Madala, 2017) utilised by baristas for a 7 g espresso shot. It is also very close to the weight of coffee in the well-known coffee capsules by Nespresso® (5.5 g/ capsule confirmed by our measurements to be 5.57 ± 0.06 g/capsule). Six grams of coffee was used in a similar study to assess the acute effect of an enriched decaf coffee with chlorogenic acid against regular decaf coffee, a placebo and a regular coffee as a positive control (Cropley et al., 2012). Therefore, 6 g of coffee wereused in the recipes and were not subject to HPLC analysis for caffeine content quantification until the recipes were finalised.

4.8.3 Step 3 Determining the treatment ingredients

4.8.3.1 Date seeds drink

This ingredient was the fundamental component of the experimental treatment but there was a lack of information regarding the preparation of any treatment containing such an ingredient. Therefore, during the development process, three different amounts were used (see Figure 41). The initial dose was 10 g as recommended by the DSD packaging, followed by 30 g, however, both these amounts resulted in a weak taste. Therefore, the supporting literature, which states 45 g of date seeds drink had been tested elsewhere for palatability (Ghnimi et al., 2015a) was utilised in the formulation of the date coffee. The taste aided in the decision that an amount exceeding 45 g may make the date seeds drink taste different from the other samples, which would have gone against our objective. More information about the gradual increase in the amount of the date seeds drink used is available in Figure 41.

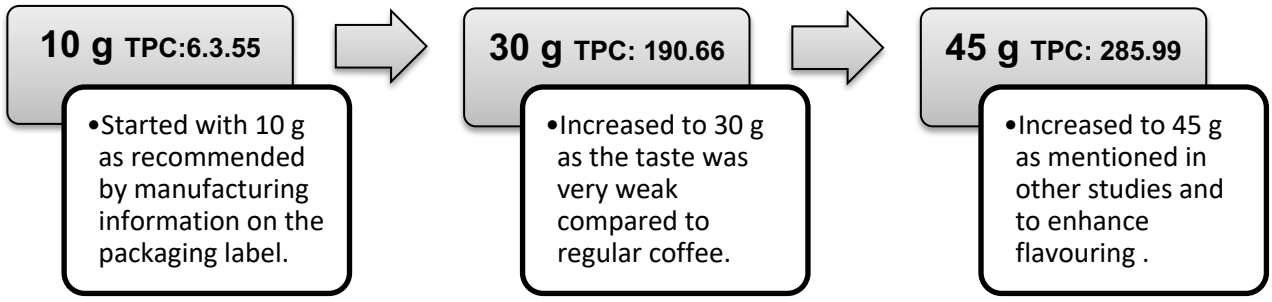


Figure 41. The three different date seed amounts used during recipe experimentation

4.8.3.2 Regular coffee

As explained in section 4.8.2, regarding the justification for the regular coffee dose of 6 g which was used in the recipe for the positive control, this would contain the desired 75 mg of caffeine.

4.8.3.3 Coffee flavouring

- *Coffee flavouring (drops)*

Initial tasting of the treatments by the researcher before panel evaluation highlighted that the date seed drink and placebo treatments were weaker in flavour than the other treatment, particularly in terms of bitterness and body. Consequently, coffee flavouring was added in an attempt to improve the depth of flavour, however, it distorted the flavour profile, so was not added to any of the treatments.

- *Decaf coffee as a coffee flavouring (3 g)*

A small amount of decaffeinated coffee was trialled for flavour within the date seed drink and placebo treatments in an attempt to improve the flavour. Decaffeinated coffee has similar organoleptic properties to regular coffee but lacks caffeine, making it an appropriate ingredient to be used as a flavour enhancer. Only 3 g of decaf coffee was used as a flavouring in both date seeds and placebo in the initial recipe and tested for sensory evaluation. The CC was low (0.81 mg/ 3 g) and was enough to enhance flavour while having no positive effect on cognition. However, the decaffeinated coffee had a TPC of 100.13 mg/ 3 g which was almost 40% higher than the TPC of the date

seeds drink (10 g) and about the 1/3 of the TPC of the 45 g amount. Therefore, the 3 g of decaf coffee was removed from recipes 2 and 3.

4.8.3.4 Brown food colouring

Seven drops of brown food colouring were initially added to the placebo, while only three drops were added to the date seeds drink to enhance the colour. The difference in the food colouring amount added to the placebo was due to the fact that it was the only ingredient added, so more was needed for colour matching. No food colouring was added to the regular coffee. Due to the removal of the 3 g of decaf coffee from DSD and placebo recipes, there was an increase in the food colouring drops in the final recipe to compensate for the observed decrease in the treatment colour. Instead of three drops utilised in both the DSD and the placebo, 7 and 10 drops were utilised in DSD and placebo respectively (see Figure 44).

Final ingredients

The date seeds drink, regular coffee, brown food colouring and hot water were the only ingredients to remain and be used in the final recipes.

4.9 Step 4 Treatment recipes and stages of development plan

It is important to highlight that the first recipe was made after some trial and errors in the pilot kitchen facility at Newcastle University. Many attempts were made to incorporate the information gathered from the literature to establish a base recipe which could be developed and improved due to the novelty of the study treatments. The brainstorming and attempts helped to overcome the taste differences between the three treatments. However, treatment recipes 2 and 3 were informed by results from the sensory evaluation and the HPLC results, and it was after this that some ingredients were removed from the recipe, while others remained with some alterations. Detailed stages and steps of the development plan and the three tables for the created recipes are summarised in Figure 42, Figure 43, and Figure 44.

Figure 44 contains the final recipe which was used in the human intervention to investigate the mood and cognitive effect in young healthy volunteers, as outlined in the next chapter.

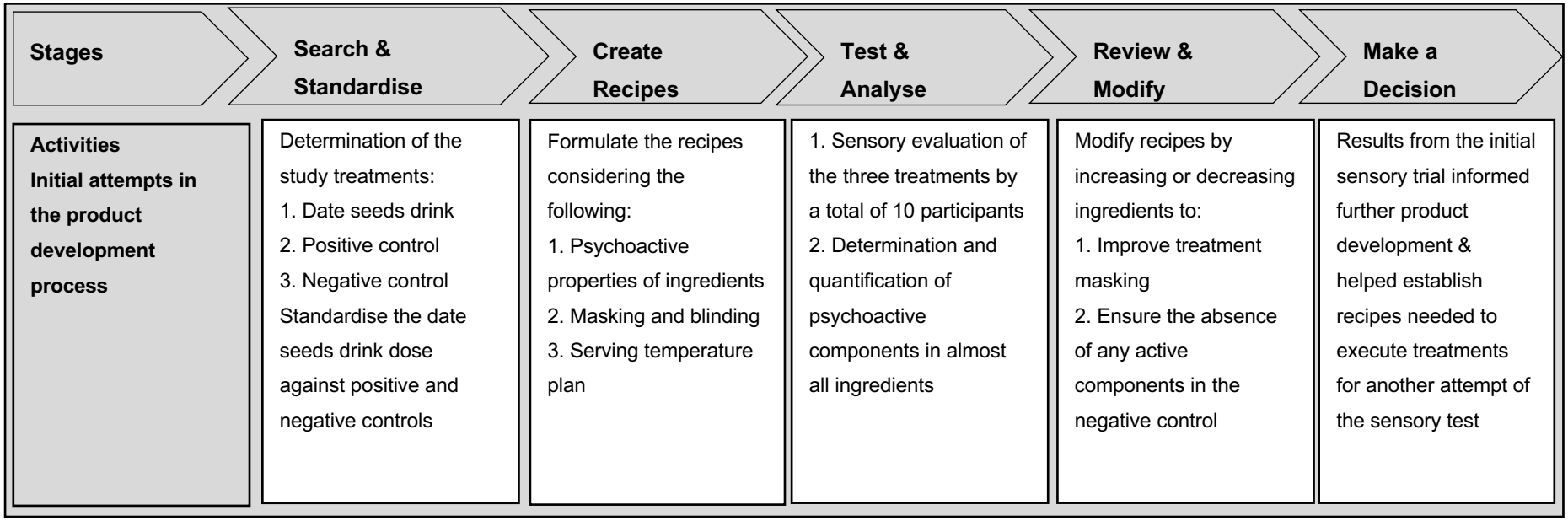
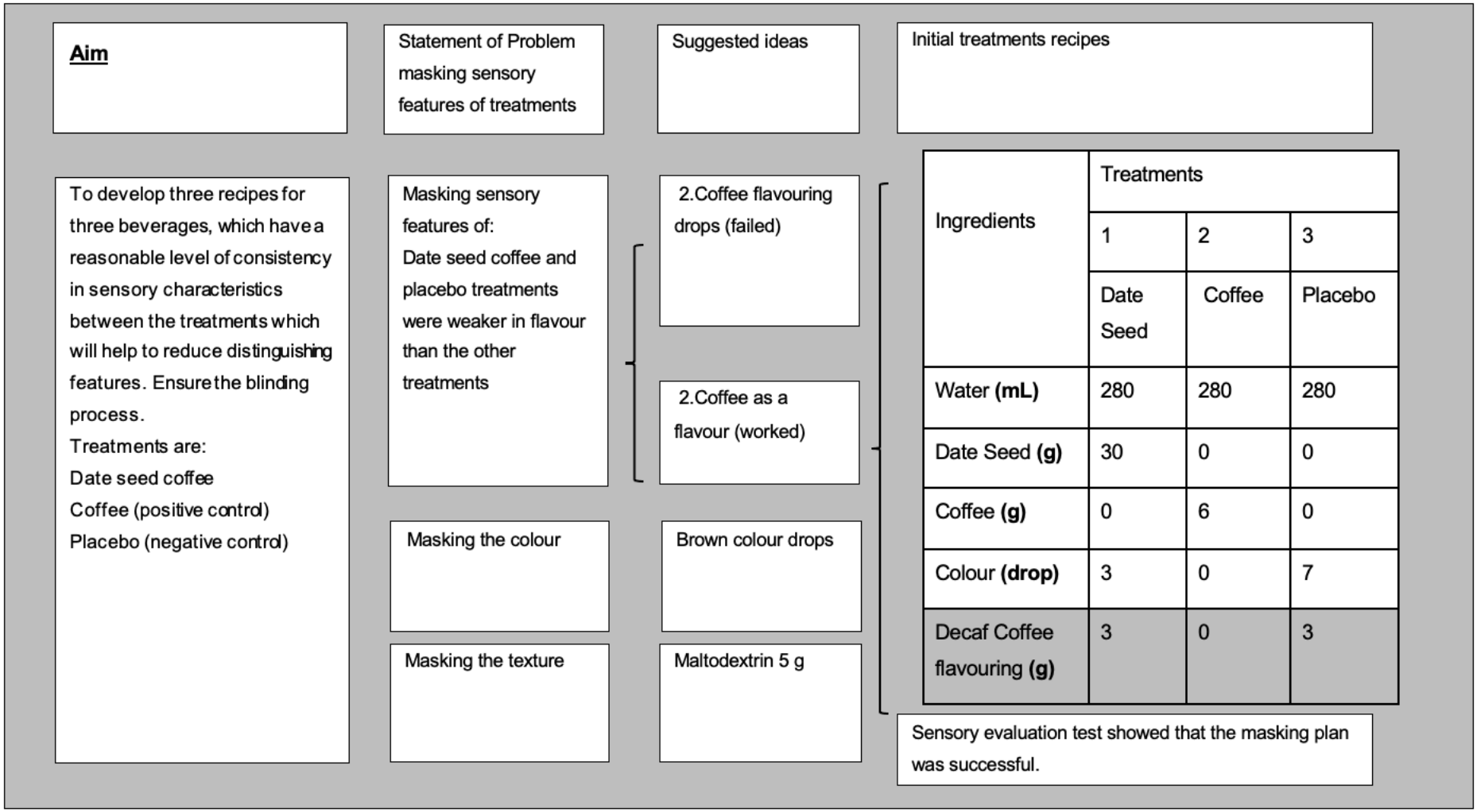


Figure 42. Stages of the product development plan



Ingredients	Treatments		
	1	2	3
	Date Seed	Coffee	Placebo
Water **(mL)**	280	280	280
Date Seed **(g)**	30	0	0
Coffee **(g)**	0	6	0
Colour **(drop)**	3	0	7
Decaf Coffee flavouring **(g)**	3	0	3

Figure 43. Product development plan including the three sensory evaluation attempts

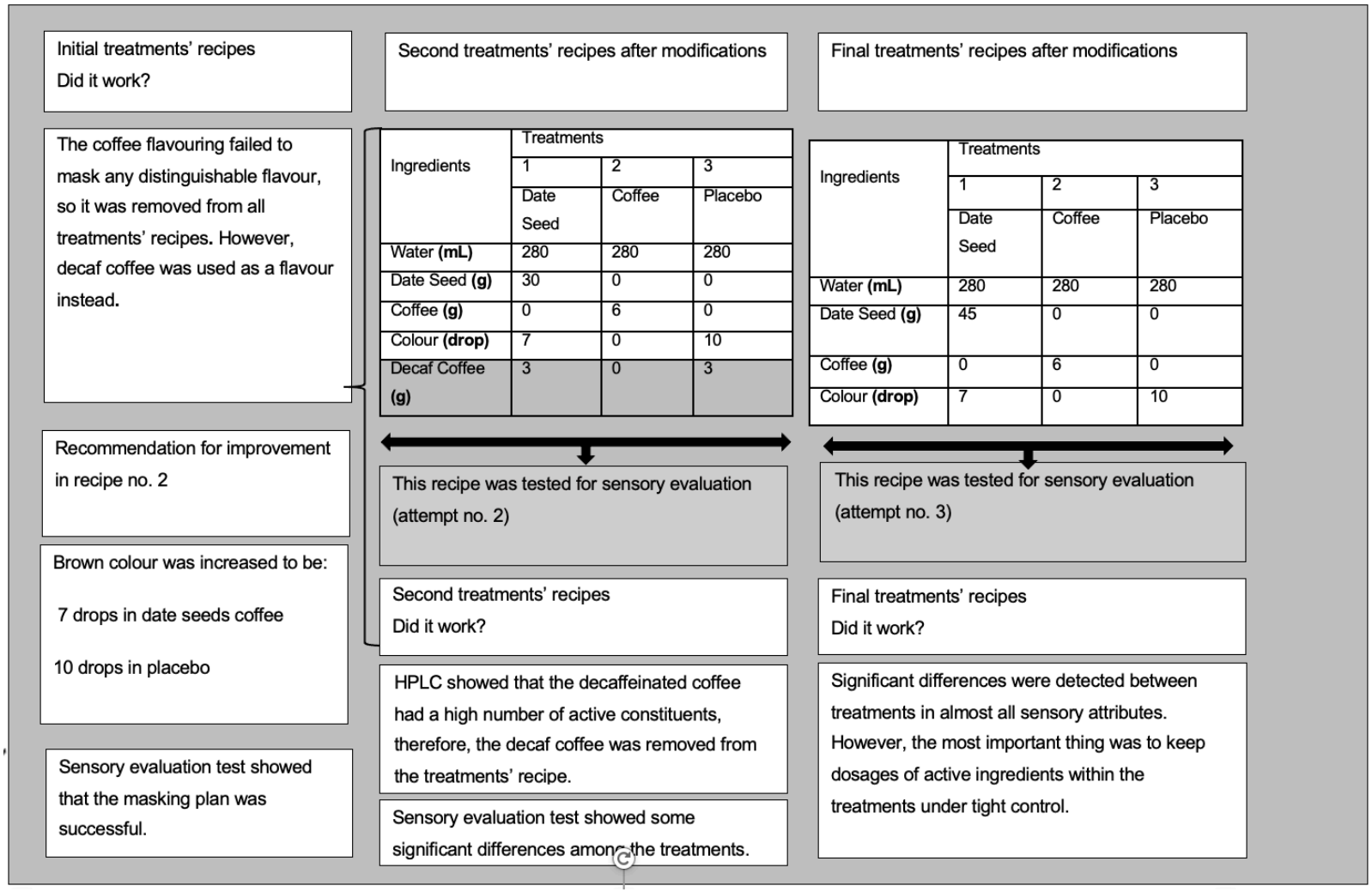


Figure 44. Product development plan including the three sensory evaluation attempts

4.10 Step 5 Sensory evaluation panels

Due to the novelty of the study design, several sensory evaluation panels were conducted as detailed later in this chapter. All steps of the treatment preparation took place in Newcastle University's NU-Food Pilot Kitchen facility.

For the purpose of the study design, all of the treatments were subjected to small sensory evaluation panel tastings to ensure that all treatments were sufficiently similar to each other in most sensory features including appearance, smell and taste. This was intended to provide a sufficient level of confusion to the participant, making them unable to identify the treatment which contained the date seed powder.

Thus, the successes of the masking and controlling procedures employed during formulating the study treatments and the placebo were tested. Three sensory panels were conducted, and each attempt involved a different cohort and different recipe.

At the NU-Food facilities of Newcastle University, the panellists tasted the coffee treatments in individual booths. The treatments were made according to a different version of the recipe shown in Figure 43 and Figure 44 and stored in thermos to keep all beverages within the average coffee temperature of 60-65C°. Small quantities (approximately 10 mL) of each treatment were poured into paper cups, then placed on paper plates divided into three sections using a marker and labelled with the treatment codes (see the top left photo in Figure 45). The treatment codes were generated using an online tool for sample randomisation (https://www.random.org/lists/).

Figure 45. Sensory analysis plates (top left), equipment used to make the coffee (bottom left) and packets containing the treatment (top and bottom right)

4.10.1 Procedure

There were three sensory evaluation panels conducted on three separate occasions; the first attempt was conducted using a paper-based questionnaire, while in the other two attempts, an electronic version was used to facilitate data collection. The use of different versions explains the difference in the scale units in Table 31.

Panellists were asked to write each of the sample codes on the line of each of the seven analytic scales which measured the following attributes: smell, sweetness, texture, flavour, colour, aftertaste and one hedonic scale measuring the overall acceptance. On the computerised questionnaire page, the instruction stated the following: “Taste the samples on your plate in sequence according to the number

labelling. Answer questions below by moving the mouse on the line as indicated in the below example".

4.10.2 Participants

For each of three attempts of the sensory tasting panels, which correspond to the three recipes, ten panellists from the School of Natural and Environmental Sciences at the University of Newcastle participated in the experiment. See Figure 46 for photos taken of the NU-Food sensory facility used for these experiments. Participants were selected based upon interest, availability and being healthy with no food or diary product allergies or milk product intolerances reported.

Participants were adults aged between 25 and 55 years old (mean age 33.9±6.6) including six females and four males. No previous training was provided to the participants. All participants were verbally briefed about the test procedure and then asked to take their places and make themselves comfortable in the sensory booths prior to the test. Water was provided throughout to cleanse the palate, as required. Each individual received a chocolate bar at the end of the trial as a gesture of gratitude for their participation.

Figure 46. Photos taken of the NU-Food sensory testing facility.

4.10.3 Statistical analysis

All statistical analysis was conducted using IBM SPSS package 25 and Microsoft Excel. One-way ANOVA was used with the Tukey test to detect any significant

differences among the three treatments: DSD, RC and P. Finally, for the sensory evaluation test, Bonferroni correction was performed to protect from Type I errors arising from the multiple ANOVAs. As there were seven different attributes measured, the alpha level of 0.05 was divided by seven to be P = 0.007.

4.10.4 Results

Table 31. Sensory evaluation attributes measured

Attempt	*Attribute*	*Sample*	*Mean*	*SD*	*P-value*
First	**Smell**	DSD [B]	3.93	2.9	0.01
		RC [A]	7.57	3.1	
		P [B]	3.36	2.7	
	Colour	DSD [B]	3.51	2.18	0.03
		RC [A]	7.08	3.2	
		P A [B]	5.91	3.12	
	Flavour	DSD	3.64	1.78	0.07
		RC	6	3.69	
		P	2.91	3.2	
	Bitterness	DSD	3.9	3.1	0.50
		RC	4.53	3.81	
		P	2.94	2.31	
	Aftertaste	DSD	4.2	2.81	0.10
		RC	5.36	3.4	
		P	2.61	1.85	
	Liking	DSD	3.44	3.25	0.85
		RC	4.4	4.59	
		P	3.89	3.38	
Second	**Smell**	DSD [A]	57.4	14.35	0.02
		RC [A B]	43.9	15.31	
		P [B]	41.5	8.53	
	Colour	DSD [A]	54	16.26	0.02
		RC [A B]	37.5	11.93	
		P [B]	49.4	9.44	
	Flavour	DSD [A]	62.2	14.33	0.001
		RC [B]	47.8	14.17	
		P B	37.1	9.63	
	Bitterness	DSD [A]	58.7	18.01	0.001
		RC [A B]	43.1	22.91	
		P [B]	25.7	12.94	
	Aftertaste	DSD [A]	59.6	17.77	0.001
		RC [A]	46.6	13.2	
		P [B]	28.8	9.72	
	Liking	DSD	29.8	19.04	0.48
		RC	39	18.17	
		P	37.9	17.79	
Third	**Smell**	DSD [A]	64.7	26.19	0.001
		RC [A]	47.3	25.73	
		P [B]	21.1	12.22	
	Colour	DSD [A]	69.5	19.59	0.001
		RC [A]	63.4	22.48	
		P [B]	25	12.61	
	Flavour	DSD [A]	66.3	23.23	0.001
		RC [A B]	51.1	20.75	
		P [B]	28.2	20.58	
	Bitterness	DSD [A]	65.1	29.05	0.01
		RC [B]	33.5	18.3	
		P [B]	30.3	24.09	
	Aftertaste	DSD [A]	71.2	25.4	0.001
		RC [A]	50.1	20.31	
		P [B]	22.2	20.29	
	Liking	DSD [A]	21.5	17.88	0.001
		RC [B]	66.2	31.11	
		P [B]	27.7	31.19	

*DSD, RC and P are the coffee type; as DSD is the date seeds drink, RC is the regular coffee and P is the placebo. Alphabetical subscript denotes post hoc significant difference at P < 0.05 according to Tukey HSD test. Significant *P* values are highlighted in grey.

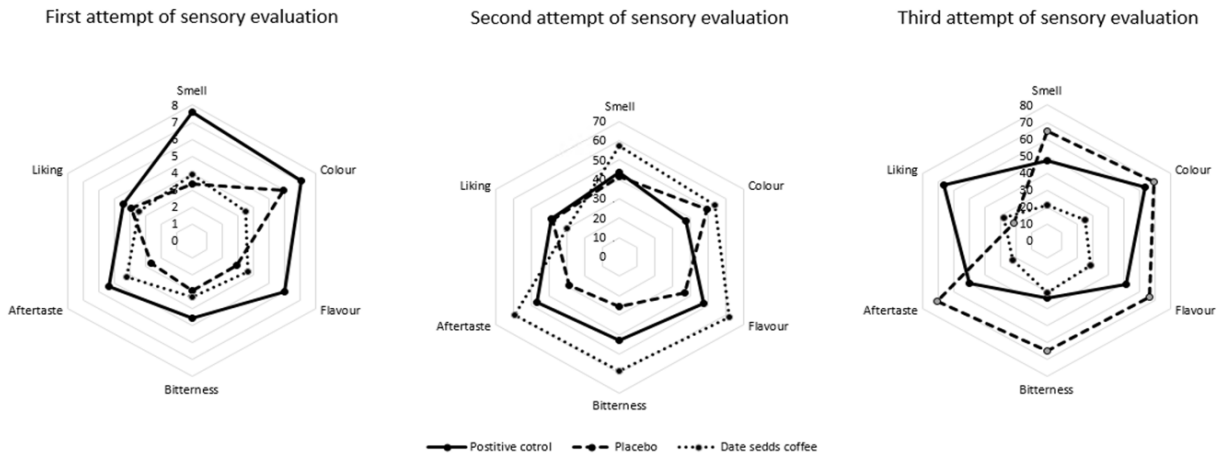


Figure 47. The sensory profile of each treatment type at each of the three attempts

4.11 Sample size and power calculation (final calculation)

Due to the limited research investigating the effects of date seeds drink on mood and cognitive function, it was challenging to find the most relevant paper to be the basis of the proposed trial power calculation. The three arms to be tested in this study are as follows; date seeds drink, regular filtered coffee, and a placebo made of coffee flavour and food colouring.

As the anticipated psychoactive component was the total catechins in the date seeds drink, it was initially decided to power this study according to the most relevant studies in this domain. However, regardless of the variation in the doses, the most enhancing effects of catechins and their derivatives were observed as physiological effects which are predictors for mood and cognitive enhancements, such as enhancing blood flow, blood pressure etc.

Wightman et al. (2012) reported that, in comparison to a placebo, the consumption of EGCG resulted in the modulation of CBF parameters in the frontal cortex during task performance. This effect was restricted to the lower (135 mg) dose of EGCG when compared to the higher dose (270 mg). Considering that to the best of our knowledge no one has investigated the effect of the drink made from roasted date seeds on mood and cognitive function, and according to our HPLC analysis to identify and quantify the

phenolic compounds in date seed ‘coffee’, we hypothesise that catechins and catechin derivatives from the date seed would acutely modulate cognitive functions and mood. There is a consistent effect of phenolic compounds in enhancing CBF, which is considered as underpinning the mechanism of action of such an effect. Also, there is no consistent effect of phenolics on mood and cognitive performance, as illustrated in Table 5. Therefore, when exploring a new or a novel food rich in phenolics, it may be justifiable to consider the mechanism of the CBF in the rationale of a new study. This rationale was used by Dietz et al. (2017) to investigate the effect of matcha tea and bars on cognitive function.

This proposed double-blind, placebo-controlled, crossover study therefore investigated the effects of a single dose of date seed drink on brain functioning and mood measured post-administration. The first attempt was to base the formulation of this single dose on the HPLC analysis of the date seeds drink extracted in water which is representive of the treatment execution, hoping to reach at least the lowest dose used in the Wightman et al. (2012).

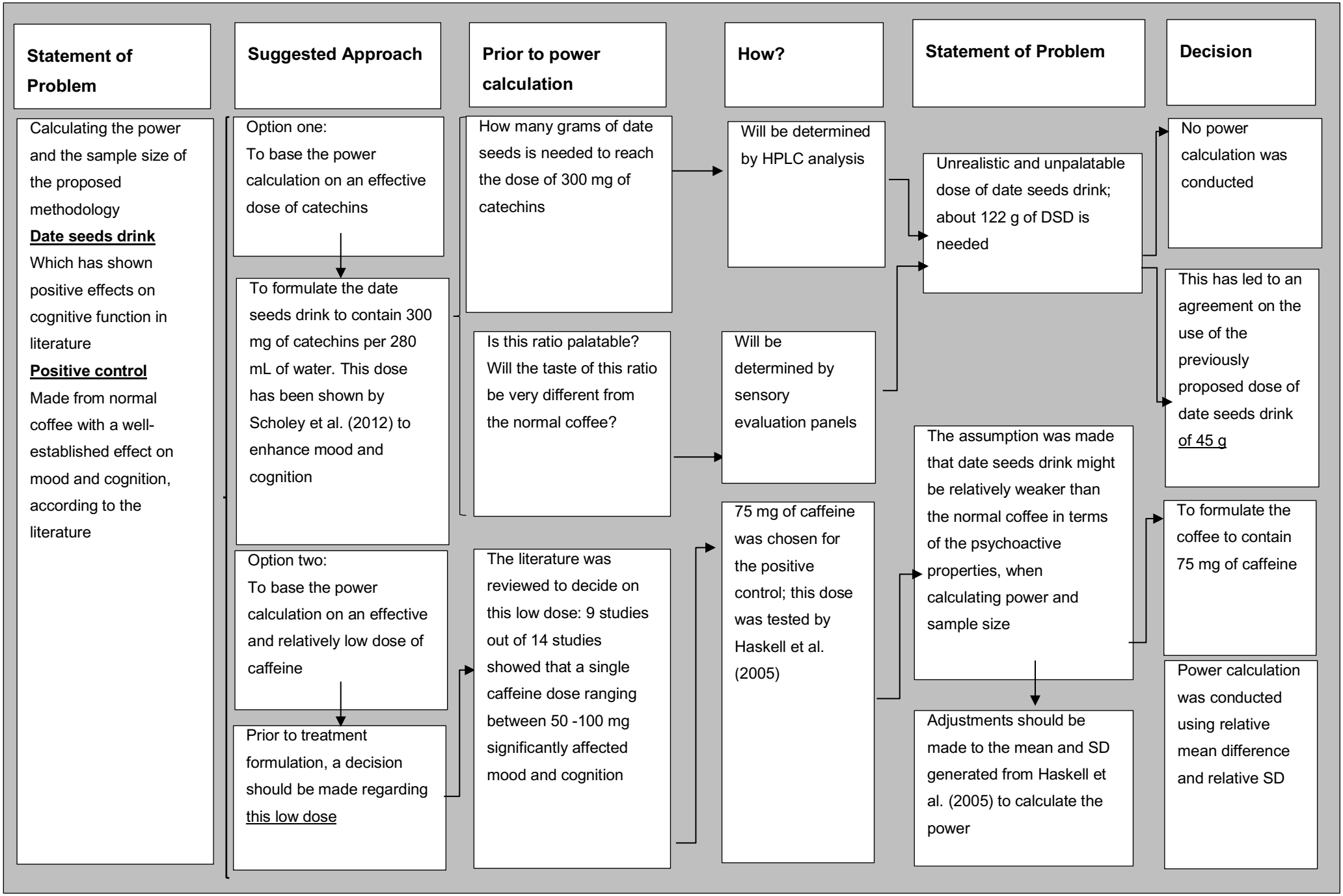


Figure 48. Overview of decision-making process.

A new approach to calculate the sample size was made by extracting all of the eighteen directly relevant cognitive measures and three mood measures from Haskell et al. (2005) to obtain a representative mean and a standard deviation to be used in the power calculation. This would be more representative and aid the detection of a comprehensive difference, rather than a random selection. Therefore, data using the dose of 150 mg was excluded and only data from the dose of 75 mg was extracted, as this dose is within the caffeine dose range chosen for the positive placebo (please see rationale for caffeine in section 4.8.2). An overview of the steps taken through the decision-making process are shown in Figure 48.

Moreover, data from both caffeine consumers and non-caffeine consumers were combined. The averages for both the placebo and the treatment (75 mg) were calculated and subtracted to determine the mean of the difference detected and the average SD.

Relative mean difference and relative SD were calculated for each measure using the following formula:

$$Relative\ mean\ difference = \text{absolute value of: } \frac{\mathrm{T} - \mathrm{P}}{(\mathrm{T} + \mathrm{P})/2}$$

$$Relative\ SD = \text{absolute value of: } \frac{\mathrm{SD}}{(\mathrm{T} + \mathrm{P})/2}$$

** Note that T is for treatment while P is for placebo*

Then, both the mean and SD were averaged for all twenty-one measures, providing a mean of 1.41 and a SD of 1.15 to be used in the power calculation. To obtain a power of 80%, the sample size at an alpha level of 5% to detect the expected difference between groups is 1.41 for the proposed study. Inputting these numbers in Minitab version 17, the results are reported in Table 32:

Table 32. Power calculation sample target outcome

Difference	***Sample size***	***Power***	***Actual Power***
1.41	*12*	*0.8*	*0.818593*

This gives a sample size of twelve participants to detect the expected difference between the placebo and the positive control. However, to be able to detect a difference that will have a weaker effect, assuming that the date seeds drink is not as potent as the positive control, the previously calculated mean was divided by two and used in the power calculation as follows:

$$New\ mean = \frac{1.41}{2} = 0.7$$

Inputting this number in Minitab version 17 gave the following results shown in Table 33.

Table 33. Power calculation sample target outcome

Difference	*Sample size*	*Power*	*Actual Power*
0.7	*44*	*0.8*	*0.805947*

This gives a sample size of 44 participants to detect a smaller difference between the placebo and the positive control.

4.12 Discussion

This chapter aimed to produce and develop an appropriate recipe for a roasted date seed beverage similar to coffee consisting of a precise quantity of presumably psychoactive properties together with sufficient levels of organoleptic properties similar to a positive control and placebo. HPLC analysis was performed to quantify the TPC and the CC for treatment creation.

4.12.1 TPC quantification

The basis of substituting the use of date seeds in the subsequent human intervention trial in place of date flesh was based on the high TPC reported in the literature. However, subsequent to trial analysis, a higher quantity was found in our results in comparison to those reported in the literature, between 2275.63 ± 3.72 to 6199.79 ± 338.31 mg of GAE /100 g which is considerably greater than the TPC quantity range of 3120 to 4430 mg of GAE /100 g stated in the literature (Al-Farsi and Lee, 2008) However, this range was attained following an extraction method utilising 20:80%

water/methanol and no information was available about the extraction method of samples in Al-Farsi and Lee (2008).

The TPC analysis of the six roasted seeds indicated that Medjool date contained the lowest TPC (2275.63 ± 3.72 mg of GAE /100 g). The commercial roasted date seeds drink which is made of Medjool seeds and extracted with only hot water, presented a lower phenolic content quantification of 635.53 ± mg of GAE /100 g. The only point of using 80% methanol was to increase the extraction efficiency and thus measure the highest possible value, even though this value is not relevant for drinks prepared using water. Thus, this indicates an association between the extraction method and TPC.

However, a parallel quantity of TPC for both the regular coffee and decaf coffee were obtained to those noted in the literature to justify the use of decaf coffee as a coffee flavouring as it contains less TPC compared to RC. The TPC of RC extracted in 100% of water and the TPC of DCP extracted in 80% methanol were 8383.02 and 3337.75 mg GAE/ 100 g, respectively. Results from a recent study which aimed to determine the TPC and antioxidant capacity of five different coffee beans found the TPC of a light to medium roasted coffee (regular) to be 54.87 to 80.51 mg/ g, equivalent to 5487 to 8051 mg GAE/100 g (Daniel and Workneh, 2017). Moreover, the TPC was quantified using the Folin-Ciocalteu method and aqueous and methanolic extractions and revealed no statistical differences between extraction methods.

The current study employed a water extraction technique to represent habitual consumption by humans. However, the solubility of phenolic compounds is widely influenced by solvent polarity, therefore the recovery of these from plant material is manipulated but a universal extraction technique is unattainable due to the variances in plants' matrix and the complexity of phenolic compounds (Alothman et al., 2009). Nonetheless, greater extraction rates due to an increased efficiency have been observed in less polar solvents such as methanol, ethanol and acetone (Lapornik et al., 2005).

Numerous factors could account for the variations detected. Most papers did not provide details of the sampling strategy, therefore, their samples may have not been representative. One such reasoning for the overestimation of the TPC within the analysed date seed types, and additional types of coffee in the chapter. In most

studies, TPC is analysed with separate peaks and both wavelength and retention are utilised explicitly for identification. A frequently utilised methodology is to calculate the equivalents, and this is comparable to the method employed in the quantification of TPC in apples by Somers (Way et al., 2020) with an absorbance at 280 nm for TPC. Abundant phenolic compounds are prominent due to compounds such as proanthocyanins, anthocyanins, hydroxycinnamic, and hydroxybenzoic acids which contain a distinguishable phenolic ring (Aleixandre-Tudo and du Toit, 2018). The method used in the present study employed ranges set for gallic acid, chlorogenic acid and epicatechin to assist with calculating the GAE, EPE and CAE. A 8-10 nm variance between the ranges permitted the incorporation of most derivatives with the specified wavelength irrespective of their retention time. This methodology considered all peaks within the 270–278 nm range as GAE, between 279–289 as EPE and 310–319 nm as CAE.

4.12.2 Other influences on TPC

However, the phenolic content of coffee is influenced by the coffee type and intensity (Król et al., 2020). The roasting time and temperature can also impact the intensity, notable as the richness of the coffee flavour, but these can furthermore affect the TPC due to thermal instability. For example, when exposed to intense roasting conditions, chlorogenic acids could potentially be almost entirely degraded to phenol derivatives (Farah and Donangelo, 2006). Commercial roasted coffee typically contains between 0.5 to 7% total chlorogenic acid content, although this depends on the processing, roasting, blend and analytical conditions. However, this content in light to medium roasted coffees is still prominent in comparison to the most other food sources (Perrone et al., 2008). Approximately 8-10% of chlorogenic acids per 1% loss of dry matter is lost due to drastic roasting conditions (Farah et al., 2006), which equates to loses as high as 95% (Trugo, 1984).

Arabica coffee has a lower total chlorogenic acids of 5780 mg/100 g in comparison to Robusta coffee which exhibited a TPC of 7002 mg/100 g of total CGAs when both were extracted in hot water (Farah et al., 2006). A combination of Arabica and Robusta with a light/medium roast was utilised in the regular coffee experiment (see Table 29) and the quantified TPC reinforced the aforementioned impact.

In contrast, the decaffeinated coffeeused instead of coffee flavouring in recipes 1 and 2 was a light/medium roast Arabica coffee. Despite the clow affeine content, the decaf coffee powder extracted in 80% methanol contained a comparatively high phenolic content to the date seeds drink extracted in water, TPC of 100 GAE mg /3 g and TPC of 110 ± 9 GAE mg /45 g respectively. Therefore, this was removed from both the placebo and the date seeds drink as demonstrated in the recipe development plan flowchart (Figure 44). Notably, this analysis in cycle 2 was only conducted on one replicate, therefore this may have influenced the robustness of the completed analysis.

4.12.3 HPLC method

HPLC analysis is a comprehensive technique widely used for separating, detecting and quantifying phenolic compounds (Kalili & De Villiers, 2011), hence, its use in the current study. Based on the published data, a higher phenolics content was expected than those established by HPLC. The results for the total phenolics of the commercial date seeds drink were sought to inform the dose of the date seed treatment, however, these were especially low that they were not applied. This is in addition to the aforementioned discrepancies produced by date cultivar, roasting methods and temperatures. The Medjool seeds incorporated in Cycle 1 of the HPLC analysis were purchased locally before being treated, roasted and ground in the pilot kitchen facilities of Newcastle University. The commercial date seeds drink produced from Medjool dates was obtained from Israel and no information on the processing and roasting of the seeds was obtainable. Further, there was also a difference in terms of solvents used to extract each sample, as the roasted Medjool seed was extracted in 80% methanol, whereas the commercial date seed was extracted in hot water only.

As briefly mentioned, the extraction methods utilised may explain why the total phenolics were substantially (3 to 4 times) lower than those reported by Habib et al. (2014a) and Al-Farsi and Lee (2008). The results for cycle 1 of the HPLC analysis demonstrated the lowest TPC for the roasted Mejdool seeds. The company supplying the commerical date seeds confirmed that they were from Mejdool dates so a third cycle of HPLC was conducted of samples extracted in 100% water, showing a large reduction in the amount of phenolics recovered compared to the Mejdool sample extracted in 20% water and 80% methanol in cycle 1.

Furthermore, discrepancies between the reported quantities of caffeine in the literature for the regular coffee and results from the study analysis were observed. A review of the lowest effective dose of caffeine in the literature, as discussed in section 4.8.2, aided in the positive controldesigned with a 6 g dose of coffee. A study by Quinlan et al. (2000) obtained 75 mg and 150 mg caffeine doses from 2.46 g and 4.91 g of instant Nescafe Gold blend respectively. However, the analysis of regular coffee conducted in the current study observed a CC of 69 ± 7 mg /6 g, a 8% reduction of the designed positive control, and although just a 6 mg difference it is important to not disregard this discrepancy. One difference is that instant coffee is fully water-soluble, while the coffee utlised in our trial was ground roasted coffee, which will have reduced caffeine solubility and thus, concentration in the served drink.

4.12.4 Sensory evaluation panels

The recipes were modified based on HPLC quantification of phenolic content and caffeine, so three sensory evaluation panels were conducted. The sensory analysis aimed to establish if any organoleptic variances were noticeable by participants, and this was specifically with reference to the different treatment attributes. Maintaining a level of blinding so that participants could not distinguish between treatments would allow their credible use in a nutritional intervention trial. Alongside the prominent importance of tight active ingredient control, this precipitated considerable challenges in recipe development, and despite the best efforts, some dissimilarities remained among the treatments, specifically in regard to the date seed drink.

In the first attempt, after the Bonferroni correction, no significant difference in all attributes was apparent. The results displayed a reasonable similarity among the organoleptic attributes for the first sensory evaluation, and further assisted in the development required before the second sensory evaluation commenced.

During the second attempt, no significant difference in smell was detected, confirming the effectiveness of the use of 3 g of decaf coffee to flavour the date seed drink and placebo. The second sensory evaluation was conducted in parallel with the completion of Cycle 2 of HPLC, both results triggered the removal of the decaffeinated coffee from the third recipe. Although the results of caffeine quantification in the decaf coffee was

confirmed as 0.27 mg/g and this was in line with the range of 0.139 to 0.22 mg/g reported in a comparable Arabica/medium roast coffee (Ashoor et al., 1983), it was much lower than the 101.5 ± 35 mg/100 g in regular coffee, equivalent to 1 mg/g according to Rodrigues and Bragagnolo (2013). Sensory evaluation results still detected a significant variance among three out of six attributes between the date seed treatment and regular coffee treatment. Furthermore, three out of six attributes showed a significant difference between the date seed treatment and placebo. These results were surprisingly dissimilar to those from the first sensory evaluation, in which the product was reformulated to increase the date seed drink strength.

The third sensory evaluation indicated an inadequacy in organoleptic consistency between treatments due to significant differences detected in almost all attributes. Nonetheless, this was determined to be the most acceptable recipe without disturbing the phytochemical elements. The dose of date seed employed (45 g/ 280 ml of water) was deemed the most feasible, without affecting palatability and consistency with a coffee beverage, while ensuring the highest possible TPC. This is due to properties of date seed ‘coffee’ such as a minor bitterness and earthly flavour being present.

Finally, the sensory evaluation panel was founded on the concept of taste testing all three treatments in succession. However, participants in the human trial will consume one treatment on each study day, and the study design employs a one-week washout period between each study visit. Therefore, this washout period may aid in ensuring participants have an inability to recall and differentiate between the treatments, since even trained panels are unable to remember details of sensory characteristics when sessions are spaced more than a week apart (Thybo et al., 2005). In addition, it was decided to conceal the colour and smell attributes further by the treatments being served in coffee cups with plastic lids for the study. The creation and development of the treatments have gone through numerous stages which were guided by recommendations on designing, conducting and reporting of studies within the human nutrition field, and some imperfections are both understood and acceptable (Welch et al., 2011). The aim was to create treatments which contain enough similar properties that they cause uncertainty and was not based on creating identical treatments. Therefore, it was decided to check or confirm this by the utilisation of a question in the

debrief of the human intervention trial asking the participants to speculate which of their three treatments they believe contained the date seed drink.

Furthermore, when evaluating the sensory features on the preference (likelihood of drinking the treatments on a regular basis), there was no significant difference among the three treatments in two of the panels. However, in the third sensory evaluation, a significant preference was observed for the regular coffee. Thus, it could be suggested that the attractiveness of the date seed drink increased when this is less intense (utilising 10 g over 45 g). Date seeds drinks have lately received a large amount of attention and research interest, with Ghnimi and Almansoori (2015) assessing and drawing comparisons of features between three commercially produced date seed samples and Arabic coffee. They elucidated a significant difference in five out of nine attributes, which is fairly consistent with the number of attributes being significantly difference in the current study, and all three date seed samples received lower scores in comparison to the Arabic coffee. Arabic coffee is brewed using a technique which is distinctive and unlike that used to prepare most other coffees. Thus, differences in scores could be accredited to the unusual brewing technique. The date seeds drink was prepared with 45 g roasted powdered seeds boiled in 100 ml of water for 2 minutes, whereas Arabic coffee powder is made by roasting the coffee beans lightly between 165–210°C and subsequently milling these beans into a powder. To construct the drink from this, 30 g of powder is boiled in 1 L of water for 20-30 minutes before saffron and cardamom are added (Habeeb and James, 2010).

In other research, the acceptability of cappuccino drinks with varying ratios of roasted date seeds was considered (Algarni, 2020), with a significant difference being observed within the 50% ratio of date seeds to regular coffee. Additionally, a sensory evaluation study by (Venkatachalam and Sengottian, 2016) looked at roasting date seeds using a similar technique to regular coffee, and adding this to 1:1 milk and 4 g of sugar once brewed in differing doses of 3%, 6% and 9%. The conventional coffee was prepared using 10 g of coffee brewed and mixed with 1:1 milk and 4 g sugar. Panellists reported no significant differences between the date seed and conventional coffee. Observations and multi-evaluations have exposed a weak taste of date seeds drink in comparison to the moderately high intensity of regular coffee. Therefore, the

addition of milk and sugar to the coffees may explain the sizeable parallel between the coffees due to successful masking.

4.13 Conclusion

The principal purpose of this chapter was to construct a novel beverage similar to coffee and a placebo to be utilised in the human nutritional trial to investigate the acute effects of consuming such treatments on mood and cognitive functioning in healthy young volunteers.

A commercially available date seed drink was bought and subsequently the contents of phenolic compounds were quantified. Due to the pre-established enhancing effect of caffeine, a positive control using regular coffee was designed. During the initial phases of development, both trial and error as well as trial and observation were employed to aid in the improvement of treatments to develop both palatability and homogeneity so that the participants could not distinguish treatments.

Although undergoing various development phases, the results of the sensory evaluation panels showed that the blinding element was not fully successful. However, the necessity for tight controls of the phenolics and caffeine presence in all treatments added a complexity to this process.

The c treatments were prepared utilising a coffee machine with a 280 ml volume, with the treatment (DSD) containing 110 ± 9 mg GAE/45 g, the positive control (RC) containing 503 ± 18 mg GAE/ 6 g of regular coffee and 69 ± 7 mg of caffeine, and a placebo (P) being made with boiled water and food colouring.

Additionally, the power calculation to establish the sample size for the human intervention trial was based on half of the effect observed for caffeine. This was accomplished founded on the average relative mean effect size for twenty-one attributes reported in a comparable study which looked at the acute effects of 75 mg of caffeine consumption. Furthermore, a clear HACCP plan was created to aid in the navigation of treatment preparation during the human trial by a third party (see Appendix LL for further information).

Chapter 5. An investigation of acute effects of mood and cognitive function following the administration of an alternative coffee made of roasted date seeds on healthy young volunteers: A double-blind, placebo-controlled, crossover design

5.1 Introduction

Chapter 4 presented data about the quantification of the phenolic content using HPLC. Statistically significant differences were detected among the six date seeds analysed, with the TPC ranging from 2397.90 ± 6.67 to 6199.79 ± 388.31 mg/100 g. Among the three phenolic equivalents measured GAE, CAE and EPE, the predominant phenolic was EPE which ranged from 2275.11 ± 501.77 to 5984 ± 338.23 mg/100 g. However, the selected commercial date seed drink has the lowest TPC (244.20 ± 14.68 mg/100 g) compared to the other six roasted seeds tested and more details about the analysis and the quantification can be found in chapter 4, section 4.6.7.3.

Studies observing the effects of the consumption of a “coffee-like” beverage made of roasted date seeds on mood and cognitive performance are absent within literature, and therefore those human interventions studying the effects of catechins were considered in this context. An improvement in cerebral blood flow, a physiological predictor, was observed post-supplementation of 135 mg of EGCG using near infrared spectroscopy (NIRS) (Wightman et al., 2012). Similarly, 300 mg of EGCG exhibited corresponding effects when determined by electroencephalogram (EEG) (Scholey et al., 2012). Within each of these studies, a methodology utilising young, healthy participants was employed, similar to the human intervention proposed for this thesis.

Several studies have explored the effects of catechins from either red or green tea, with results relating to mood and significant effects on cognitive tests. Both a single 336.4 mg dose and a daily intake over 12 weeks of decaffeinated green tea catechins (GTC), within a parallel design, displayed an improvement in both memory tasks and attention tasks (Baba et al., 2020). In line with this, significant improvements were presented in tasks which assessed both basic attention and psychomotor speed after supplementation of 4 g of matcha tea in contrast to the control in a randomised

placebo-controlled, single blind study (Dietz et al., 2017). However, these treatments contained 136 mg caffeine and 67 mg theanine in addition to 280 mg of EGCG. As discussed in chapter 4, section 4 4.8.1, the use of multiple stimulants makes it difficult to attribute observed effects to each compound and complicates utilising this in support of the rationale.

Suitable and accurate comparisons are challenging to draw between studies due to the variety of methods employed, such as the dissimilar cognitive assessment tools. However, the considered literature indicated that a variety of polyphenol sources may influence distinctive cognitive domains. Markedly, both spatial memory and immediate word recognition appear to be enhanced by the consumption of polyphenols, as explained in detail in Table 5.

Many different mechanisms have been suggested for the effects of polyphenol consumption upon mood and cognitive function. Most prominently, this was anticipated to be due to the antioxidant capacity (Rice-Evans et al., 1996), yet it has been disputed that this cannot be solely as a result of this capacity. A proposal has been made that polyphenols may contribute to neuron function protection and integrity, as perceptible by augmentations to the CBF and therefore aid in the overall health of neurons overtime (Williams et al., 2008, Sorond et al., 2008).

Aquas date seed extract (DSE) is a strong antioxidant and free radical scavenger (Vayalil, 2012). It has been tested in animal-based studies and showed some therapeutic effects which include hepatorenal protective (Ahmed et al., 2015, El-Far et al., 2016), cerebroprotective (Kalantaripour et al., 2012b, Hasan and Mohieldein, 2016), anti-diabetic (Hasan and Mohieldein, 2016) and normalised elevated cholesterol in rats with high-fat diet' (Takaeidi et al., 2014). Most studies examined the prevention or therapeutic effects of date seeds using a raw seed (Majid et al., 2008a, Kalantaripour et al., 2012a, Habib and Ibrahim, 2011), instead of a roasted seed.

A recent study revealed that the TPC of the brew ranged between 8778.61 to 15510 of GAE mg/100 mg DW when it was roasted for 10-30 min at 160ºC, quantified using the Folin-Ciocalteu method (Fikry et al., 2019). Although, the Folin-Ciocalteu method is known to overestimate the TPC, however, in case of this study, the reported values seem to be incorrect. However, the consideration of the effect of roasting in increasing

the TPC of the date seeds motivated new research to examine this newly promoted alternative, free of caffeine coffee instead (see section 1.12 for more information).

The antioxidant capacity of DSE was considered on the basis of the positive effects observed, when utilised as either a protective or mitigative treatment against neurodegenerative diseases in animal studies. Kalantaripour et al. (2012) investigated the cerebroprotective effects of aqueous date seed extract on rats, observing a significant increase in the antioxidant enzyme activity (SOD) and antioxidant levels. Although, the mechanistic insight by which date seed induces a cerebroprotective effect remains unknown, it has been attributed to the phenolic compounds within the date seed. However, no information was available about the phenolic content of the administered date seed treatment (Kalantaripour et al., 2012a).

Furthermore, an acute (150 mg/kg single dose) and subacute (150 mg/kg/day for 7 days) study was performed to evaluate the neuropharmacological effects of the DSD against a regular commercial coffee extract as a control. The neuropharmacological activity was evaluated using the open field test (locomotive activity) and phenobarbital sodium (a popular hypnotic and sedative drug) induced sleeping time test (Farag et al., 2019). Hence, DSD had an alertness effect on the experimental animal. However, pre-treatment with DSD and the commercial DSD showed no significant stimulant effect on the central nervous system in mice locomotive activity (which reflects alertness and wakefulness of mental activity) compared to the group pre-treated with RC, and this may be due to the absence of caffeine in DSD (Farag et al., 2019). The 150 mg/kg mouse dose corresponds to a dose of about 800 mg for a human (using a weight of 70 kg and a dose translation factor of 12), which allows a comparison between the mouse dose to our dose of DSD. This has revealed that the DSD dose of 110 ±9 mg/45 g utilised in this thesis is deemed to be much lower than the dose used by Farag et al. (2019).

In conclusion, there is an inconsistency amid polyphenol sources, doses and correlated efficacy, as shown in Table 5. DSD presents a high phenolic content, however, there is a lack human research examining the effects of DSD on mood and cognitive function. Therefore, this provides a worthy proposal for exploring the acute effects of DSD on mood and cognitive function within a human intervention.

The aim of this aspect of the current study therefore focused on the impact of standardised date seed drink upon various cognitive functioning indices and mood. The form that was used was a commercially available roasted date seeds drink. This coffee will be tested against regular coffee (positive control) and a negative control (placebo). Both drinks were brewed using the same method. All drinks were similar to some extent in terms of colour and flavour but differing in composition. Due to the phenolic compounds being hypothesised as the major active compounds in date seeds, the cognitive assessments were conducted with the phenolic levels within the maximum concentration (Cmax), as explained previously in section 3.1, between 45- and 90-minute post-supplementation. Biofluids were not included in collection or analysis, and consequently were not used to consider the bioavailability of the phenolic content.

5.2 Study design

The study investigated the acute effects on human cognitive function and mood after the administration of a single dose of a 'coffee-like beverage' made out of roasted date seeds against a positive control made of regular coffee and a negative control which were all matched for flavour, colour and appearance. The study followed a double-blind, counterbalanced, placebo-controlled, repeated measures design. Treatment orders were counterbalanced with the use of a Latin square. The primary objectives of the current study focused upon the impact of a standardised for roasted date seed powder upon cognitive functioning and mood. The date seed powder was commercially available and purchased as ground roasted date seed powder. The dose of date seed drink was 45 g extracted in 280 ml of hot water, which yielded a TPC of 110 ± 9 mg of GAE/45 g/280 ml.

5.2.1 Study design development

Extra attention was paid in controlling the background noises during the performance of the cognitive tests compared to when the first trial was conducted. Additional signs declaring "cognitive test in progress, please be quiet" were positioned around the facilities and disposable earplugs were provided to the participants to restrict any repercussions from noise disruption.

5.3 Methods

5.3.1 Ethical approval

The study was approved by the FMS Ethical committee Newcastle University, application number 164/9711/2019, and registered on ClinicalTrial.gov (number NCT04009564) (see Appendix W, Appendix AA andAppendix BB).

5.3.2 Participants

A total of 52 healthy participants aged between 18 and 35 were recruited through advertisement via poster and flyer from Newcastle, including 38 females and 11 males (see Table 34 for more details). Each participant received a £40 voucher as an honorarium.

Table 34. Descriptive characteristics of the participants[18].

Descriptive Statistics	*Min*	*Max*	*Mean*	*SD*
Height (cm)	148	187	165.4	8.8
Weight (kg)	42.6	115	65.5	14.2
BMI	18	30	24	3.6
Age	20	35	26.6	4.9

5.3.2.1 Participant criteria

All participants signed a consent form and reported themselves to be healthy, not pregnant, not consuming any over the counter medication or supplementations, with no food allergies or sensitivity to the treatment constituents. All were non-tobacco users and had a body mass index below 35/m^2 but no lower than 18/m^2.

Screening for any contraindications to the study was completed with the use of an exclusion questionnaire and a case report form (see Appendix DD, pages S3 and S4).

[18] *Values are presented in mean ± SD, (n=49; 38 females and 11 males).

The anthropometric data obtained is shown in Table 34. Sample size was determined and illustrated in detail in chapter 4, section 4.11. Accordingly, 48 participants were utilised to detect a true difference between the treatment groups with a power of 85%. It is important to mention that this study was initially powered to 90% with a total of 56 participants required to reach this power. However, due to Covid-19 and the difficulties in resuming the trial during the lockdown period, the power was decreased to 85%. Recruitment was stopped after the breakout of Covid-19 and the subsequent lockdown and restrictions by the UK government to limit face-to-face activities, so data was collected for 52 individuals.

In total, 70 participants were screened, of which 13 dropped out, due to the long-time commitments required for the study. A total of 57 participants were supposed to complete the study, however, due to the Covid-19 pandemic, only 52 participants successfully completed the trial. The detailed recruiting process flow chart is available in Figure 49.

5.3.3 Study treatments

The study followed an acute dose crossover, placebo-controlled, double-blind, randomised design, with three treatment conditions: DSD, RC and placebo. The DSD treatment consisted of 45 g of date seed drink, while the RC consisted of 6 g of regular coffee (see Table 35). It might be important to mention that the reducing sugar content in date seeds was low compared to the optimal dose of 25 g glucose to be effective to enhance cognitive functions in humans (Owen et al., 2012), and thus, it is unlikely to contribute additional cognitive effects. Hossain et al. (2014) reported the percentage of glucose and fructose content in dates pits to be 2.180±0.152 and 2.287±0.074, respectively, which contribute to 0.98 and 1.02 g of glucose and fructose/45 g of the date seed dose utilised in this study.

Brown colouring was added to maintain treatment blinding. The TPC and the caffeine quantity of each treatment was quantified as described in chapter 4, section 4.6.7.3. The placebo treatment consisted of boiled water and food colouring only, with no TPC. Both DSD and RC treatments were in powdered form and were prepared using a filter coffee machine and administered as a normal hot coffee drink in paper cups with a lid

to maintain treatment blinding. The coffee powders were weighed prior to the study day according to the doses, before being air vacuumed, sealed and stored frozen within the walk-in freezer at the NU-Food facility at Newcastle University until the day of use.

Table 35. Total phenolic content (TPC) of the three study treatments

Treatment	***Date seed drink DSD 280 ml***	***Regular coffee RC 280 ml***	***Placebo 280 ml***
TPC (GAE+CAE+EPE)	110 ± 9 mg/280 ml	503 ± 18 mg/280 ml	NA
CC mg	ND	69 ± 7 mg/280 ml	NA

*Date seeds drink (DSD; 45 g in 280 ml of hot water) and regular coffee (RC; 6 g in 280 ml of hot water) and placebo. TPC is the total phenolic content, GAE is gallic acid equivalent, CAE is chlorogenic acid equivalent, EPE is epicatechin equivalent and CC is caffeine content and ND is not detected.

Treatments were made by adding 280 ml of boiled water and contained no added milk or sugar. The three treatments were coded and a randomisation schedule for each subject was generated using Williams Design (see Appendix KK for more details) by a third party who had no other involvement in the study. No member of the investigation team was aware of the coding of the treatments until the blind-data review was completed after the statistical analysis and the determination of any significant difference was completed. Food safety during treatment execution was ensured according to the HACCP plan, available in chapter 4, Appendix LL.

The sensory evaluation panel conducted in chapter 4 showed that sensory differences existed between the date seed drink, the regular coffee and the placebo. However, this did not necessarily constitute a failure of the blinding procedure, since determining which treatment is received as a single treatment is much more difficult than detecting minor sensory differences in a direct comparison. Therefore, a further question was added to the written debrief of the current study, which was given to the participants after the completion of the trial, as follows:

“Which of the three treatments you had was the date seeds drink?” The aim of this question was to check if the participants were able to recognise the treatment identities.

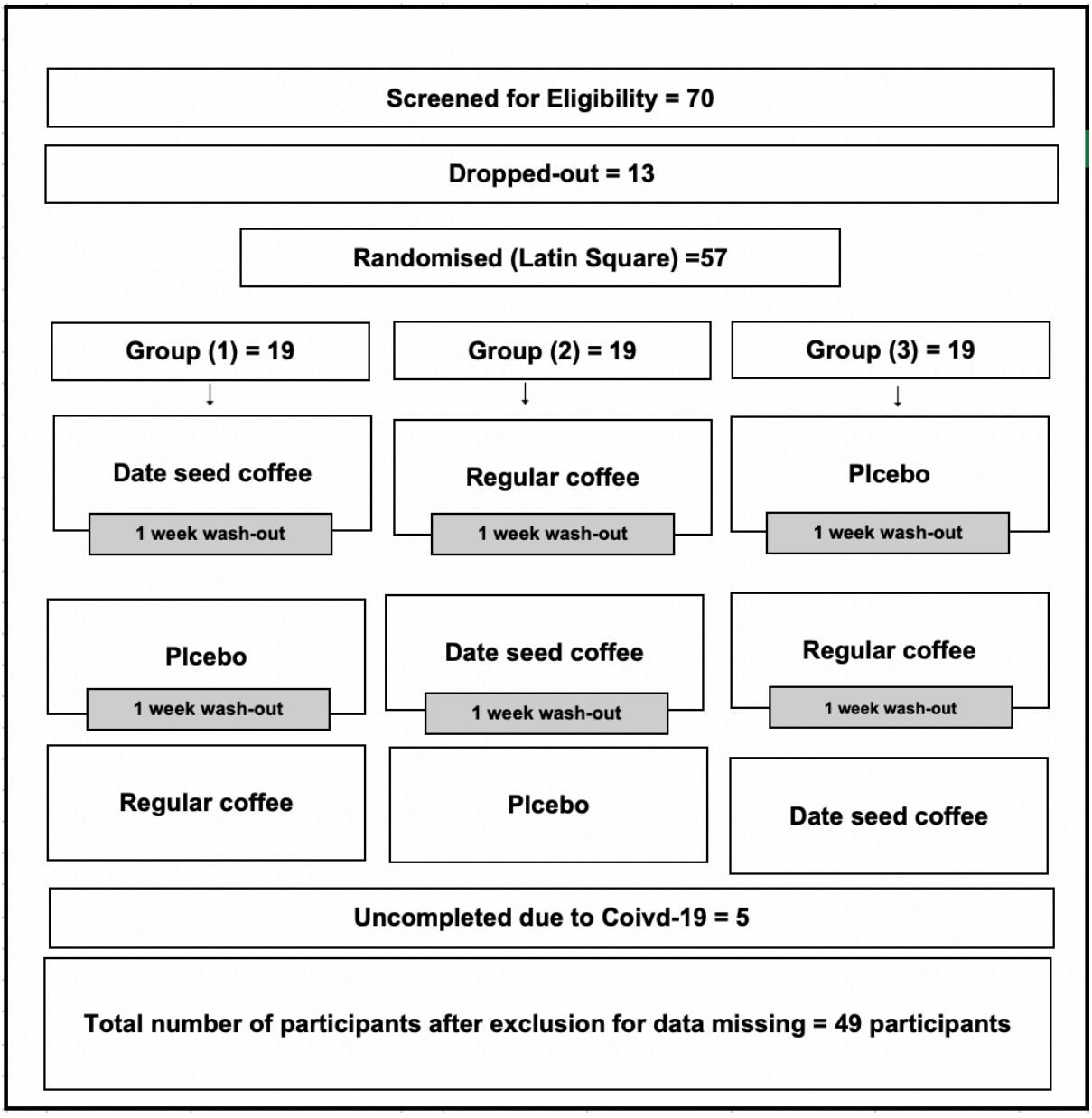


Figure 49. The recruitment process of participants date seeds drink DSD, regular coffee RC and placebo P

5.3.4 Cognitive function test CogTrack

A series of nine online cognitive tests (cognition) within the CogTrack system were used to assess cognitive function. This method was used in chapter 3 to assess the acute effect of the administration of date fruit on mood and cognitive function on young healthy volunteers. The following tasks are described in the order in which they were administered in chapter 3, section 3.3.4.

- Immediate word recall
- Pattern separation
- Simple reaction time
- Digit vigilance
- Choice reaction time
- Spatial working memory
- Numeric working memory
- Delayed word recall
- Word recognition

The selected nine tasks were combined to calculate eight core measures of cognitive indices according to certain equations: Attentional Intensity Index, Sustained Attention Index, Attentional Fluctuation Index, Memory Retrieval Speed Index, Cognitive Reaction Time, Working Memory Capacity index, Episodic Memory Capacity Index and Quality of Memory Index. The calculation of each index and a full description of the equation of each index can be found in Chapter 3, section 3.3.4.11, Figure 25 and in Appendix EE.

5.3.5 Mood scales

5.3.5.1 Bond-Lader VASs of mood and alertness

The sixteen visual analogue scales of the Bond-Lader were converted into an electronic version by an expert member of staff from the Faculty of Natural and Environmental Sciences at the University of Newcastle. Scores from the sixteen Bond-Lader visual analogue scales were combined, as recommended by the authors, to

form three mood factors: ‘alert’, ‘calm’ and ‘content’ (Bond & Lader, 1974) as described in chapter 3, section3.3.5 and in Appendix FF.

5.3.5.2 Caffeine research VASs

This questionnaire was designed to capture mood changes associated with caffeine and has shown sensibility in detecting changes in mood following the consumption of caffeine in previous studies such as Rogers et al. (2003), Scholey and Kennedy (2004), and Camfield et al. (2013). It consists of seven descriptors (‘relaxed’, ‘alert’, ‘jittery’, ‘tired’, ‘tense’, ‘headache’ and ‘overall mood’) with each followed by a 100 mm horizontal line scale labelled ‘not at all’ (left-hand end) and ‘extremely’ (right-hand end); except for the line for overall mood which was labelled ‘very bad’ and ‘very good’. Volunteers were instructed to ‘mark the lines according to how you feel right now.’ Ratings were scored from 0–100.

5.3.5.3 Caffeine consumption Questionnaire

Caffeine habits for the previous day were assessed using a caffeine consumption table developed by Bühler et al. (2014). This questionnaire has been used to assess caffeine consumption of volunteers during the day within several studies, such as Gonçalves et al. (2017) and Wesnes et al. (2017a), and also in assessing the effect of caffeine consumption on sport, such as Wilk et al. (2019) and Saunders et al. (2017). At the screening visit and on each study day, participants were asked to complete the table to determine their individual caffeine habits.

5.3.6 Procedure

After recruitment, the volunteers attended a training/familiarisation session during which they performed the CogTrack system on three successive occasions to ensure that any effects of practice, familiarity or test anxiety had been overcome prior to the first study day (Wesnes and Pincock, 2002). Before the screening day commenced, they were asked to sign the informed consent form. See Figure 50 for an illustration of the study timeline.

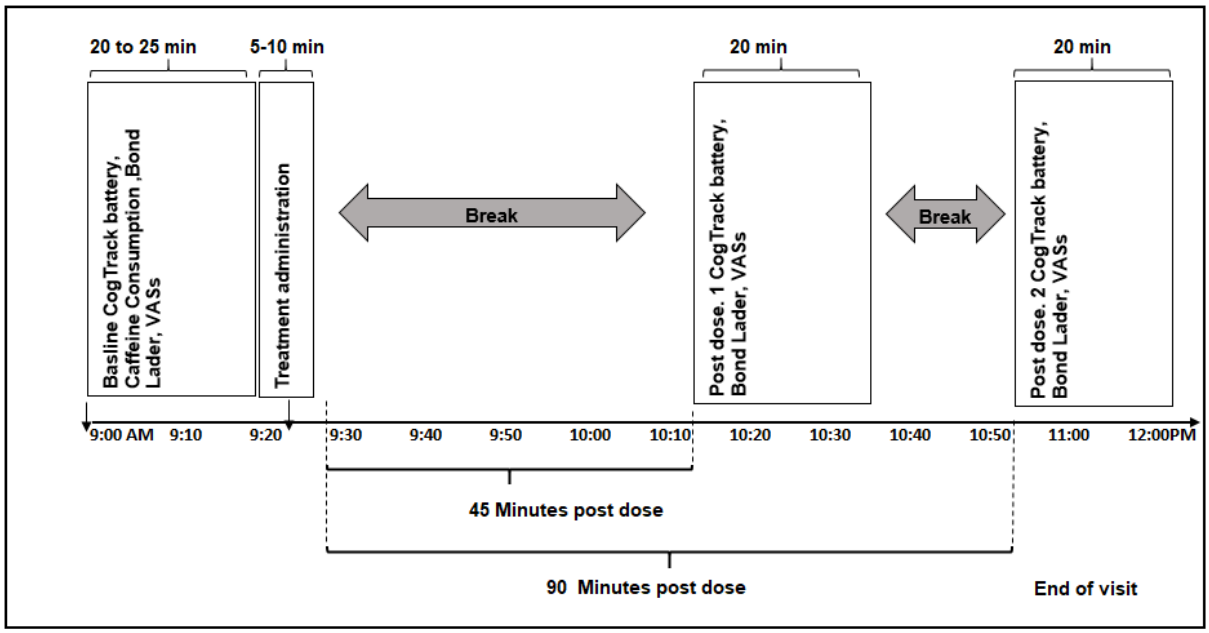


Figure 50. The timeline of each study da

5.3.6.1 Dietary restrictions and nonintervention foods

The volunteers were instructed to limit their consumption of tea and certain fruits (such as oranges, apples, grapes, peaches, grapefruit juice, cherries, blueberries, pomegranate juice, raspberries, cranberries, black elderberries, blackcurrants, plums, blackberries, strawberries, apricots) the day before each visit to one portion or less (as these are rich in phenolic content), and to avoid alcohol for the 24 h prior to arrival, as well as to have fasted and not consumed caffeine since 21:00 the previous evening. On each study visit, they were required to attend the laboratory at 09:00, and each visit was separated by seven days.

Baseline assessments for the CogTrack, caffeine consumption questionnaire, Bond-Lader and caffeine research visual analogue scales (VASs) were completed. The volunteers were then given the allocated treatment drink and a cup of water, with all of the treatment having to be consumed within five to ten minutes. The Bond-Lader and VASs were taken after each performance of the CogTrack system and were repeated at 45 and 90-min post-consumption of the treatment. However, the caffeine consumption questionnaire was only completed at the baseline assessment. The timeline of each study day is illustrated in Figure 50 above.

After the completion of the three visits, the participants were given a £40 voucher to thank them for their participation and a written debrief containing the question regarding treatment recognition (see Appendix P).

5.3.7 Statistical analyses

The baseline scores over the three study conditions for each outcome measure (CogTrack, Bond-Lader mood scales and Caffeine research VASs) were compared using MMRM analyses of variance (ANOVAs) to ensure that there were no systematic variations over the study conditions. Change from baseline for each outcome measure (CogTrack, Bond-Lader mood scales and Caffeine research VASs) was calculated by subtracting each post-dose (45 min and 90 min) scores from the baseline scores of each measure. Linear mixed model analyses (MML) were conducted on the change from baseline data for all outcomes. Study treatments (three levels), time of testing (two levels), and the interaction between study treatment and time of testing were fitted as fixed factors. In addition, for consistency, interpretability and agreement with other studies (Watson et al., 2015, Wesnes et al., 2017b, Watson et al., 2019), the cognitive tests were combined to create the eight measured indices of cognitive function (as described previously in Chapter 3, section 3.3.4.11). All analysis was conducted using the SPSS statistical package version 24 for Windows.

As explained in chapter 3, section 3.6, two statistical approaches were performed to express caution in the interpretation of the results due to the novelty of this research. Linear mixed model analyses (MML) were conducted on the change from baseline data for all outcomes. Study treatments (three levels), time of testing (two levels), and the interaction between study treatment and time of testing were fitted as fixed factors. The analysis was conducted using the SPSS statistical package version 24 for windows. The MML was conducted twice as follows: a less cautious approach for all outcomes from CogTrack: individual tasks and indices in addition to all mood outcomes of Bond-Lader scales and Caffeine research VASs. For measures which had significant main effects of treatment and/or interactions, between-condition pairwise comparisons were performed using simple MMRM analyses of variance ANOVAs with multiple comparisons. Moreover, a more cautious approach for the same outcomes but with Bonferroni corrections was performed to identify the degree

of any significant differences between the study treatment conditions and to protect from Type I errors identified by the ANOVAs. The alpha level of 0.05 was divided by the number of variables in each measure, therefore, divided by thirty for the thirty individual cognitive tasks, divided by eight for the eight measures for the CogTrack indices, divided by seven for measures of Caffeine research VASs, and divided by three for the three measures for the Bond-Lader mood scale.

The total caffeine intake as mg/day for each participant was calculated from the data gathered within the consumption questionnaire (Table 36). Consumption was then categorised into high and low caffeine daily intake as follows: (41- 240 mg/day) and (0-40 mg/day). A similar categorisation of participants with low and high caffeine consumption was utilised in another study by Haskell et al. (2005). Quantities of 0 and 240 mg were the lowest and the highest amounts estimated from the data reported by the participants within the questionnaire. To test the effect of caffeine consumption on the outcome measures, the same MML model was conducted on the change from baseline data with the same factors (treatments and repetitions), but the caffeine consumption category was fitted as a third fixed factor this time.

Finally, in case there were no significant effects detected and to be able to see a general direction for each treatment effect, the direction of change from baseline for each treatment for the total number of participants were pooled and tested using Chi-square. Summing the data of each treatment could cause result reinforcement, which cannot be seen in ANOVA. Chi-square again was calculated for each measure individually only when clear variations among treatments were observed.

5.4 Results

5.4.1 Missing data

Three participants had partially missing data on one day. All data sets for the participants with IDs AD52, AD54 and AD56 were entirely excluded from the data sheet prior to the analysis of all measures: Bond-Lader and Caffeine research VASs. Therefore, the number of participants decreased from 52 participants to 49 participants. Detailed information about the specific tasks in which the data were missing is available in Appendix HH.

5.4.2 Caffeine consumption questionnaire

The caffeine-containing products which the volunteers reported taking on the day before the laboratory visits were in descending order of frequency: coffee, chocolate bars, tea, cola-type fizzy drinks, and energy drinks. The percentage of the volunteers which reported not taking any caffeine-containing product prior to each of the four visits screening, visit 1, visit 2, visit 3 and visit 4 were as follows: 12.2%, 16.3%, 18.4% and 18.4% respectively. The consumed caffeine products as described as portions/day were 1.37 ± 0.1, with the range being 0–5 (see Table 36). The percentage of volunteers who reported consuming five portions prior to each visit was: 2%, 2%, 4% and 0%. The percentage of the most frequently consumed of caffeine products are illustrated in Appendix JJ.

Table 36. Summary of the average amount of caffeine consumed /day in mg by the participants

Statistics	***Average amount of caffeine consumed/day (mg)***
Mean	61.89
SD	49.73
Min	0
Max	240

*Obtained from the caffeine consumption questionnaire at the start of each study visit.

5.4.3 Baseline

For the baseline of each of the three visits, for CogTrack measures, Bond-Lader mood scales' factors and Caffeine research VASs, the MMRM ANOVA's methodology was undertaken by using the actual scores obtained at the baseline of each study visit. None of the measures approached significance (all $p>0.05$), and thus the MML was conducted as planned.

5.4.4 CogTrack individual tasks' outcomes

5.4.4.1 A less cautious approach

Delayed word recall task

There was a significant main treatment effect on the percentage of the correct words of the delayed word recall task (F=3.34, P=0.037). A pairwise comparison revealed that the attenuation of the decline was significant for the placebo (P=0.01) but not for date seed drink nor regular coffee, with no significant difference between regular coffee and date seed drink detected (P=0.56).

Pattern separation task

There was a significant main treatment effect on the original stimuli average speed (msec) of the pattern separation task (F=8.60, P=0.027). A pairwise comparison revealed that the decrease was not significant for both the date seed drink (P= 0.127) and for regular coffee (P=0.244), but there was a significant difference between date seeds drink and regular coffee (P=0.007). However, for both task outcomes, data visualisation in Figure 51 suggests the occurrence of Type I errors.

5.4.4.2 A more cautious approach

As explained in section 5.3.7, a cautious approach was also used to increase the certainty about the previously reported tasks that had reached a significant level. Thus, the alpha level for the CogTrack tasks was corrected by Bonferroni correction by dividing the alpha level of 0.05 by 30 (number of outcomes) = 0.0017. Therefore, the results were readjusted, but none of the two tasks outcomes reached the significance level (all p>0.0017) required.

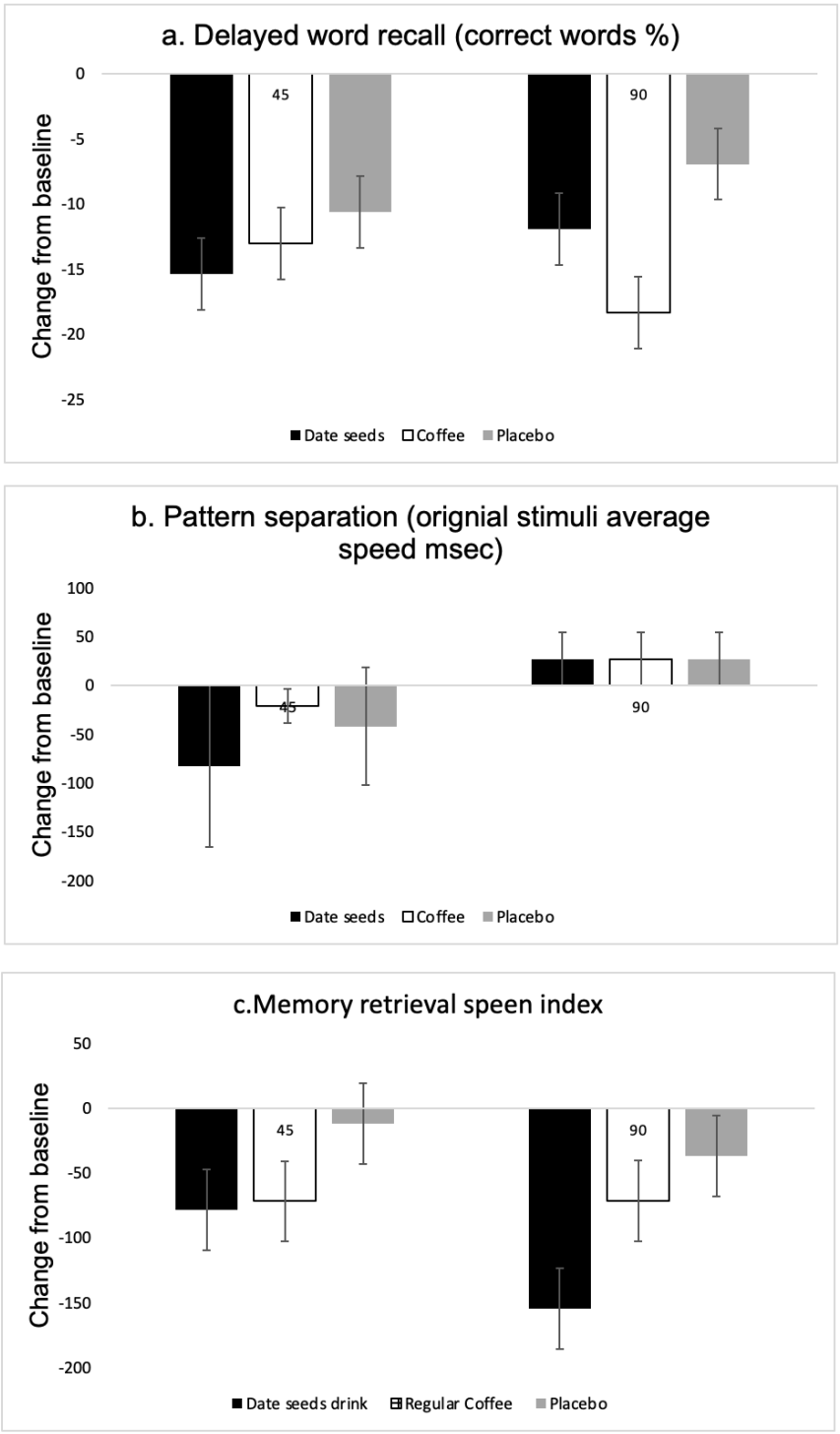


Figure 51. Main effects of change from baseline scores for core measures

(a) Percentage of delayed word recall task, (b) average speed for original stimuli for pattern separation task, and (c) memory retrieval speed index. Scores are presented as means and SE. Descending scores reflects impairments compared to baseline levels, ascending scores reflect improvements, except for average of speed where descending and ascending scores indicate the opposite.

5.4.5 CogTrack, Bond-Lader and Caffeine research Visual Analogue Scales

5.4.5.1 A less cautious approach

For the eight CogTrack indices, there was no significant main effect of treatment, neither of repeated post-treatment test sessions, nor an interaction between treatments, with the exception of one index of CogTrack. There was a significant main treatment effect on the memory retrieval index (F =4.42, P=0.01). A pairwise comparison revealed that the decrease was significant for the date seed drink (P= 0.003) but not for regular coffee (P= 0.12), and there was no significant difference between date seeds drink and regular coffee (P= 0.14). However, the data visualised in Figure 51 indicates Type I errors. The overall changes from pre-dose values for all CogTrack, Bond-Lader and Caffeine research VASs measures are presented in Table 40.

5.4.5.2 A more cautious approach

As explained in section 5.3.7, a cautious approach was also used to increase the certainty about the previously reported tasks that had reached a significant level. Thus, the alpha level for the CogTrack indices was corrected by Bonferroni correction by dividing the alpha level of 0.05 by eight (number of CogTrack composites outcomes) = 0.00625. Therefore, the results were readjusted, and it was found that memory retrieval speed index did not reach significance (all p>0.00625).

5.4.6 Effect of habitual caffeine consumption

The MML with caffeine consumption category fitted as a fixed factor showed that there was a significant main effect of the caffeine consumption category, but not for treatment and caffeine consumption category interaction, in one CogTrack index outcome and two outcomes of the caffeine research VASs as follows: Sustain Attentional index (F=11.399, P=0.001), Tense (F=4.721, P=0.031) and Headache (F =4.755, P=0.03). To increase the level of certainty about the two indices which had reached a significant level, the alpha level for the CogTrack indices was corrected by Bonferroni correction, for the CogTrack indices by dividing the alpha level of 0.05 by eight (number of measures) = 0.00625, and divided by seven (number of measures)

= 0.00714 for measures of Caffeine research VASs. Therefore, the result was readjusted, with only the sustained attention of the CogTrack indices reaching significance (all P >0.00625). Data visualisation in Figure 52, 53 and Figure 54 illustrate the observed effect of the caffeine consumption factor.

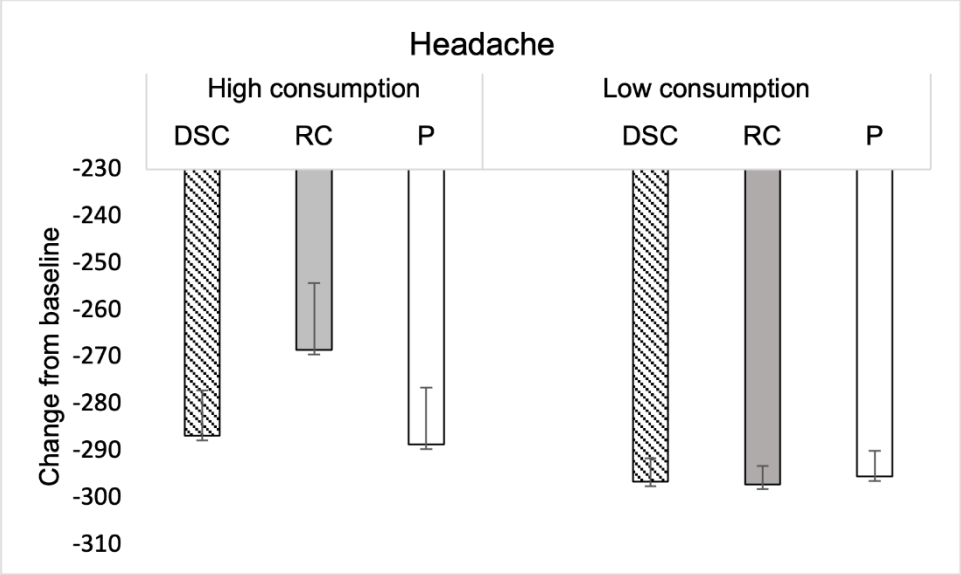


Figure 52. Headache (caffeine research VAS).

*Date seeds drink (DSD), regular coffee (RC) and placebo (P).

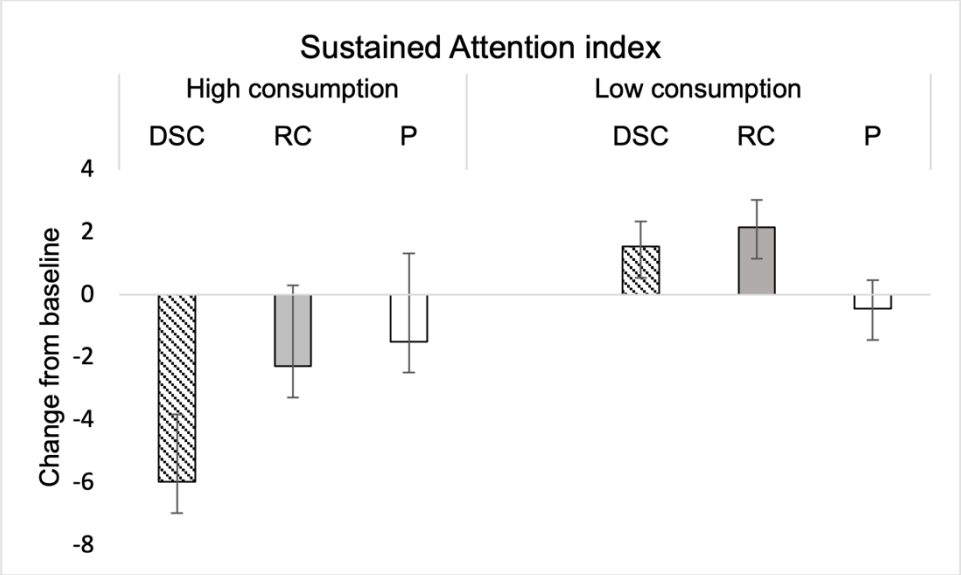


Figure 53. Sustained attention index (CogTrack).

*Date seeds drink (DSD), regular coffee (RC) and placebo (P).

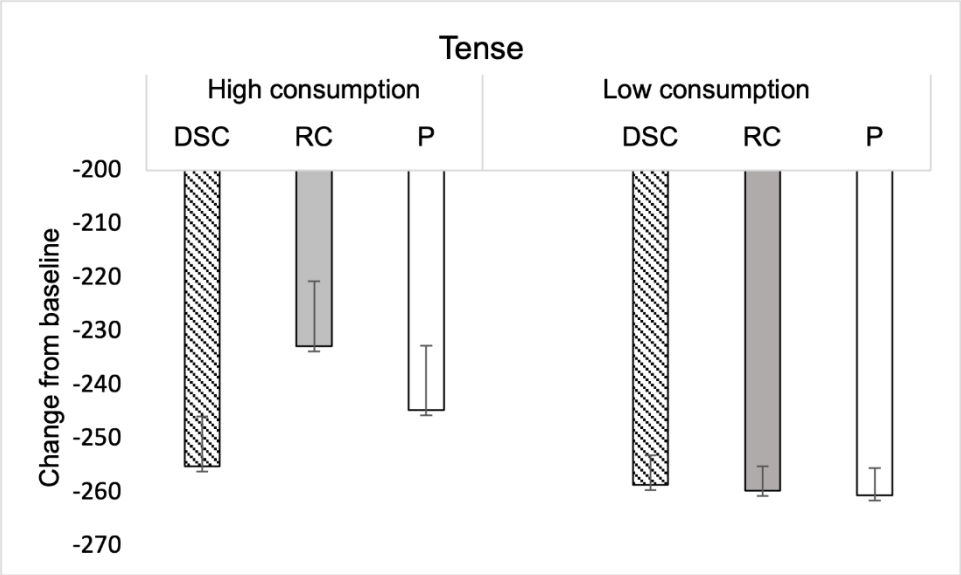


Figure 54. Tense (Caffeine research VAS)

*Date seeds drink (DSD), regular coffee (RC) and placebo (P).

5.4.7 Treatment guesses (Chi-square)

The calculated Chi-square of the true and false answers for the survey question: which of the three treatments you had was the date seeds drink?

The Chi-square test for the participants who guessed the date seed drink correctly did not differ from those who did not, $X^2_{(1)} = 0.702$ ($p = 0.401$), which means that the number of expected true or false answers was not significantly different from the observed answers. Therefore, the number of participants who were not able to recognise which treatment was the DSD was not statistically significantly different from the number of participants who were able to do so; 59% of the participants could not differentiate the study treatments (see Table 37).

Table 37. Values and percentages for observed correct and false answers vs expected correct and false answers

Observed correct answers	***Observed false answers***	***Expected correct answers***	***Expected false answers***
20	29	16	33
40.81 %	59.18 %	33 %	67 %

5.4.8 Effect direction (Chi-square)

The calculated Chi-square of the positive and negative outcomes for the CogTrack and Bond-Lader measures of all the data summed together showed no statistically significant difference between the DSD, RG and placebo, with a statistically significant difference in one of the Bond-Lader mood scale indices Alertness. The date seeds drink and regular coffee had a positive direction in alertness when compared to the placebo; alertness $X^2_{(1)} = 6.36$ ($p = 0.02$) $p < 0.041$. Table 38 shows that 33% and 34% of participants were more alert following the consumption of DSD and RC respectively.

Table 38. Number of participants who reported greater or lower alertness scores for the different treatments

Measure	*Treatments*[19]	*Observed Negative*	*Observed Positive*	*Expected Negative*	*Expected Positive*
Alertness	DSD	16	33	24.5	24.5
	P	26	23	24.5	24.5
	RC	15	34	24.5	24.5

[19] DSD is date seeds drink, RC is regular coffee, P is the placebo.

Table 39. Mean ± SE for pre-dose baseline and change from baseline and *ANOVA* for the individual tasks of CogTrack of date seeds drink, regular coffee, and placebo

(n= 49; 38 females and 11 males)

Task	Measure	Treatment	Baseline	Post-dose change		Effect of treatment		Effect of treatment* time intervals	
			0 Min	45 min	90 min	F	P	F	P
Immediate Word Recall	Correct words %	Date seeds	48.71 ± 2.33	-5.17 ± 2.40	-3.81 ± 2.40	0.82	0.44	1.33	0.27
		Coffee	50.48 ± 2.02	-5.44 ± 2.40	-9.65 ± 2.44				
		Placebo	49.80 ± 2.09	-7.36 ± 2.42	-3.91 ± 2.46				
	Number of incorrect words (errors)	Date seeds	0.37 ± 0.08	0.04 ± 0.13	0.04 ± 0.13	0.14	0.87	0.17	0.85
		Coffee	0.43 ± 0.11	0.02 ± 0.13	0.14 ±0.13				
		Placebo	0.39 ± 0.09	0.02 ± 0.13	0.00 ± 0.13				
Simple Reaction Time	Average Speed (msec)	Date seeds	340.54 ± 12.66	1.04 ± 12.84	2.18 ± 12.84	0.76	0.47	0.47	0.63
		Coffee	374.53 ± 36.49	6.19 ± 12.97	21.10 ± 12.97				
		Placebo	375.13 ± 20.87	-14.53 ± 12.84	11.62 ± 13.11				
Digit Vigilance	Average Speed (msec)	Date seeds	465.36 ± 6.05	3.77 ± 4.79	3.29 ± 4.79	0.07	0.94	0.80	0.45
		Coffee	464.39 ± 5.45	7.40 ± 4.79	10.89 ± 4.79				
		Placebo	475.56 ± 6.37	3.88 ± 4.79	1.35 ± 4.79				
	Targets Detected %	Date seeds	90.16 ± 1.77	0.40 ± 1.45	-0.13 ± 1.45	1.08	0.34	0.20	0.82
		Coffee	90.39 ± 1.65	0.31 ± 1.45	-0.13 ± 1.45				
		Placebo	87.98 ± 1.90	-1.67 ± 1.45	0.99 ± 1.45				
	False Alarms	Date seeds	2.71 ± 0.40	-0.46 ± 0.47	-0.44 ± 0.47	1.86	0.16	0.04	0.96
		Coffee	2.94 ± 0.53	-0.59 ± 0.47	-0.34 ± 0.47				
		Placebo	2.20 ± 0.33	0.32 ± 0.47	0.34 ± 0.47				
Choice Reaction Time	Accuracy %	Date seeds	95.47 ± 0.59	0.16 ± 0.60	-0.69 ± 0.60	1.81	0.16	0.16	0.85
		Coffee	94.20 ± 0.77	0.89 ± 0.60	0.73 ± 0.60				
		Placebo	95.02 ± 0.66	0.16 ± 0.60	-0.32 ± 0.60				
	Average Speed (msec)	Date seeds	500.93 ± 40.59	-28.10 ± 24.51	-27.14 ± 24.51	0.90	0.41	0.07	0.93
		Coffee	469.40 ± 11.64	11.13 ± 24.51	-3.75 ± 24.51				
		Placebo	471.01 ± 11.61	-3.65 ± 24.51	-3.17 ± 24.51				
Spatial Working Memory	Original Stimuli Accuracy %	Date seeds	93.11 ± 1.25	0.51 ± 2.32	-0.51 ± 2.32	1.13	0.33	0.37	0.69
		Coffee	93.24 ± 1.06	1.53 ± 2.32	-1.65 ± 2.32				
		Placebo	93.75 ± 0.93	-1.65 ± 2.32	-2.42 ± 2.32				
	New Stimuli Accuracy %	Date seeds	95.51 ± 1.18	-0.30 ± 1.49	-0.93 ± 1.50	1.15	0.32	0.02	0.98
		Coffee	95.92 ± 1.48	0.61 ± 1.49	-0.10 ± 1.49				
		Placebo	96.73 ± 0.71	-1.93 ± 1.49	-2.04 ± 1.49				
	Original Stimuli Average Speed (msec)	Date seeds	697.40 ± 24.11	1.67 ± 36.22	-16.37 ± 36.60	1.66	0.19	0.20	0.82
		Coffee	708.01 ± 46.81	-81.34 ± 36.22	-56.00 ± 36.22				
		Placebo	648.51 ± 33.24	-12.25 ± 36.22	-21.47 ± 36.22				
		Date seeds	787.07 ± 30.75	-8.85 ± 71.57	-53.34 ± 72.31	1.56	0.21	0.12	0.89

Task	Measure	Treatment	Baseline	Post-dose change		Effect of treatment		Effect of treatment* time intervals	
			0 Min	45 min	90 min	F	P	F	P
	New Stimuli Average Speed (msec)	Coffee	825.38 ± 119.42	-146.49 ± 71.57	-129.56 ± 71.57				
		Placebo	687.56 ± 28.11	-34.03 ± 71.57	-18.45 ± 71.57				
	Average Speed	Date seeds	748.35 ± 26.17	-4.58 ± 43.58	-37.20 ± 44.03	1.59	0.20	0.20	0.82
		Coffee	755.20 ± 68.16	-99.30 ± 43.58	-78.24 ± 43.58				
		Placebo	670.64 ± 27.76	-24.96 ± 43.58	-24.96 ± 43.58				
Numeric Working Memory	Original Stimuli Accuracy %	Date seeds	91.43 ± 1.13	2.85 ± 1.51	0.69 ± 1.52	1.28	0.28	0.29	0.75
		Coffee	91.70 ± 1.35	0.40 ± 1.51	0.00 ± 1.51				
		Placebo	91.97 ± 1.36	0.68 ± 1.51	-1.90 ± 1.51				
	New Stimuli Accuracy %	Date seeds	96.19 ± 0.78	0.00 ± 1.05	-1.38 ± 1.07	2.18	0.12	2.53	0.08
		Coffee	95.10 ± 1.06	0.68 ± 1.05	1.90 ± 1.05				
		Placebo	96.19 ± 0.78	1.22 ± 1.05	-2.31 ± 1.05				
	Original Stimuli Average Speed (msec)	Date seeds	638.32 ± 16.22	-5.90 ± 30.34	-32.24 ± 30.65	0.33	0.72	0.54	0.58
		Coffee	614.14 ± 16.30	-36.92 ± 30.34	-0.37 ± 30.34				
		Placebo	640.63 ± 38.19	-39.22 ± 30.34	-39.22 ± 30.34				
	New Stimuli Accuracy msec	Date seeds	730.66 ± 24.11	-16.31 ± 18.53	-50.82 ± 18.53	2.13	0.12	0.59	0.55
		Coffee	664.91 ± 15.95	-6.15 ± 17.96	-4.61 ± 17.96				
		Placebo	653.57 ± 20.62	3.14 ± 17.96	1.67 ± 17.96				
	Average Speed (msec)	Date seeds	686.21 ± 18.97	-19.84 ± 19.59	-53.22 ± 19.79	0.90	0.41	0.92	0.40
		Coffee	640.72 ± 13.81	-21.81 ± 19.59	-2.17 ± 19.59				
		Placebo	645.67 ± 22.31	-14.37 ± 19.59	-17.64 ± 19.59				
Delayed Word Recall	Correct words %	Date seeds	39.59 ± 2.46	-15.37 ± 2.75	-11.94 ± 2.78	3.34	0.04	1.72	0.18
		Coffee	42.72 ± 2.50	-13.06 ± 2.75	-18.36 ± 2.75				
		Placebo	38.50 ± 2.10	-10.61 ± 2.75	-6.94 ± 2.75				
	Number of incorrect words (errors)	Date seeds	0.94 ± 0.35	0.28 ± 0.19	0.06 ± 0.19	0.46	0.63	1.01	0.36
		Coffee	0.67 ± 0.13	0.00 ± 0.19	0.28 ± 0.19				
		Placebo	0.63 ± 0.13	-0.02 ± 0.19	-0.04 ± 0.19				
Word Recognition	Original Stimuli Accuracy %	Date seeds	76.74 ± 2.51	-0.27 ± 2.36	0.83 ± 2.39	0.03	0.97	0.63	0.53
		Coffee	75.51 ± 2.58	-0.54 ± 2.36	0.54 ± 2.36				
		Placebo	74.42 ± 2.60	-3.13 ± 2.36	2.58 ± 2.36				
	New Stimuli Accuracy %	Date seeds	91.97 ± 1.64	-2.85 ± 2.02	-6.80 ± 2.04	2.73	0.07	0.25	0.78
		Coffee	90.48 ± 2.00	-0.13 ± 2.02	-2.64 ± 2.04				
		Placebo	89.93 ± 2.25	0.28 ± 2.06	-0.74 ± 2.11				
	Original Stimuli Average Speed (msec)	Date seeds	803.19 ± 30.22	42.59 ± 35.56	4.51 ± 36.71	1.10	0.33	1.01	0.37
		Coffee	758.03 ± 21.48	63.91 ± 35.93	88.19 ± 35.56				
		Placebo	770.55 ± 29.92	10.36 ± 35.93	73.43 ± 35.56				
	New Stimuli Average Speed (msec)	Date seeds	879.89 ± 29.63	11.03 ± 35.65	-6.86 ± 36.02	0.77	0.46	0.20	0.82
		Coffee	830.62 ± 33.35	30.35 ± 35.65	-0.55 ± 35.65				
		Placebo	789.57 ± 23.32	38.83 ± 35.65	51.91 ± 35.65				
	Average Speed (msec)	Date seeds	843.46 ± 27.68	9.55 ± 34.83	64.42 ± 35.19	0.13	0.88	0.67	0.51
		Coffee	796.85 ± 26.23	67.51 ± 34.83	41.42 ± 34.83				
		Placebo	779.58 ± 23.49	40.09 ± 34.83	55.36 ± 34.83				

Task	Measure	Treatment	Baseline	Post-dose change		Effect of treatment		Effect of treatment* time intervals	
			0 Min	45 min	90 min	F	P	F	P
Pattern Separation	Original Stimuli Accuracy %	Date seeds	78.16 ± 2.31	0.51 ± 1.90	-2.81 ± 1.92	0.36	0.70	1.23	0.29
		Coffee	78.67 ± 2.44	-2.65 ± 1.90	-2.85 ± 1.90				
		Placebo	76.84 ± 2.54	-3.46 ± 1.90	-0.81 ± 1.90				
	New Stimuli Accuracy %	Date seeds	78.57 ± 2.02	-3.36 ± 2.02	-4.06 ± 2.04	1.07	0.34	0.05	0.95
		Coffee	77.55 ± 2.20	-1.63 ± 2.02	-3.06 ± 2.02				
		Placebo	77.86 ± 2.31	-5.20 ± 2.02	-5.40 ± 2.02				
	Original Stimuli Average Speed (msec)	Date seeds	1015.91 ± 35.30	-68.96 ± 40.12	-17.63 ± 40.53	0.04	0.96	1.05	0.35
		Coffee	960.08 ± 28.94	-23.46 ± 40.12	-77.71 ± 40.12				
		Placebo	956.61 ± 35.77	-33.12 ± 40.12	-77.19 ± 40.12				
	New Stimuli Average Speed (msec)	Date seeds	1076.88 ± 39.02	-82.84 ± 27.27	-82.84 ± 27.27	3.65	0.03	0.12	0.89
		Coffee	981.26 ± 33.62	-21.07 ± 27.27	-17.33 ± 27.27				
		Placebo	977.02 ± 32.72	-41.78 ± 27.27	-60.26 ± 27.27				
	Average Speed (msec)	Date seeds	1042.17 ± 31.97	-74.03 ± 26.68	-63.10 ± 26.95	0.71	0.49	0.42	0.66
		Coffee	968.46 ± 28.45	-25.48 ± 26.68	-47.89 ± 26.68				
		Placebo	965.30 ± 29.56	-34.68 ± 26.68	-71.32 ± 26.68				

Table 40. Mean ± SE for pre-dose baseline and change from baseline and *ANOVA* for CogTrack, Bond-Lader mood and Caffeine research VASs following the administration of date seeds drink, regular coffee and placebo. (n= 49; 38 females and 11 males).

Measures	Treatments	Baseline	45 min post-dose change	90 min post-dose change	Effect of treatment		Effect of treatment* time intervals	
					F	*P*	*F*	*P*
Attentional Intensity Index	Date seeds drink	1219.09±19.88	15.73±12.72	10.77±12.72	0.22	0.8	0.18	0.84
	Regular Coffee	1222.97±19.77	10.10±12.72	13.79±12.72				
	Placebo	1245.44±20.01	0.19±12.72	10.48±12.72				
Sustained Attention Index	Date seeds drink	87.53±1.34	0.89±1.21	-0.26±1.21	1.39	0.25	0.25	0.7
	Regular Coffee	86.13±1.45	1.71±1.21	1.05±1.21				
	Placebo	86.56±1.23	-1.04±1.21	-0.21±1.21				
Attentional Fluctuation Index	Date seeds drink	74.95±7.90	-5.33±10.24	-2.12±10.24	1.43	0.24	0	0.98
	Regular Coffee	76.42±6.08	5.15±10.24	8.25±10.24				
	Placebo	87.35±9.91	-12.72±10.24	-8.32±10.24				
Memory Retrieval Speed Index	Date seeds drink	3002.49±61.48	-78.15±31.02	-154.56±31.02	4.42	0.01	0.79	0.45
	Regular Coffee	2830.51±49.88	-71.61±31.02	-71.28±31.02				
	Placebo	2747.38±49.64	-11.63±31.02	-36.70±31.02				
Cognitive Reaction Time	Date seeds drink	134.68±7.60	1.68±11.26	-4.09±11.26	0.63	0.53	0.33	0.72
	Regular Coffee	127.40±10.26	-1.40±11.26	-23.18±11.26				
	Placebo	113.79±15.14	1.64±11.26	-4.70±11.26				
Working Memory Capacity index	Date seeds drink	276.24±2.95	3.06±3.67	-2.52±3.67	1.96	0.14	0.14	0.86
	Regular Coffee	275.96±3.63	3.23±3.67	0.14±3.67				
	Placebo	278.65±2.72	-1.69±3.67	-8.68±3.67				
Episodic Memory Capacity Index	Date seeds drink	-117.24±9.97	4.95±10.77	-4.33±10.77	2.22	0.11	1.31	0.27
	Regular Coffee	-125.59±8.31	12.81±10.77	32.63±10.77				
	Placebo	-128.85±8.38	22.63±10.77	13.96±10.77				
Quality of Memory Index	Date seeds drink	31.23±11.14	23.31±12.50	3.81±12.50	2.09	0.12	1.86	0.16
	Regular Coffee	18.51±10.99	26.93±12.50	49.67±12.50				
	Placebo	21.23±10.17	29.66±12.50	11.06±12.50				
Bond-Lader Mood (Alertness)	Date seeds drink	57.70±2.41	-5.18±2.20	2.33±2.20	0.74	0.48	0.99	0.37
	Regular Coffee	59.59±2.52	-3.47±2.20	-1.27±2.20				
	Placebo	58.92±2.60	-0.76±2.20	1.31±2.20				
	Date seeds drink	62.20±2.38	-1.83±1.67	0.67±1.67	1.4	0.25	0.24	0.79

Measures	Treatments	Baseline	45 min post-dose change	90 min post-dose change	Effect of treatment		Effect of treatment* time intervals	
					F	*P*	*F*	*P*
Bond-Lader Mood (Contentment)	Regular Coffee	65.40±2.41	-3.52±1.67	-2.12±1.67				
	Placebo	62.49±2.53	-0.34±1.67	-0.16±1.67				
Bond-Lader Mood (Calmness)	Date seeds drink	58.59±2.57	4.94±2.16	-2.19±2.16	0.07	0.94	0.24	0.79
	Regular Coffee	57.81±2.33	4.27±2.16	-0.30±2.16				
	Placebo	58.28±2.32	3.52±2.16	-1.02±2.16				
Caffeine questionnaire (Alert)	Date seeds drink	49.80±23.67	12.18±3.98	8.65±3.98	1.2	0.29	0.51	0.6
	Regular Coffee	53.86±25.93	5.16±3.98	9.63±3.98				
	Placebo	54.76±21.47	4.08±3.98	4.27±3.98				
Caffeine questionnaire (Relaxed)	Date seeds drink	60.73±3.20	0.71±2.87	-2.73±2.87	0.75	0.47	0.16	0.85
	Regular Coffee	59.78±3.08	2.22±2.87	2.04±2.87				
	Placebo	57.00±3.07	2.86±2.87	1.00±2.87				
Caffeine questionnaire (Jittery)	Date seeds drink	34.14±3.37	6.16±2.95	7.63±2.95	1.25	0.29	0.08	0.92
	Regular Coffee	29.94±2.83	2.45±2.95	3.47±2.95				
	Placebo	32.63±3.15	3.14±2.95	2.35±2.95				
Caffeine questionnaire (Tired)	Date seeds drink	52.76±3.47	-11.43±4.09	-4.55±4.08	0	1	0.87	0.42
	Regular Coffee	48.49±3.51	-2.57±3.51	-5.22±3.81				
	Placebo	49.71±3.46	-4.69±3.35	-5.18±3.80				
Caffeine questionnaire (Tense)	Date seeds drink	36.94±3.36	1.80±2.88	0.43±2.88	0.77	0.46	0.09	0.97
	Regular Coffee	37.12±3.34	0.39±2.88	-0.86±2.88				
	Placebo	36.39±3.13	2.92±2.88	3.69±2.88				
Caffeine questionnaire (Headache)	Date seeds drink	27.55±3.95	-1.00±3.33	4.57±3.33	0.94	0.39	1.12	0.33
	Regular Coffee	23.71±3.57	5.73±3.33	1.57±3.33				
	Placebo	26.20±3.65	-0.31±3.33	-1.49±3.33				
Caffeine questionnaire (Overall Mood)	Date seeds drink	62.55±3.01	4.06±55.18	-0.24±55.18	0	1	0.88	0.42
	Regular Coffee	64.92±2.98	0.69±55.18	3.06±55.18				
	Placebo	64.96±2.72	0.98±55.18	1.45±55.18				

5.5 Discussion and conclusion

The current study found no evidence of a significant alteration of cognitive performance following the administration of date seeds drink in comparison to a positive control and placebo.

5.5.1 Treatment creation

The justification for conducting this research trial, as discussed within the literature review of section 5.1, was founded on animal literature. These studies included many analyses employing a date seed containing diet consumed by the test animals within each investigation. However, similar to the lack of research testing the direct effect of date fruit in enhancing cognitive functions in animals, in comparison, there is an even fewer published studies about DSE. Most of the cited work in this chapter has indirectly referred to either the neuroprotection or the therapeutic potential of DSE due to its high phenolic content. Although the novelty of this research has been a challenge, inconsistency in the methodology used in other research and the missing information in the few available studies was a further challenge. Indeed, there were difficulties in translating any dose ranges from animal experimental evidence to human experiments as a result of the differences in how the date seeds drink is selected, quantified for phenolics, and finally prepared. Thus, a comparison of dose is irrelevant to some extent. However, the DSE dose utilised in Habib and Ibrahim (2011) was used as an example (as this study contained all relevant information) to capture the large difference in TPC between doses (range of 1722–3444 mg GAE to 110 mg GAE). Although the DSE used in Habib and Ibrahim (2011) was a non-roasted date seed form, a large difference can still be observed, which may explain the absence of effect.

The DSD utilised in the current study was prepared from roasted date seeds and this helped directly in facilitating the treatment execution, and indirectly in increasing the TPC. The increase in the total phenolic contents during roasting could be attributed to the development of Maillard reaction products during roasting (Özdemir and Devres, 2000). Such effects were previously noticed for sesame seed extract (Rizki et al., 2015) and a coffee-like maize beverage (Youn and Chung, 2012). It has been suggested that the roasting process could cause evaporation of intracellular water,

triggering chemical reactions which may change the lignocellulosic structure and promote protein denaturation, which could result in a greater availability of the phenolic compounds in the matrix (Rizki et al., 2015). However, data from coffee studies contradicts this suggestion. A decrease in the TPC of Robusta coffee measured using the Folin-Ciocalteau method (from 28.2 to 18.6 g of GAE/100 of DW) outlines the possible loss of phenolic compounds during roasting (Vignoli et al., 2014). Roasting also decreased the TPC in coffee in another study by Bobková et al. (2020). Therefore, the increased Folin-Ciucalteu TPC in our results might be just an artefact caused by the Maillard melanoidins.

The results obtained for the TPC of the six varieties roasted in the current study (see Table 26) in accordance to Rahman et al. (2007) at 220ºC for 15–20 min may provide an explanation for the results, regardless of the variation of the cultivar type, being in line with the results from Fikry et al. (2019) estimating the phenolic content of roasted date seed to range from 8778.61 to 15510 GAE mg/100 mg DW. Since no information was available regarding the temperature used in roasting the commercial DSD utilised within the study, it could be speculated that the date seeds were roasted at a lower temperature for a long time. However, another study by Ahmed et al. (2016) used exactly the same method as Rahman et al. (2007) when roasting three different cultivars (Ajwah, Aseel and Hallawi), reporting a TPC range of 843.54 to 1204.7 mg/100 g which is different to that reported in Fikry et al. (2019). However, and as previously mentioned in section 5.1, the TCP reported by Fikry et al. (2019) was estimated using the Folin-Ciocalteu method, which can be considered less accurate than other methods such as HPLC or LC-MS, and this should be taken into consideration when planning future research.

In light of this, quantifying a large number of commercial DSD in the future may aid in choosing the right product with the highest phenolic content for future research. Alternatively, creating a DSD within the pilot kitchen, after screening different seeds and roasting under tight control of temperature and time, could be considered. However, the lack of information regarding date cultivar used within most of the studies is problematic and causes difficulty for capturing ideas about which cultivars can be considered rich in phenolics and which are poor.

5.5.2 Direction of effects

The supplementary Chi-square analysis of the positive and negative values of the measures, which was conducted to find out a direction of the date-containing treatments in comparison to placebo, showed that the date coffee was equally effective as regular coffee and differed from placebo (see section 5.4.8). However, to confirm the equivalent effect observed between RC and DSD, alternative cognitive paradigms should be considered when designing a future study, those paradigms which have formerly displayed sensitivity to flavonoid-rich nutritional interventions in rats and humans during sustained mental effort. Some cognitive batteries with high intensity paradigm have been used in many of the published studies investigating the acute effect of polyphenols on mood and cognitive performance, such as Watson et al. (2015) and Field et al. (2011). This paradigm consists of several (3-7) repetitions of the attention tasks. These same high intensity tasks were used in studies to assess the effect of caffeine on cognitive function, such as Yeomans et al. (2002) and Haskell et al. (2005).

5.5.3 Power, effect size and statistics

The current study was powered on the basis of an established effect from an effective dose of caffeine. The chosen dose of caffeine was made upon a mini systematic review conducted in sections 4.8.2 and in 4.11 to identify a low effective dose of caffeine to be comparable with the anticipated moderate to low effect of date seeds drink. Therefore, the current study was powered for half of the effect of a small dose of 75 mg caffeine. However, even the positive control, which was thus expected to exhibit the full enhancing effect on mood and cognitive function, showed no effect. As discussed in section 4.8.2, among fourteen studies testing a single caffeine dose ranging from 50 to 100 mg, nine showed that this dose significantly affected mood, mainly in alertness index. Furthermore, the fact that only five studies from fourteen utilised a caffeine dose range of 50-100 mg and showed no effect, made us wonder whether these studies has considered to correct their *p*-value using Bonferroni correction to count for the multiple outcomes measured, or the number of well-designed but unpublished studies which were unable to detect any significant effects of caffeine consumption. More specifically, and as a supportive example of this

assumption, it has been concluded that 75 mg of caffeine in 150 ml of water had minimal effects on cognitive functions in comparison to 75 mg of caffeine combined with 75 mg of glucose within the same vehicle (Adan and Serra-Grabulosa, 2010).

Scholey and Kennedy (2004) demonstrated that the administration of an energy drink made mainly of caffeine (75 mg) and glucose (37.5 g), and some herbs for flavouring, could improve cognitive function; specifically in speed of attention and secondary memory. Most importantly, that the caffeine dose in isolation resulted in no significant effect. These two studies were published to show the significant effect of a “like energy drink” which consisted of glucose and caffeine together. However, such non-significant results were not included in a review of the impact of caffeine on mood and cognitive function (Ruxton, 2008). Furthermore, many of the available studies had small sample sizes, although the body of evidence was considerable for most measures of mood and cognitive function. In comparison, the dose of 75 mg is considered a moderate dose and has been utilised in a relatively large number of studies (Smit and Rogers, 2000), and thus this was the chosen dose. As a result, it may be indicated that the power calculation could be deemed as insufficient or inappropriate for such an exploratory study. However, this is the nature of underpowered studies, when it is generally assumed that this dose has this effect, the most positive results are considered normal, and average results are considered a failure and not published, so the published data give an incorrect (too positive) basis for the power calculation. This is not a fault of the power calculation. Nonetheless, it is inappropriate for authors and editors who uncritically published studies describing the effects as significant and important even though their overall data did not show any effects (after Bonferroni).

The effect of caffeine was not the main interest of the current study but its inclusion was for the purpose of incorporating a positive control to test the robustness of our methodology. However, it may be relevant to briefly discuss the issue of whether caffeine merely reverses withdrawal symptoms or confers a real cognitive benefit as this remains controversial and is hard to resolve because most people are now exposed to some dietary caffeine (Heatherley et al., 2006). Supplementary analysis of data was conducted, whereby participants’ caffeine consumption was used to categorise the participants into two groups: participants with low caffeine consumption and participants with high consumption. The categorisation of participants into two

groups was based on the average, minimum and maximum amounts of caffeine as calculated from the caffeine consumption questionnaire (Table 36). This analysis helped in speculating a similar pattern to that reported in the literature in this domain. Participants with low caffeine consumption showed enhancements in their sustained attentional index following the administration of both DSD and RC relative to placebo, while a lesser attenuation in the same index was observed in the participants with high caffeine consumption following the RG (Figure 53). A noticeable attenuation was observed in two measures of the caffeine research VASs, Tense and Headache, following the administration of RG in the high caffeine consumption group and an even greater attenuation in the same measures in the low caffeine consumption group (see Figure 53 and Figure 54). Although, as described in section 5.4.6, those outcomes did not reach a significant level after Bonferroni correction, it may demonstrate that the data is confirming, to some degree, the same pattern mentioned in Christopher et al. (2005) and Haskell et al. (2005) regardless of the Bonferroni correction. The reported pattern for the caffeine effect has shown enhancements in cognitive performance in non-consumers and modulations in mood in consumers. However, it is important to refer to the fact that there was some variation in the method used in categorising the participants according to their caffeine consumption between the current study and the aforementioned studies. To be precise, in checking potential volunteers' eligibility, the participants were asked to report their caffeine consumption prior to the screening visit. However, participants in Haskell et al. (2005), for example, were catagorised as follows: 'habitual non-consumer; consumes less than 50 mg/day (not from tea or coffee)' while 'habitual consumer: consumes more than 50 mg/day (mainly from tea and/or coffee). Subsequently, the ranges of both groups were 0-47 mg and 60-800 mg respectively, whereas in the recent study, the caffeine data was analysed afterwards, and the ranges for the same groups were different and relatively higher (0-40 mg/day and 41-240 mg/day, respectively).

Moreover, although Christopher et al. (2005) for example, showed there was a statistically significant effect of caffeine relative to the placebo on both mood (alertness) and number of cognitive performance measures (focus attention task, repeated digit task and (categoric search task), there was some information missing that may affect the robustness of the used methodology. Firstly, the caffeine dose was 2 mg/kg, yet no data on the average weight of the participant was included in the

anthropometric measurement section. Secondly, unlike most studies, during which the participants abstain from caffeine-containing foods or drinks, the participant baseline measures were taken after consumption of their normal breakfast, while the post-dose measures were taken when participants returned to the university and after consuming their normal quota of caffeine.

5.6 Conclusion

In conclusion, the results of the current study exhibited no overall effects of either date seeds drink or regular coffee administration on cognitive performance within a “young and healthy” adult cohort. The Chi-square analysis showed that the date coffee was equally effective as regular coffee and differed from placebo in increasing alertness. The current study employed a robust design and method regardless of the challenges regarding, for example, treatment creation and replication of phenolic doses reported in the literature etc. It is important that trials which may be considered ‘unsuccessful’ are still published to prevent a “file drawer” bias resulting from non-significant results being viewed as unfavourable and therefore not published. This is important to avoid further researchers from reinvestigating the same thing (Young and Bang, 2004) which could be detrimental to the scientific community, and to ensure the entirety of clinical research data is accessible (Kennedy, 2004).

Chapter 6. Discussion and conclusion

6.1 General discussion

The acute supplementation of treatments incorporating date fruit flesh or date seeds and quantifying the consequences of these on mood and cognitive function in a healthy, young population was the principal aim of the current study. Chapter 2 outlined the development of innovative date-containing treatments and a parallel placebo, and successfully quantified their phenolic content, GI, GL, and assessed them for both palatability and sensory properties. Chapter 3 employed these treatments in an acute trial on a young, healthy population, however, only small overall significant effects on mood but not on cognitive performance were observed. Chapter 4 described the utilisation of the roasted date seeds to produce a "coffee-like" beverage, as well as developing placebo and positive control treatments, in addition to quantifying the phenolic content, caffeine content, palatability and sensory properties. Chapter 5 subsequently investigated the acute mood and cognitive effects of this treatment in comparison to the positive control and negative control, in healthy, young adults.

As date fruit and date seeds were reported to be a source of phenolic compounds (Al-Farsi and Lee, 2008), it was postulated that the phenolic content may have an enhancing effect on cognitive performance and mood. Therefore, all treatments were created originally to conduct two human interventions, which were made of either date fruits or date seeds and were formulated to contain the highest phenolic content feasible. However, an important outcome of the experiments in this thesis was increasing the author's in-depth understanding of how to critically evaluate the work of others, since treating each published result as evidence can be very misleading, especially for new researchers. This has occurred on several occasions during this research as follows:

- The initial rationale for conducting this research was based on the above-mentioned published claim that date fruit and date seeds are a "rich" source of phenolic compounds. However, after conducting our own chemical analysis in

more reliable manner compared to analysis undertaken by other studies, we determined that fresh fruit flesh, which was the highest cultivar for phenolics, yielded 1.60 and 3.69 mg of GAE/g FW of Barhi and Khassab respectively. By comparison, fresh blueberry, as an example of another fruit which is also claimed to be “rich” in phenolics, yielded 19.6 mg/g. This comparison reveals that not every classification made by a paper is accurate and reliable and the potential for exaggeration or misinterpretation must always be taken into account.

- Moreover, most of the thesis rationale was mainly based on animal studies due to the novelty of the research topic. The number of studies investigating the effect of both date fruit and date seed is very low compared to other fruits. However, as it was demonstrated several times in this thesis, serious methodological problems were present in previous research studies. For example, studies attributed most of the neuroprotective or cerebroprotective effects to the “high” phenolic content of date fruit, without providing any chemical quantification for the “high” phenolic content and only referred to literature on this matter, specifically the same literature (Al-Farsi & Lee, 2008), in which inaccuracies were identified, as discussed above. An extreme example of literature that fails to achieve the basic level to be scientifically reproducible research is the study highlighted in section 3.8.3. It includes incorrect information provided in three different articles, which claim that the animals’ date diet was produced by Research Diet Inc. (NJ, USA), while the company’s response to our enquiry demonstrated that this was not accurate.

- Another aspect that should have been considered carefully in the rationale of this thesis is that the positive effects reported about physiological effects of phenolic compounds on the brain, like neuroimaging outcomes (e.g., fMRI and EEG) and enhancing cerebral blood flow, are not by themselves evidence of positive effects on cognitive function (Lamport and Williams, 2020). The effects of phenolics on physiological aspects were reported in many studies and are suggested mechanisms of phenolic actions and considered an explanation for the memory-enhancing effect following the phenolics consumption (Lamport et al., 2012). However, enhancing a physiological function will only improve cognitive function if this physiology (e.g. sub-optimal cerebral blood flow) is the

limiting factor (Lamport and Williams, 2020). Therefore, a new researcher should differentiate between the two different types of outcomes and not consider them equivalent, even when some studies included both types of assessments in the same study like (Wightman et al., 2012), (Watson et al., 2019) and(Francis et al., 2006).

- A clear positive relationship between the consumption of foods rich in polyphenols and cognitive enhancements is not definitive and the impression conveyed in the literature may be assumed to be stronger than in reality. This has been identified as a result of the inconsistent use of different doses, designs (acute and chronic), durations or sensitivity of populations and, most importantly, utilisation of different cognitive tests. Therefore, the amount of research which has reported statistically significant findings should be interpreted with caution. The *p-value* in these studies should have been corrected, e.g. using Bonferroni corrections, to protect the results from errors of multiple outcomes. Some studies did not correct their *p-value* including Haskell et al. (2017) and Field et al. (2011), while other studies corrected their *p-value* partially or in incorrectly (Watson et al., 2015). In this context, we must distinguish between using Bonferroni, LSD or Tukey tests when making multiple comparisons of different treatments in the same trial, and between using the Bonferroni to correct the *p*-value when using different outcome measurements assessing the same overall effect (Vickerstaff et al., 2019). The study by Haskell et al. (2017) will be used to demonstrate the important effect of Bonferroni test on the significancy of a study outcome. The authors reported a significant effect following the consumption of a single dose of blackcurrant juice as revealed by ANOVA ($F(1.8) = 5.40$, $p= 0.028$). However, the corrected alpha level for this study, after the Bonferroni correction, would be 0.0071 instead of 0.05, which means that there was no statistically significant effect of the blackcurrant juice. A minor error resulted in an invalid study outcome.

- As a result, if all published findings were handled statistically in a more appropriate way and the *p*-value was corrected using Bonferroni, many more non-significant results would have been reported. This has contributed to less interest in “riding the wave” by more careful assessments of the effect of

polyphenols on neuroprotective or cerebroprotective mechanisms. More precisely, with some foods that are well-known to be low in phenolic content, such as bananas, which have been associated with antianxiety and antidepressant effects while invigorating memory performance (Samad et al., 2017) or dates, as we demonstrated in the present study to have no effects.

- The same observation, with regard to the application of the Bonferroni to correct the *p*-value for multiple outcomes, applied to the three studies (Quinlan et al., 2000, Hindmarch et al., 2000, Haskell et al., 2005) which were previously mentioned in section 4.8.2 for utilising the dose of 75 g of caffeine. None of these studies had corrected their *p*-value using the Bonferroni.

- Moreover, the utilised assessment tool to measure cognitive function was the CogTrack system. This system is like many other batteries (CDR or COMPASS) and was developed to assess both enhancement and impairments in cognitive functions. Taking into account the important overview of Lamport and Williams (2020) in their recent review of current evidence about the effects of phenolic content on cognitive function, there is some support for enhancement effects of phenolic compounds on cognition. Conversely, they mentioned that most of the reviewed systematic reviews and meta-analysis expressed caution and showed that benefits were not consistent across all studies. Additionally, the same review also indicated that the positive effect was observed more in an elderly population compared to a young healthy population. Although the CogTrack model has been validated and shown to be sensitive to detect changes in cognitive performance, further research to investigate its sensitivity with a young healthy population may be beneficial.

- Furthermore, another observation needs to be considered when reviewing the literature, the ‘high baseline’ or the ‘ceiling performance’ among healthy young humans. It basically means that their cognitive function is already almost perfect, therefore measuring a genuine enhancing effect will be difficult unless the baseline is lowered by imposing stress or fatigue on younger people to lower their performance. Although there is then a risk that the cognitive performance really measures something else, such as resistance to stress, which may explain the better results when testing older people with some pre-

existing impairment. A small change in cognitive performance may simply be very difficult to measure.

Separately, the current study results showed a small enhancing effect of dates consumption and no enhanced effect of date seeds consumption on cognitive performance in a "young and healthy" adult cohort. Non-significant results are acknowledged to be commonly unfavourable and can trigger a "file drawer" bias in publication, with unsuccessful trials not being published. However, this can be detrimental to the scientific enterprise as it impacts other researchers when conducting similar analyses (Young and Bang, 2004) and hence the principal aim is to ensure data from all clinical research is made available to the research community (Kennedy, 2004).

A survey by Kennedy (2004) established several motives for not completing a manuscript for unsuccessful trials, including a perceived incapability to publish the manuscript, time concerns, awareness of flawed methods or design, incapability of interpreting outcomes, and the conclusion that the results are unimportant. Nevertheless, the current study was proposed after consideration of the guidelines for planning, accomplishing and reporting of human intervention studies to appraise the health benefits of foods (Welch et al., 2011). This created a robust methodology and study design despite many studies cited containing methodological issues which could affect result reliability and having utilised these to generate a rationale for the conduction, to be explained in section 6.2.

Finally, it is important to mention that date fruit and its products is an important component of diet for people in the Middle East and they have their own strong beliefs that this "super food" has an enhancing effect on mood, memory, and concentration. Although these beliefs have not been proven scientifically, however, it was the original motive to carry on this research. The absence of effects demonstrated in this thesis after the consumption of date fruit would not necessarily contribute to abolish these beliefs. Likewise, the inconsistency in results reported about the effect of caffeine to enhance cognitive ability will not result in making people think to skip their morning cup of coffee.

6.2 Potential methodological limitations

Both investigations were designed to allow the effects of phenolics to be evaluated while the phenolic acid levels were at maximum concentration (Cmax): between 45- and 135-minute post-supplementation in the date study (with three post-dose assessments repetitions) and between 45- and 90-minute post-supplementation (in the date seed study with two post-dose assessments repetitions). Moreover, in both studies, three arms of treatments were utilised, which resulted in three study visits. Counting the screening and training visit, this makes the total number of visits for each study to be four, so the total number of visits/hours involved in both studies was relatively high; this should be considered for any future research studies. Additionally, there was an observed correlation between the value of the voucher given to the participants and their compliance to the study visits, 32% of participants dropping out when £20 vouchers were given, while only 19% dropped out when £40 vouchers were provided. Indeed, while the original motive for the participant was their contribution to research and science, the substantial effort invested in the trial should be generously compensated.

The GI and GL assessment in chapter 2 were conducted on a small cohort of ten participants and were used to define the glucose responses of the date and yoghurt treatments. However, this assessment had some limitations in terms of methodologies. Technically speaking, the trial was performed according to the glycaemic response and health guidelines published by International Life Sciences Institute ILSI (SERIES). However, the date-containing treatments were tested against a reference food of 50 g of glucose dissolved in water, while the placebo created to be tested in the first human intervention was not tested in the GL and GL study. The error in this assessment was in the exclusion of the placebo, but this was done due to the consideration of the large number of finger pricks involved in each visit; the addition of the placebo as a testing arm would have made the total number of blood sugar finger pricks to exceed 32 pricks, so in consideration of the participants, the placebo was omitted. The reason for conducting the GI study was to consider the glycaemic profile of the treatments when analysing the cognitive data afterwards and consequently, the exclusion became problematic for this analysis. Thus, from a statistical point of view, this data was not useful due to the absence of the placebo

data. However, since no differences were detected among all treatments, this did not significantly impact the study. However, it may be useful to consider using an advanced and more comfortable procedure when measuring glucose levels, such as a continuous glucose monitor in future research studies and this equipment offers the advantage of not missing any peaks in data between measurements.

Another limitation of the study was to how the sensory evaluation panels were conducted to assess the masking of the created treatments and their matching placebo for both human investigation of date fruit and date seed. All four panels conducted involved three samples (two treatments and placebo) served to the participants as a trio. This method is a standard profiling test and allowed the participants to directly compare the samples and recognise differences. It would have been much more difficult to recognise a sensory attribute if the samples were served on different days, so, if the panellists had been asked to score only one sample each day, and been given different samples on different days, which is called the recognition test, the differences would have been less significant. A similar approach to this suggestion was performed indirectly, when we asked the participants to guess the identity of the treatment retrospectively in a written question in the debrief of both trials.

Another limitation of the study was related to the absence of the quantification of the phenolic content of the negative placebo (brown food colouring added to hot water) in the date seed study. Although, it was predicted to be very low, it would have improved the study if the phenolic content had been assessed in case the value was affected by one of the placebo ingredients such as the brown food colouring.

An observation regarding the nature of some cognitive test tasks could be considered a limitation. The typing speed can be viewed as a problem in the functions of the immediate and delayed word recalls. In these tasks, the CogTrack system allows the participants one minute to recall all words in any order by typing them using the computer keyboard. The participants' speed is reflected in the number of words correctly recalled and those not in the list (errors) recorded in every visit for each session, terefore, if the participant does not have sufficient time to write all the words that they can recall, a change in typing speed might be misinterpreted as a change in word recall (memory). However, an adjustment to the recall period may improve the accuracy by increasing the chance of recalling more words.

6.3 Recommendations for future research

As previously discussed, a primary direction for future research would include the assessment of the chronic supplementation of date fruit and seeds on young, middle-aged and elderly adults while employing alternative cognitive paradigms, in particular those which have formerly displayed sensitivity to flavonoid-rich nutritional interventions in rats and humans during sustained mental effort.

One proposal could be the incorporation of a chronic trial, as discussed in the introduction to chapter 4, section 4.1, including the contemplation of physiological measures such as a non-invasive measure of cerebral blood flow, for example, NIRS (Wightman et al., 2012) or the assessment of the effects on brain wave activities using a simplified electroencephalogram (EEG), as used in (Okello et al., 2016).

Finally, conducting a systematic review in the future regarding which cognitive tasks are correlated or independent and sensitive to phenolics manipulations simultaneously could help provide definitive guidance for choosing the appropriate statistical approach, which may allow us to reconsider the use of the Bonferroni correction to express our caution in the interpretation of the thesis data.

6.4 Conclusion

The treatments created to appraise the impact of date fruit or seeds on healthy participants did not provide any evidence of positive impact after the consumption of single doses.

To summarise, the principal novel knowledge which has been contributed as a result of the research reported in this thesis is as follows:

- The primary development of a standardised date-containing treatment with a quantified phenolic content and a placebo.
- The first known assessment of the effects of the consumption of treatments made from date fruit and yoghurt on mood and cognitive function.
- The creation of a standardised “coffee-like” beverage, made using roasted date seeds, and a placebo with a quantified phenolic content.

- The first assessment of the effects of the consumption of treatments made from date seeds on mood and cognitive function.
- The use of a positive control treatment to provide context and reference data for the assessment of data from the new treatment increased the robustness of the study design.

Appendices

Appendix A. GI and GL Clinical trial protocol

ClinicalTrials.gov PRS
Protocol Registration and Results System

ClinicalTrials.gov PRS DRAFT Receipt (Working Version)
Last Update: 03/20/2018 08:23

ClinicalTrials.gov ID: [Not yet assigned]

Study Identification

Unique Protocol ID:	DFGI
Brief Title:	Assessing the Glycaemic Index of Two Different Cultivars of Date Fruit
Official Title:	Assessing the Glycaemic Index of Two Different Cultivars of Date Fruit When Mixed With 0% Fat Yogurt on Healthy Volunteers.
Secondary IDs:	

Study Status

Record Verification:	March 2018
Overall Status:	Not yet recruiting
Study Start:	April 1, 2018 [Anticipated]
Primary Completion:	May 31, 2018 [Anticipated]
Study Completion:	June 1, 2018 [Anticipated]

Sponsor/Collaborators

Sponsor:	Newcastle University
Responsible Party:	Sponsor
Collaborators:	

Oversight

U.S. FDA-regulated Drug:	No
U.S. FDA-regulated Device:	No
U.S. FDA IND/IDE:	No
Human Subjects Review:	Board Status: Pending Board Name: FMS Faculty Ethics Commitee Board Affiliation: Newcastle university Phone: 0191 208 5633 Email: Kimberley.Sutherland@newcastle.ac.uk Address: Kimberley Sutherland Faculty Support Assistant Research & Innovation Office

- Page 1 of 5 [DRAFT] -

Faculty of Medical Sciences

Newcastle University

Tel: 0191 208 5633

Data Monitoring: No

FDA Regulated Intervention: No

Study Description

Brief Summary: Fruit of the date palm (P. dactylifera) may be considered as an emerging and potential candidate for the development of health-promoting foods, owing to its high nutritional values.

Furthermore, aqueous extracts of dates have previously been shown to have potent antioxidant activity, because they inhibit in vitro lipid and protein oxidation and possess free radical scavenging capacity.

Although the high sugar content of date fruit has always been a concern, date fruit has been regarded as a low-GI to medium-GI food. However, very limited, inconsistent and contradictory information is available on the glycaemic index values of different date varieties, which may be attributed to both the methodology as well as other food factors. Date consumption is high among people of Arabic origin, where it's very common for them to be eaten with coffee or yoghurt. Therefore, in view of these concerns, the objective of this trial is to evaluate the glycaemic response of two different varieties of dates, named Birhi & Khassab, in an early maturation stage (Rutab stage), when mixed with 0% fat yogurt, on ten healthy participants aged between 18 and 45.

Detailed Description: Introduction Although, the fruit of the date palm (P. dactylifera) may be considered an emerging and potential candidate for the development of health-promoting foods owing to its high nutritive values (Juhaimi, Ghafoor et al. 2012), the high sugar content of date fruit has always been a concern. Sun-dried dates, which is the well-known ripening stage of date fruit, can be regarded as low-GI to medium-GI food. However, very limited, inconsistent and contradictory information is available on the glycaemic Index values of different date varieties. This variation could be attributed to either the methodology or the food factors (Ahmed et al. 1991)

Rational and objective Nowadays, low-GI foods have often been found to induce beneficial effects on risk factors for certain non-communicable chronic diseases (Alfenas and Mattes 2005; Galgani et al. 2006). As the chemical composition of dates can vary depending on cultivar, soil conditions, agronomic practices as well as the ripening stage (Al-Hooti et al. 1997). It is important to know the GI of the local/regional date varieties, and in different date products such as dates with yoghurt. Date consumption is high among people of Arabic origin, where it is very commonly eaten with coffee or yoghurt. During the first trial of this PHD project, treatments containing 150g of 0% fat yoghurt and two different freeze dried date powders, depending on if it was a Birhi treatment or Khassab treatment, were formulated. These were used to assess acute effects of date fruit and yoghurt on mood and cognitive performance in healthy volunteers, as per the ethical approval from Newcastle University. These exact treatments will be used again, and the trial will aim to evaluate their glycaemic index on 10 healthy participants.

Participants Ten healthy participants aged between 18 and 45 will be recruited. Participants will be required to undergo a screening visit, followed by three study visits. The trial will last for a month in total. Glycaemic indexes will be

calculated using standard methods. Results will be calculated using means and standard deviations.

Design

A standard experimental study involving the measurement of the glycaemic responses of the ingestion of two different varieties of dates, when mixed with 0% fat yoghurt, and a placebo treatment.

Conditions

Conditions: Glycaemic Index of Two Different Cultivars of Date Fruit

NOTE : "glycaemic index of two different cultivars of date fruit" is not a recognized condition

Keywords:

Study Design

Study Type: Interventional

Primary Purpose: Basic Science

Study Phase: N/A

Interventional Study Model: Crossover Assignment

Number of Arms: 3

Masking: None (Open Label)

Allocation: Non-Randomized

Enrollment: 10 [Anticipated]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Birhi + YEO 0% fat yoghurt 47g of total carbohydrate in which contains 43.6g of Freeze-dried of Birhi powder+ 150g of 0% fat yoghurt	Procedure/Surgery: Glycaemic index The GI will be calculated according to Wolever et al (1991). Capillary blood samples will be taken using the finger prick method. According to the method of Wolever et al (1991), blood Glucose will be recorded at baseline, and then at 15 minutes intervals post consumption of the treatment of the day, for a duration of 2 hours and 15 min in total.
Experimental: Khassab + YEO 0% fat yoghurt 47g of total carbohydrate in which contains34.6g of freeze-dried Khassab powder+ 150g of 0% fat yoghurt	Procedure/Surgery: Glycaemic index The GI will be calculated according to Wolever et al (1991). Capillary blood samples will be taken using the finger prick method. According to the method of Wolever et al (1991), blood Glucose will be recorded at baseline, and then at 15 minutes intervals post consumption of the treatment of the day, for a duration of 2 hours and 15 min in total.
Experimental: 50g of Glucose dissolved in 100 ml of water (standard food) 50g of pure glucose dissolved in 100ml of water	Procedure/Surgery: Glycaemic index The GI will be calculated according to Wolever et al (1991). Capillary blood samples will be taken using the finger prick method. According to the method of Wolever et al (1991), blood Glucose will be recorded at baseline, and then at 15 minutes intervals post

Arms	Assigned Interventions
	consumption of the treatment of the day, for a duration of 2 hours and 15 min in total.

NOTE : Arm/Group Label should have no more than 40 characters.
NOTE : No interventions have been included in Arm Description for 'Birhi + YEO 0% fat yoghurt'
NOTE : No interventions have been included in Arm Description for 'Khassab + YEO 0% fat yoghurt'
NOTE : No interventions have been included in Arm Description for '50g of Glucose dissolved in 100 ml of water (standard food)'
NOTE : Intervention 'Glycaemic index' has not been included in any Arm/Group Descriptions.

Outcome Measures

Primary Outcome Measure:

1. Assessing the glycaemic index of two different cultivars of date fruit when mixed with 0% fat yogurt on healthy volunteers.
   On each of the study days, blood glucose will be measured at baseline, 15 min post-dose, 30 min post-dose, 45 min post-dose, 60 min post-dose, 75 min post-dose, 90 min post-dose, 105 min post-dose and 120 min post- dose.

   Each of the three treatment study visits will last approximately 2 hours 15, and there will be a one week wash out period between each treatment visit.

   [Time Frame: Change from baseline every 15 minutes post supplementation for 8 post-dose intervals.]

Eligibility

Minimum Age: 18 Years
Maximum Age: 45 Years
Sex: All
Gender Based:
Accepts Healthy Volunteers: Yes
Criteria: Inclusion Criteria:

- Participants A total of 10 healthy participants aged between 18 and 45 will be recruited through advertisement via poster and flyer. This is an internationally recognised standard protocol which recommends using 10 volunteers or replicates for each foodstuff, and all participants will be required to undergo a screening visit.

Inclusion Criteria Healthy participants aged 18-45 with a BMI >18 <36 will be recruited from the Newcastle Upon-Tyne area.

Exclusion Criteria:

Participants will be considered ineligible to participate in the study if they meet any of the following criteria:

1. They have any metabolic diseases such as type 1 or type 2 diabetes.
2. They have a BMI above 35kg/m2 or lower than 18kg/m2
3. They are taking any illicit or prescribed drugs.
4. They are using dietary supplements, over the counter medicine or recreational drugs
5. They are females who are pregnant or seeking to become pregnant.
6. They have allergies to any food products.
7. They have any dairy intolerances.

Contacts/Locations

Central Contact Person:	Prf. Chris Seal, Prof Telephone: +44 (0) 191 208 7650 Email: Chris.seal@ncl.ac.uk
Central Contact Backup:	Dr. Anthony Watson, Phd Telephone: 01912086935 Email: Anthony.watson@ncl.ac.uk
Study Officials:	NOTE : Study Official is required by the WHO and ICMJE.
Locations:	

IPDSharing

Plan to Share IPD:	Undecided

References

Citations:	
Links:	
Available IPD/Information:	

Appendix B. GI and GL ethical Approval

Faculty of Medical Sciences
Newcastle University
The Medical School
Framlington Place
Newcastle upon Tyne
NE2 4HH United Kingdom

Duaa Altuwairki
Institute of Cellular Medicine

FACULTY OF MEDICAL SCIENCES: ETHICS COMMITTEE

Dear Duaa,

Title: Assessing the glycaemic index of two different cultivars of date fruit when mixed with 0% fat yogurt on healthy volunteers.
Application No: 1490/4474/2018
Start date to end date: 16/04/2018 to 31/05/2018

On behalf of the Faculty of Medical Sciences Ethics Committee, I am writing to confirm that the ethical aspects of your proposal have been considered and your study has been given ethical approval.

The approval is limited to this project: **1490/4474/2018**. If you wish for a further approval to extend this project, please submit a re-application to the FMS Ethics Committee and this will be considered.

During the course of your research project you may find it necessary to revise your protocol. Substantial changes in methodology, or changes that impact on the interface between the researcher and the participants must be considered by the FMS Ethics Committee, prior to implementation.*

At the close of your research project, please report any adverse events that have occurred and the actions that were taken to the FMS Ethics Committee.*

Best wishes,

Yours sincerely
K. Sutherland
Kimberley Sutherland
On behalf of Faculty Ethics Committee

cc.
Professor Daniel Nettle, Chair of FMS Ethics Committee
Mrs Kay Howes, Research Manager

*Please refer to the latest guidance available on the internal Newcastle web-site.

tel: +44 (0) 191 208 6000
fax: +44 (0) 191 208 6621

www.ncl.ac.uk

Appendix C. GI and GL Case Report Form CRF

Participant **DFGI**

Participants initials:

nu-food
Food & Consumer Research Facility

Study code: DFGI

<Assessing the Glycaemic Index of different varieties of Date Fruit in Healthy Adults>

CASE REPORT FORM

Participant ID:

Trial Sponsor: NEWCASTLE UNIVERSITY

Final Version Date: 22/02/2018

CRF completion Instructions

When completing the CRF please ensure:

- Black ink should be used.
- Each section is completed fully.
- Any corrections made to any data in the CRF are initialled and dated.
- The consent form is signed, dated and the name of signatory is clearly printed by all parties.
- The date of consent recorded in the CRF is the date the Participant signs the consent form.

Taking Consent

The participant must be eligible and have given consent before entering the study.

For the Participant to give consent they must sign and date two original consent forms after they have completely read the Participant information sheet and have fully understood what the study entails. The Participant must clearly print their name on the consent and the date must be the date the Participant signs the form. No study procedures can occur prior to the Participant signing the consent form.

As well as the Participant signing the consent form, it is necessary for the person explaining the study to the Participant to sign the consent form. By signing the consent form the person explaining the study confirms that they have witnessed the Participant give consent and that the Participant fully understands what the study entails.

Please ensure that two copies of the consent form are signed – one copy to be given to the Participant, one copy to remain at NU-Food Research (this copy should be stored in a locked filing cabinet, separate from all participant data).

VISIT 0 - SCREENING Date: D D M M Y Y Y Y

Participant initials:
Participant number:

PARTICIPANT ELIGIBILITY CHECKLIST - TRIAL ENTRY

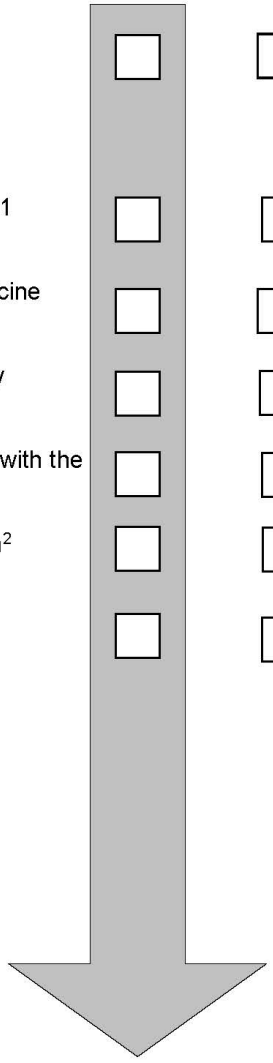
Please tick:	YES	NO
Has the Participant given written informed consent?	☐	☐
Is the Participant:		
In good health?	☐	☐
Aged between 18 and 45 years?	☐	☐
Orientated to person, place and time and has the ability to communicate with study staff?	☐	☐
Motivated to participate in and complete the study as instructed and to attend visits in a well-rested state?	☐	☐
Does the Participant:		
Intend to comply with the 12 hour fasting restriction prior to each study session?	☐	☐

If NO the Participant is ineligible for the trial. Please only complete pages S1 – S5.

VISIT 0 - SCREENING Date: D D M M Y Y Y Y

Participant initials:
Participant number:

	Please tick: YES	NO
Is the Participant:		
Pregnant or seeking to become pregnant?	☐	☐
Does the Participant:		
Have a history of metabolic diseases like type 1 or type 2 diabetes?	☐	☐
Currently take a pharmaceutical product/medicine (except contraception?)	☐	☐
Have any known allergies or intolerance to any ingredients in the study preparation?	☐	☐
Have a Body Mass Index (BMI) above 40 kg/m^2 (severely obese)?	☐	☐
Do you have allergies to **ANY** food product?	☐	☐



If YES the Participant is ineligible for the trial.
At Screening visit, please only complete pages S1 – S5.

VISIT 0 - SCREENING Date: D D M M Y Y Y Y

Participant initials:
Participant number:

DOCUMENTATION OF INFORMED CONSENT

IMPORTANT: Informed consent must be obtained from the Participant BEFORE any trial procedures are started.

Has the Participants' written informed consent been obtained?

Yes ☐
No ☐ (Please tick)

If NO: The Participant is not eligible for the trial

If YES: Keep the site consent form with the Participant's notes

Date of Consent: D D M M Y Y Y Y

Is the Participant eligible for this trial?

Yes - Eligible ☐
No - Screen failure ☐ (Please tick)

If NO: please state main reason:

Fails to meet inclusion / exclusion criteria ☐
Participant has withdrawn consent ☐ (Please tick)

RR's signature

Date: D D M M Y Y Y Y

If the Participant does not satisfy ALL of the eligibility criteria or has withdrawn consent then please only complete pages S1 – S5. If the Participant satisfies all of the eligibility criteria and has provided appropriate consent please proceed.

VISIT 0 - SCREENING Date: D D M M Y Y Y Y

Participant initials:
Participant number:

PARTICIPANT DEMOGRAPHICS

Date of Birth: D D M M Y Y Y Y

Age: Years_____ Months_____

Sex: male (M) ☐ or female (F) ☐

Race:
- Maori ☐
- Black ☐
- Oriental ☐
- Caucasian ☐
- Other ☐ please specify _____

Does the Participant require glasses/contact lenses to use a computer?

YES ☐
NO ☐
(Please tick)

Which hand does the Participant use to write with? RIGHT ☐ LEFT ☐

Is the participant vegetarian? Yes ☐ No ☐

How many portions of fruit and vegetables does the participant eat in a typical day? [Portion= one piece of fruit, a handful of vegetables or a glass of fresh fruit juice (each additional glass of juice does not count as extra)]

_____ Portion (s).

How many years of full time education has the participant had? _____

What is the highest level of qualification achieved?_____

Height ☐☐☐ cm

Weight ☐☐☐.☐☐ kg

BMI ☐☐.☐ kg/m^2

Blood Pressure

Systolic ☐☐☐

Diastolic ☐☐☐

Heart Rate ☐☐☐ BPM

What was the Participant's blood glucose level?

☐☐ Mmol/L

CONCOMITANT MEDICATION:

Is the Participant receiving any concomitant medications, therapies and/or vitamin supplementation?

Yes ☐
No ☐ (Please tick)

If YES: Please complete the concomitant medication record on page 17.

MEDICAL HISTORY (Within the past 5 years)

Specify Diagnosis	1 = Past 2 = Present	Severity 1 = mild 2 = moderate 3 = severe	Concomitant Treatment 1 = Yes * 2 = No	Details
1.				
2.				
3.				
4.				
5.				
6.				
7.				
8.				
9.				
10.				

* If Yes please complete concomitant medication record on page 17.

Please note that the volunteer may not eligible to participate if taking or intending to take any prescription pharmaceutical product during the study (except for contraception for females and some topically applied therapeutic agents). Please refer to the protocol for the specific guidelines for the study.

Study Day

Date: D D M M Y Y Y Y

Participant initials:
Participant number:

Study Day 1

Participant number allocated:

Have there been any changes to the subject's concomitant medications, therapies and/or vitamin supplementation since their last visit?

No ☐ Yes ☐ (if Yes complete concomitant medication record)

Has the participant experienced any adverse events (illness) since the last visit?

No ☐ Yes ☐ (if Yes complete adverse event record)

Has the participant fasted from 10pm yesterday? (If No, re-schedule their study day)

No ☐ Yes ☐

Baseline Samples

What were the Participant's blood pressure and heart rate?

First reading:

Blood Pressure

Systolic MM Hg

Diastolic MM Hg

Heart Rate BPM

Second reading:

Blood Pressure

Systolic MM Hg

Diastolic MM Hg

Heart Rate BPM

Study Day

Date: D D M M Y Y Y Y

Participant initials:

Participant number:

What was the Participants' blood glucose level?

☐☐ **Mmol/L**

Treatment: Treatment taken ☐ Time taken _____

Post dose 1 Blood Glucose15 minutes post treatment

☐☐ **Mmol/L**

Post dose 2 Blood Glucose 30 minutes post treatment

☐☐ **Mmol/L**

Post dose 3 Blood Glucose 45 minutes post treatment

☐☐ **Mmol/L**

First reading:

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

Second reading:

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

Post dose 4 Blood Glucose 60 minutes post treatment

☐☐ **Mmol/L**

Post dose 5 Blood Glucose 75 minutes post treatment

☐☐ **Mmol/L**

Study Day

Date: D D M M Y Y Y Y

Participant initials:
Participant number:

Post dose 6 Blood Glucose 90 minutes post treatment

Mmol/L

Post dose 7 Blood Glucose 105 minutes post treatment

Mmol/L

Post dose 8 Blood Glucose 120 minutes post treatment

Mmol/L

First reading:

Blood Pressure

Systolic MM Hg

Diastolic MM Hg

Heart Rate BPM

Second reading:

Blood Pressure

Systolic MM Hg

Diastolic MM Hg

Heart Rate BPM

D D M M Y Y Y Y

Date:

RR's signature

Study Day

Date: D D M M Y Y Y Y

Participant initials: ☐☐☐

Participant number: ☐☐☐

Study Day 2

Have there been any changes to the subject's concomitant medications, therapies and/or vitamin supplementation since their last visit?

No ☐ Yes ☐ (if Yes complete concomitant medication record)

Has the participant experienced any adverse events (illness) since the last visit?

No ☐ Yes ☐ (if Yes complete adverse event record)

Has the participant fasted from 10pm yesterday? (If No, re-schedule their study day)

No ☐ Yes ☐

Has participant confirmed they have consumed no caffeine or alcohol since 10pm yesterday? (If No, re-schedule their study day)

No ☐ Yes ☐

Baseline Samples

What were the Participant's blood pressure and heart rate?

First reading:

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

Second reading:

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

What was the Participant's blood glucose level?

☐☐ **Mmol/L**

Treatment: Treatment taken ☐ Time taken _____

Study Day :

Date: D D M M Y Y Y Y

Participant initials:

Participant number:

Post dose 1 Blood Glucose15 minutes post treatment

Mmol/L

Post dose 2 Blood Glucose 30 minutes post treatment

Mmol/L

Post dose 3 Blood Glucose 45 minutes post treatment

Mmol/L

First reading:

Blood Pressure

Systolic MM Hg

Diastolic MM Hg

Heart Rate BPM

Second reading:

Blood Pressure

Systolic MM Hg

Diastolic MM Hg

Heart Rate BPM

Post dose 4 Blood Glucose 60 minutes post treatment

Mmol/L

Post dose 5 Blood Glucose 75 minutes post treatment

Mmol/L

Post dose 6 Blood Glucose 90 minutes post treatment

Mmol/L

Study Day :

Date: D D M M Y Y Y Y

Participant initials:

Participant number:

Post dose 7 Blood Glucose 105 minutes post treatment

☐☐ **Mmol/L**

Post dose 8 Blood Glucose 120 minutes post treatment

☐☐ **Mmol/L**

First reading:

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

Second reading:

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

RR's signature

Date: D D M M Y Y Y Y

Study Day :

Date: D D M M Y Y Y Y

Participant initials:

Participant number:

Study Day 3

Have there been any changes to the subjects' concomitant medications, therapies and/or vitamin supplementation since their last visit?

No ☐ Yes ☐ (if Yes complete concomitant medication record)

Has the participant experienced any adverse events (illness) since the last visit?

No ☐ Yes ☐ (if Yes complete adverse event record)

Has the participant fasted from 10pm yesterday? (If No, re-schedule their study day)

No ☐ Yes ☐

Has participant confirmed they have consumed no caffeine or alcohol since 10pm yesterday? (If No, re-schedule their study day)

No ☐ Yes ☐

Baseline Samples

What were the Participant's blood pressure and heart rate?

First reading:

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

Second reading:

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

Study Day :

Date: D D M M Y Y Y Y

Participant initials:

Participant number:

What was the Participant's blood glucose level?

☐☐ **Mmol/L**

Treatment: Treatment taken ☐ Time taken _____

Post dose 1 Blood Glucose15 minutes post treatment)

☐☐ **Mmol/L**

Post dose 2 Blood Glucose 30 minutes post treatment)

☐☐ **Mmol/L**

Post dose 3 Blood Glucose 45 minutes post treatment)

☐☐ **Mmol/L**

First reading:

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

Second reading:

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

Study Day :

Date: D D M M Y Y Y Y

Participant initials:
Participant number:

Post dose 4 Blood Glucose 60 minutes post treatment)

Mmol/L

Post dose 5 Blood Glucose 75 minutes post treatment)

Mmol/L

Post dose 6 Blood Glucose 90 minutes post treatment)

Mmol/L

Post dose 7 Blood Glucose 105 minutes post treatment)

Mmol/L

Post dose 8 Blood Glucose 120 minutes post treatment)

Mmol/L

First reading:

Blood Pressure

Systolic MM Hg

Diastolic MM Hg

Heart Rate BPM

Second reading:

Blood Pressure

Systolic MM Hg

Diastolic MM Hg

Heart Rate BPM

RR's signature

Date: D D M M Y Y Y Y

Study Day :

Date: D D M M Y Y Y Y

Participant initials:

Participant number:

Study Day 4

Have there been any changes to the subject's concomitant medications, therapies and/or vitamin supplementation since their last visit?

No ☐ Yes ☐ (if Yes complete concomitant medication record)

Has the participant experienced any adverse events (illness) since the last visit?

No ☐ Yes ☐ (if Yes complete adverse event record)

Has the participant fasted from 10pm yesterday? (If No re-schedule their study day)

No ☐ Yes ☐

Has participant confirmed they have consumed no caffeine or alcohol since 10pm yesterday? (If No re-schedule their study day)

No ☐ Yes ☐

Baseline Samples

What were the Participant's blood pressure and heart rate?

First reading:

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

Second reading:

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

Study Day :

Date: D D M M Y Y Y Y

Participant initials:

Participant number:

What was the Participant's blood glucose level?

☐☐ **Mmol/L**

Treatment: Treatment taken ☐ Time taken _____

Post dose 1 Blood Glucose15 minutes post treatment)

☐☐ **Mmol/L**

Post dose 2 Blood Glucose 30 minutes post treatment)

☐☐ **Mmol/L**

Post dose 3 Blood Glucose 45 minutes post treatment)

☐☐ **Mmol/L**

First reading:

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

Second reading:

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

Post dose 4 Blood Glucose 60 minutes post treatment

☐☐ **Mmol/L**

Post dose 5 Blood Glucose 75 minutes post treatment

☐☐ **Mmol/L**

Study Day :

Date: D D M M Y Y Y Y

Participant initials:

Participant number:

Post dose 6 Blood Glucose 90 minutes post treatment

Mmol/L

Post dose 7 Blood Glucose 105 minutes post treatment

Mmol/L

Post dose 8 Blood Glucose 120 minutes post treatment

Mmol/L

First reading:

Blood Pressure

Systolic MM Hg

Diastolic MM Hg

Heart Rate BPM

Second reading:

Blood Pressure

Systolic MM Hg

Diastolic MM Hg

Heart Rate BPM

RR's signature

Date: D D M M Y Y Y Y

Study Day :

Date: D D M M Y Y Y Y

Participant initials:

Participant number:

TRIAL OUTCOME (please tick appropriate box):

Completed trial: ☐

If trial not completed, please complete:

Date of subject withdrawal from the study: D D M M Y Y Y Y

Provide main reason for premature termination (one reason only):

adverse event	☐	withdrawn consent	☐
did not co-operate	☐	refused treatment	☐
administrative reason	☐	lost to follow-up	☐
protocol violation	☐	other	☐

if other please specify: _____

_____ Date: D D M M Y Y Y Y

Investigator's signature

ADVERSE EVENTS

Date: D D M M Y Y Y Y

Participant initials:
Participant number:

Adverse Events

Were there any Adverse Events? ☐ 1 NO ☐ 2 YES, please complete all sections below, cross appropriate number.

	if **yes***		**Date**	**Severity**	**Relation to Study Drug**	**Action(s) Taken** *(several statements are possible)*	**Outcome of Event**
Adverse Event, specify Please list ONE event per line.	**Serious** 1 No 2 Yes *	**Reason** *(several statements are possible)* 1. Results in Death 2. Life-threatening 5. Hospitalization – new / prolonged 6. Congenital anomaly/birth defect 10. Persistent or significant disability/incapacity 11. Important medical event	**Start / Stop** If ongoing update at next visit dd / mm / yy	1. Mild 2. Moderate 3. Severe	**Possible?** 1. No 2. Yes	1. None 2. Dose of study drug reduced 3. Study drug discontinued and restarted 4. Study drug discontinued permanently 5. Remedial drug therapy, specify on concomitant medication page 6. Other (specify below) 7. Infusion rate of study drug educed 8. Hospitalization required or prolonged	1. Resolved 2. Improved 3. Unchanged 4. Worsened 6. Death 7. Insufficient Follow-up
1.	1 2* ☐☐	1 2 5 6 10 11 ☐☐☐☐☐☐	/ / / /	1 2 3 ☐☐☐	1 2 ☐☐	1 2 3 4 5 6 7 8 ☐☐☐☐☐☐☐☐	1 2 3 4 6 7 ☐☐☐☐☐☐
2.	1 2* ☐☐	1 2 5 6 10 11 ☐☐☐☐☐☐	/ / / /	1 2 3 ☐☐☐	1 2 ☐☐	1 2 3 4 5 6 7 8 ☐☐☐☐☐☐☐☐	1 2 3 4 6 7 ☐☐☐☐☐☐
3.	1 2* ☐☐	1 2 5 6 10 11 ☐☐☐☐☐☐	/ / / /	1 2 3 ☐☐☐	1 2 ☐☐	1 2 3 4 5 6 7 8 ☐☐☐☐☐☐☐☐	1 2 3 4 6 7 ☐☐☐☐☐☐
4.	1 2* ☐☐	1 2 5 6 10 11 ☐☐☐☐☐☐	/ / / /	1 2 3 ☐☐☐	1 2 ☐☐	1 2 3 4 5 6 7 8 ☐☐☐☐☐☐☐☐	1 2 3 4 6 7 ☐☐☐☐☐☐

Further Details of Adverse Events: _____

D D M M Y Y Y Y

_____ Investigator's Signature Date: ☐☐☐☐☐☐☐☐

CONCOMITANT MEDICATION RECORD

Date: D D M M Y Y Y Y

Participant initials:
Participant number:

Please complete the following information fully for any concomitant medication, therapies and/or vitamin supplementation:

*** If Indication is due to a new/worsening AE, please complete the AE form (pg 18).**

Concomitant Treatment (please use generic name)	Indication	Single Dose	Total Daily Dose	Units	Frequency (e.g. BID, PRN)	Route code	Start and Stop Dates DD MM YY
1.							Start: / /
							Stop: / /
2.							Start: / /
							Stop: / /
3.							Start: / /
							Stop: / /
4.							Start: / /
							Stop: / /
5.							Start: / /
							Stop: / /
6.							Start: / /
							Stop: / /
7.							Start: / /
							Stop: / /
8.							Start: / /
							Stop: / /
9.							Start: / /
							Stop: / /

Appendix D. GI and GL Consent Form

nu-food
Food & Consumer Research Facility

Newcastle University

Assessing the glycaemic index of two varieties of date fruit when mixed with 0% fat yoghurt (DFGI)

CONSENT BY VOLUNTEER TO PARTICIPATE IN A NUTRITIONAL STUDY

Participant ID_____

		Please initial box
1.	I confirm that I have read and understood the information sheet for the above study and have had the opportunity to ask questions.	☐
2.	I understand that my participation is voluntary and that I am free to withdraw at any time.	☐
3.	I understand that I need to give 4 finger prick blood samples during each visit	☐
4.	I confirm that I have no allergies or intolerances to any food or drink	☐
5.	I agree to take part in the trial	☐

_____	_____	_____
Name of Volunteer *(Please print)*	Date	Signature
_____	_____	_____
Name of Research Team Member (Please print)	Date	Signature

Appendix E. GI and GL Participants Information

Assessing the Glycaemic index of two different varieties of date fruit consumption when mixed with 0% fat yoghurt on healthy volunteers

Information Sheet for Participants

Investigator: Duaa Altuwairki

Supervisors:

Pro. Chris Seal

Dr. Anthony Watson

Newcastle University

Faculty of Medical Sciences

ICM

Newcastle upon Tyne

NE1 7RU

For further information please contact:

Email: D.altuwairki2@ncl.ac.uk

Telephone: Researcher 07402033360

http:www.ncl.ac.uk/hnrc

Participation Information Sheet Version Duaa Altuwairki (PGR)

22/02/2018

You are being invited to take part in a research study. Before you decide to take part it is important you understand why the research is being conducted, and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please ask us if there is anything that is not clear, or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this.

What is the purpose of this study?

Date fruits are considered a functional food due to their high nutritional value and possible properties against neurodegenerative diseases, and for improving brain functions. However, the high sugar content of date fruit has always been a concern. A glycaemic index is the ability of the carbohydrate based food to increase blood glucose levels.

Foods with carbohydrates that break down quickly during digestion and release glucose rapidly into the bloodstream tend to have a high glycaemic index; foods with carbohydrates that break down more slowly, releasing glucose more gradually into the bloodstream, tend to have a low glycaemic index. The concept was developed by Dr. David J. Jenkins and colleagues in 1980–1981 at the University of Toronto in their research to find out which foods were best for people with diabetes.

Date fruit has been regarded as a low-GI to medium-GI food, however, very limited, inconsistent and contradictory information is available about the glycaemic index values of the different date varieties.

Therefore, this study will assess the glycaemic responses of the consumption of two different varieties of Date fruit when mixed with 0% fat yoghurt on healthy volunteers.

Why have I been chosen?

We are looking for young volunteers who are not over the age of 45 years, if they are females they should not be pregnant, or seeking to become pregnant. Participants should have no history of, or currently have allergies to any food products, have a BMI below 35kg/m^2 and are using no dietary supplements, over the counter medicine or recreational drugs. They should have no history of any metabolic diseases such as type 1 or type 2 diabetes to take part in this study. We will be recruiting 10 volunteers in total from the Newcastle Upon Tyne area.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be asked to sign a consent form on your 'Screening Visit'; you will be given a copy of this to keep.

However, you will be free to withdraw from the study without giving a reason anytime up to the end of your final visit. Shortly after this, all data will be fully anonymised, and therefore, from this point forward it will not be possible to withdraw any data from the study.

What will happen to me if I take part?

If you agree to take part we will ask you to visit **the NU-Food research facility, Newcastle University** on Four occasions. The first visit is a screening visit to assess your suitability for the project. We will ask you a series of questions to ensure you're ok to take part, you will also have the opportunity to discuss the project with the research team. If you are suitable, there will be three further visits. At each visit, you need to come at 9:30 am to the **NU-Food research facility, Newcastle University**, and you will complete a series of finger pricks which will assess your blood glucose levels. This will be conducted 9 times per visit. One time at the baseline, followed by the treatment of the day, and then repeat the measurement at 15 min, 30 min, 45 min, 60 min, 75 min, 90 min, 105 min and 120 min post consumption of the study foods. Each visit will last approximately two hours and 15mins. Also your blood pressure will be assessed three times, in each visit using a blood pressure machine. The finger prick method is a procedure that helps to assess the blood glucose level with only a drop of blood. The procedure is basically about; taking one of your fingers, quickly and gently puncturing with a sterile lancet. Then a drop of your blood that should be formed at the puncture site will be applied to the analysis strip. Then we will provide you with an adhesive plaster to apply on the punctured finger.

What else do I have to do?

If you agree to take part, we will ask you to make short visits to NU-Food, Newcastle University, on four occasions. The first visit is a screening visit to assess your suitability for the project. On the evening before these visits, you will need to fast from 10 pm; this means that you should not eat or drink anything except water until you complete your visit the following morning.

What will happen to the samples I provide?

Only a few drops of your blood will be collected using finger prick, for the purpose of blood glucose level analysis.

Appendix F. level 2 Food safety and hygiene certificate

LEVEL 2 FOOD SAFETY AND HYGIENE FOR CATERING

DUAA ALTUWAIRKI

has successfully completed a programme of training in food safety and hygiene at level 2 and an assessment which concluded the course

one TRAINING SERVICES
Food Safety UK

Examinations Officer

Director - One Training Services Ltd

AWARDED	13 June 2019
CENTRE	2N56BD
CERTIFICATE NUMBER	71795-156-044-9329

RoSPA Approved E-learning course

CPD CERTIFIED The CPD Certification Service

Training delivered by: ONE Training Services Ltd https://food-safety.org.uk

Appendix G. The sheet used for sensory evaluation by participants

Newcastle University

nu-food
Food & Consumer Research Facility

Sensory Evaluation of Yoghurt

Instructions:

1. Please take a spoon full of the yoghurt.
2. Taste all samples in sequence according to the number labelling.
3. To score the yoghurt, place the sample number on the line as indicated in the example below
4. If you think 2 sample are the same or very similar please ad the sample numbers below and above the line to avoid overlap

Example

Size

Very Small — µ ¥ ¶ — Very Big

Smell

Very Strong — Very Weak

Sweetness

Very Sweet — Very Sour

Texture

Very Smooth — Very Gritty

Flavour

Very Intense — Very Mild

Colour

Pale Red — Dark Red

Aftertaste

Strong Aftertaste — Weak Aftertaste

Overall Acceptability

Delicious — Not Acceptable

Thank you for participating

Appendix H. SPSS output for HPLC data analysis one-way ANOVA

Oneway

[DataSet0]

Descriptives

TPC

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Between-Component Variance
						Lower Bound	Upper Bound			
Barhi		3	163.5200	18.17290	10.49213	118.3760	208.6640	144.60	180.84	
Khassab		3	369.1100	11.74733	6.78232	339.9280	398.2920	357.50	380.99	
Barhi + yoghurt		3	191.5100	2.25107	1.29965	185.9180	197.1020	189.43	193.90	
Khassab + yoghurt		3	207.1133	9.09986	5.25381	184.5080	229.7186	197.02	214.69	
Total		12	232.8133	84.39446	24.36258	179.1917	286.4350	144.60	380.99	
Model	Fixed Effects			11.79119	3.40382	224.9641	240.6626			
	Random Effects				46.31847	85.4073	380.2194			8535.25684

Test of Homogeneity of Variances

TPC

Levene Statistic	df1	df2	Sig.
1.621	3	8	.260

ANOVA

TPC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	77234.408	3	25744.803	185.172	.000
Within Groups	1112.258	8	139.032		
Total	78346.666	11			

Post Hoc Tests

Multiple Comparisons

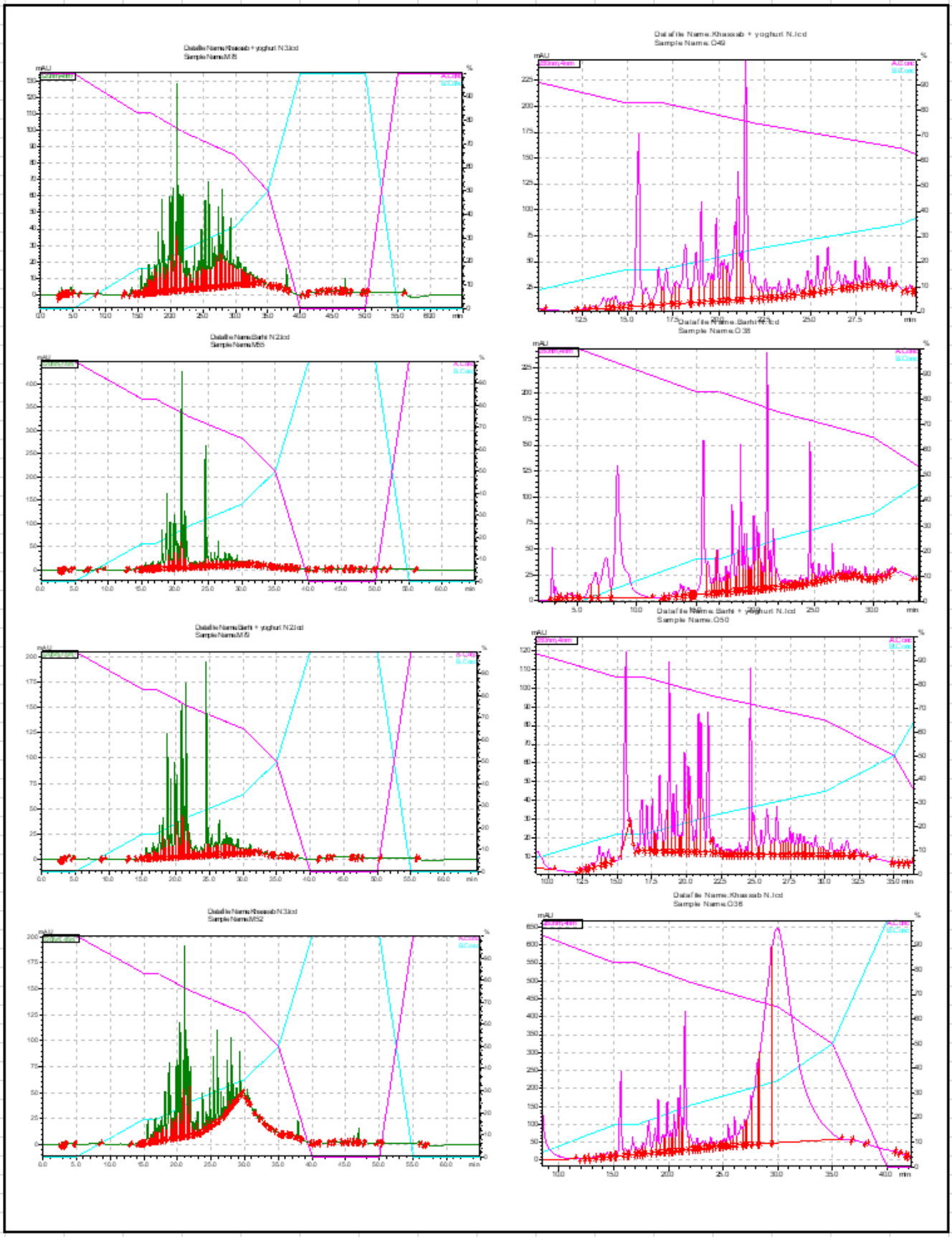
Dependent Variable: TPC

Tukey HSD

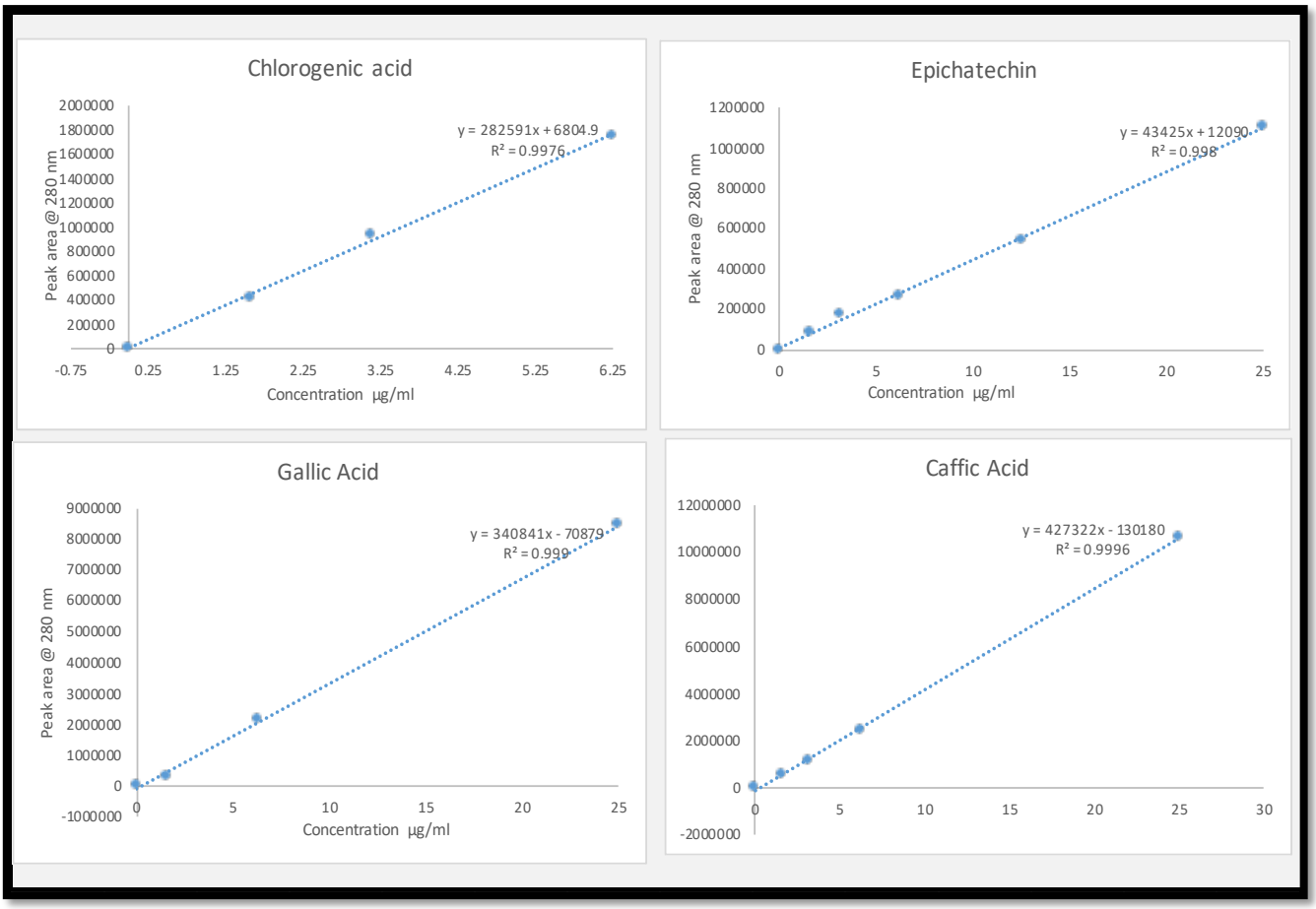
(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Barhi	Khassab	-205.59000*	9.62747	.000	-236.4205	-174.7595
	Barhi + yoghurt	-27.99000	9.62747	.076	-58.8205	2.8405
	Khassab + yoghurt	-43.59333*	9.62747	.008	-74.4239	-12.7628
Khassab	Barhi	205.59000*	9.62747	.000	174.7595	236.4205
	Barhi + yoghurt	177.60000*	9.62747	.000	146.7695	208.4305
	Khassab + yoghurt	161.99667*	9.62747	.000	131.1661	192.8272
Barhi + yoghurt	Barhi	27.99000	9.62747	.076	-2.8405	58.8205
	Khassab	-177.60000*	9.62747	.000	-208.4305	-146.7695
	Khassab + yoghurt	-15.60333	9.62747	.420	-46.4339	15.2272
Khassab + yoghurt	Barhi	43.59333*	9.62747	.008	12.7628	74.4239
	Khassab	-161.99667*	9.62747	.000	-192.8272	-131.1661
	Barhi + yoghurt	15.60333	9.62747	.420	-15.2272	46.4339

*. The mean difference is significant at the 0.05 level.

Appendix I. Chromatogram for date samples



Appendix J. Calibration curves for standards used in the HPLC analysis.



Appendix K. Study Protocol registration on Clinical trial

ClinicalTrials.gov PRS
Protocol Registration and Results System

ClinicalTrials.gov Protocol Registration and Results System (PRS) Receipt
Release Date: September 21, 2018

ClinicalTrials.gov ID: NCT03350100

Study Identification

Unique Protocol ID:	DFCPM1
Brief Title:	The Effect of Date Fruit on Mood and Cognition in Healthy Adults
Official Title:	An Investigation Into the Acute Effects of Date Fruit (Phoenix Dactylifera L.) on Mood and Cognitive Performance in Healthy Volunteers.
Secondary IDs:	

Study Status

Record Verification:	September 2018
Overall Status:	Completed
Study Start:	October 31, 2017 [Actual]
Primary Completion:	March 29, 2018 [Actual]
Study Completion:	April 1, 2018 [Actual]

Sponsor/Collaborators

Sponsor:	Newcastle University
Responsible Party:	Sponsor
Collaborators:	

Oversight

U.S. FDA-regulated Drug:	No
U.S. FDA-regulated Device:	No
U.S. FDA IND/IDE:	No
Human Subjects Review:	Board Status: Approved Approval Number: 1408/118/2017 Board Name: FMS Faculty Ethics Commitee Board Affiliation: Newcastle university Phone: 0191 208 5633 Email: Kimberley.Sutherland@newcastle.ac.uk Address: Kimberley Sutherland Faculty Support Assistant

Research & Innovation Office

Faculty of Medical Sciences

Newcastle University

Tel: 0191 208 5633

Data Monitoring:

Study Description

Brief Summary: A number of studies have considered the neuroprotective effects of date fruit on neurodegenerative diseases in animals. However, so far no study has addressed the acute effects of date fruit on mood and cognitive performance in humans. This study will investigate the acute effects of two different cultivars of Saudi dates on mood and cognitive performance into healthy volunteers.

This study will follow a double blind, randomised, placebo controlled, repeated measures, cross over design with two active treatment arms versus placebo. Treatment orders will be counterbalanced with the use of a Latin Square design.

Thirty six healthy participants aged between 18 and 35 will be recruited. Participants will be required to undergo a screening/training visit, followed by three measurement visits at weekly intervals. The trial will last for 3 months in total.

Detailed Description: Introduction Fruit of the date palm (P. dactylifera) may be considered an emerging and potential candidate for the development of health-promoting foods owing to its high nutritive values (Juhaimi, Ghafoor et al. 2012). Recently, scientific studies have revealed the medicinal properties of P. dactylifera in the different ripening stages by examining anti-inflammatory, anti-angiogenic and antibacterial activity. The anti-inflammatory activity of various parts of P. dactylifera have been evaluated (Shabani, Zangiabadi et al. 2013); it is evident from these studies that, both in vivo and in vitro, date fruits display anti-inflammatory activity, strongly linked to secondary metabolites and antioxidant behaviour.

More than 16 published studies have investigated the physiological and the psychological effects of P. dactylifera on the brain using animal models. Although this domain of research is in its infancy , the findings are quite promising and suggest that date fruits have the potential to become, on the one hand, a neuroprotective agent in ischemic stroke (Majid, Marzieh et al. 2008), brain damage (Kalantaripour, Asadi-Shekaari et al. 2012), and Alzheimer's disease and (Essa, Akbar et al. 2016), on the other hand, a brain enhancer by attenuating impairment in cognition caused by neurodegenerative diseases (Subash, Essa et al. 2015).

Aim The aim of this study is to investigate the acute effects of two different cultivars of P. dactylifera commonly consumed in Saudi Arabia on mood and cognitive performance of healthy volunteers.

Design The project will investigate the acute effects on human cognitive function and mood after the administration of a single dose of two different date fruits using a yogurt as the carrier medium against a control yoghurt matched for sugar, flavour, volume, fibers, taste and appearance. The study will follow a double-blind, counterbalanced, placebo controlled, repeated measures design, with two active treatment arms versus placebo, with one week between each measurement visit. Treatment orders will be counterbalanced with the use of a Latin Square design (Williams 1949). Participants will be randomly allocated to a treatment order as selected through a Williams Latin Square.

Participants will be required to undergo a screening/training visit, followed by three measurement visits at weekly intervals. The trial will last for 3 months in total, which is about 2-3 weeks for each participants.

Treatment Five different cultivars of date fruit were initially considered and screened for testing against cognitive performance and mood according to the following criteria; popularity, representation of the different maturation stages, and representation of the different colours, which has a great an effect on the total phenolic content. Therefore from the Bisir maturation stage Khasab and Birhi dates were chosen, from the Rutab maturation stage Sukari dates and Ajwah and Khalas dates were chosen from the Tamer maturation stage.

After a chemical analysis of polyphenol content of the five different cultivars, the study was narrowed down to two different cultivars of date fruits: Khasab and Birhi. According to our initial analysis Khasb and Birhi cultivars contained the highest amount of polyphenols, and while the total sugar in all varieties was similar it was lower in these two varieties compared with the others tested.

Taking the stickiness of the fruit and the high content of fibres, a total of 115g of date powder will be mixed into one portion of low fat yogurt (e.g. Yeo yogurt 150 g BigFish® which has 240kJ/56kcal), with consideration to the wet weight variation prior to freeze drying process. The naturally occurring sugars in the date will be quantified. The total volume of the treatment yogurt will be up to 265 mg. The control yogurt will be a placebo mix containing glucose, fructose, sucrose, fibres and food dye in order to be closely matched for volume, taste, appearance, texture, and energy with the treatment. Yogurts will be coded and prepared freshly each morning by a third party who shall not take further part in the running of the study. No member of the investigation team will be aware of the coding of the drinks until a blind-data review is completed. Both the treatments and the placebo will be provided to the participants in plastic cups of similar appearance.

Participants A total of 36 healthy participants aged between 18 and 35 will be recruited through advertisement via poster and flyer. All participants will be required to undergo a screening visit.

Study procedures:

1. Screening All participants will be screened for any contraindications prior to the beginning of the study. The eligible participants will then be asked to complete the Cog-Track Battery four times to remove practice effects. On each of the study days, cognitive assessments will be conducted at baseline, 45 min post dose, 90 min post dose and 135 min post dose. After completing each cognitive assessment, participants will be asked to complete Visual Analogue Scales to assess their mood status.
2. Study day The volunteers will be briefed about the study protocol and will sign a written informed consent (see appendix). The participants will arrive at the NU-Food research center at about 9:30 am after a 12 hour overnight fast. Measurements of height and weight will be taken again in order to track any change, and this will be followed by a completion of the baseline Cog-Track Battery, Bond-Lader Visual Analogue Scales, Profile of mood states questionnaire and finger-prick blood glucose test. The participants will then consume their treatment for that day (as allocated by the Williams Latin square) and be asked to sit quietly (participants may work quietly, read or watch TV as they wish). They will be asked to start the second period of cognitive performance tasks 45 min after treatment administration to coincide with another blood glucose test. The same tasks (cognition and blood glucose) will be collected again at 90 min and 135 min post dose. Each of the three study visits will last approximately 3 hours. Participants will be required to stay in the research center during breaks between each test/blood sample. Participants will have access to a comfortable waiting room. At the

end of the testing day all participants will receive a standard meal (A sandwich and a drink). (A detailed scheduling for screening and study days can be found in appendixes).

Conditions

Conditions: Mood and Cognitive Performance

Keywords:

Study Design

Study Type: Interventional

Primary Purpose: Basic Science

Study Phase: N/A

Interventional Study Model: Crossover Assignment

Number of Arms: 3

Masking: Double (Participant, Investigator)

Allocation: Randomized

Enrollment: 36 [Actual]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Birhi date cultivar A 48.46 g of freeze dried powder of Birhi date Cultivar which is equivalent to a 115g of fresh dates, contains 14.72 g of total sugar in which 4.6 g Glucose, 2.49 fructose and 3.4 sucrose, and a total phenolic content of 162.8 mg/100 g of GAE. and 0.80 g of fibres, will be mixed into one portion of low fat yogurt (e.g. Yeo yogurt 150 g BigFish®, which gives a total energy of 299 KJ.	Dietary Supplement: Acute effect of two Saudi cultivars of date fruit on mood and cognitive performance Acute effect of two Saudi cultivars of date fruit on mood and cognitive performance at baseline, 45 post-dose, 90 min post-dose and 135 min post-dose
Experimental: Khassab date cultivar A 34.5 g of freeze dried powder of Khassab date Cultivar which is equivalent to A 115g of fresh dates, contains 14.72 g of total sugar in which 4.6 g Glucose, 2.49 fructose and 3.4 sucrose, and a total phenolic content of 91.52 mg/100 g of GAE. and 0.80 g of fibres,will be mixed into one portion of low fat yogurt (e.g. Yeo yogurt 150 g BigFish®, which gives a total energy of 299 KJ.	Dietary Supplement: Acute effect of two Saudi cultivars of date fruit on mood and cognitive performance Acute effect of two Saudi cultivars of date fruit on mood and cognitive performance at baseline, 45 post-dose, 90 min post-dose and 135 min post-dose
Placebo Comparator: placebo A 14.72 g of total sugar in which 4.6 g Glucose, 2.49 fructose and 3.4 sucrose, and 0.80 g of fibres, will be mixed into one portion of low fat yogurt (e.g. Yeo yogurt 150 g BigFish®, which gives a total energy of 299 KJ.	Dietary Supplement: Acute effect of two Saudi cultivars of date fruit on mood and cognitive performance Acute effect of two Saudi cultivars of date fruit on mood and cognitive performance at baseline, 45 post-dose, 90 min post-dose and 135 min post-dose

Outcome Measures

Primary Outcome Measure:

1. cognition: Simple Reaction Time
   Cog-track an online set of nine cognitive tests (www.wesnes.com).

   [Time Frame: Change from baseline, 45 min pos-dose, 90 min post-dose and 135 postdose]
2. Cognition: Digit Vigilance
   Cog-track an online set of nine cognitive tests (www.wesnes.com).

   [Time Frame: Change from baseline, 45 min pos-dose, 90 min post-dose and 135 postdose]
3. Cognition: Choice Reaction Time
   Cog-track an online set of nine cognitive tests (www.wesnes.com).

   [Time Frame: Change from baseline, 45 min pos-dose, 90 min post-dose and 135 postdose]
4. Cognition: Numeric Working Memory
   Cog-track an online set of nine cognitive tests (www.wesnes.com).

   [Time Frame: Change from baseline, 45 min pos-dose, 90 min post-dose and 135 postdose]
5. Cognition: Spatial Working Memory
   Cog-track an online set of nine cognitive tests (www.wesnes.com).

   [Time Frame: Change from baseline, 45 min pos-dose, 90 min post-dose and 135 postdose]
6. Cognition: Immediate Word Recall
   Cog-track an online set of nine cognitive tests (www.wesnes.com).

   [Time Frame: Change from baseline, 45 min pos-dose, 90 min post-dose and 135 postdose]
7. Cognition:Delayed Word Recall
   Cog-track an online set of nine cognitive tests (www.wesnes.com).

   [Time Frame: Change from baseline, 45 min pos-dose, 90 min post-dose and 135 postdose]
8. Cognition:Word Recognition
   Cog-track an online set of nine cognitive tests (www.wesnes.com).

   [Time Frame: Change from baseline, 45 min pos-dose, 90 min post-dose and 135 postdose]
9. Cognition:Pattern Separation
   Cog-track an online set of nine cognitive tests (www.wesnes.com).

   [Time Frame: Change from baseline, 45 min pos-dose, 90 min post-dose and 135 postdose]
10. Mood scales Bond Lader
    The Bond and Lader Visual Analogue Scales (1974)

    [Time Frame: Change from baseline, 45 min pos-dose, 90 min post-dose and 135 postdose]
11. The Profile of Mood States (POMS)
    The Profile of Mood States (POMS; McNair et al., 1992)

    [Time Frame: Change from visit 1 to visit 2 to visit 3]
12. Blood Glucose level test
    Finger prick

    [Time Frame: Change from baseline, 45 min pos-dose, 90 min post-dose and 135 postdose]

Eligibility

Minimum Age:	18 Years
Maximum Age:	35 Years
Sex:	All
Gender Based:	

Accepts Healthy Volunteers: Yes

Criteria: Inclusion Criteria:

A total of 36 healthy participants aged between 18 and 35 will be recruited through advertisement via poster and flyer. All participants will be required to undergo a screening visit.

Exclusion Criteria:

Healthy participants aged 18-35 with a BMI >18 <36 will be recruited from the Newcastle Upon-Tyne area

Participants will be considered ineligible to participate in the study if they meet any of the following criteria:

1. They have a BMI above 35kg/m2 or lower than 18kg/m2
2. They smoke or consume tobacco products
3. They are taking any illicit or prescribed drugs
4. They are using dietary supplements, over the counter medicine or recreational drugs
5. They have a history of or currently abuse alcohol
6. They have a history of dyslexia, ADHD, learning difficulties or color blindness
7. They are females who are pregnant, seeking to become pregnant or do not use
8. They have allergies to any food products.
9. They have any metabolic diseases such as type 1 or type 2 diabetes.

Contacts/Locations

Central Contact Person: Prf. Chris Seal, Prof
Telephone: +44 (0) 191 208 7650
Email: Chris.seal@ncl.ac.uk

Central Contact Backup: Dr. Anthony Watson, Phd
Telephone: 01912086935
Email: Anthony.watson@ncl.ac.uk

Study Officials:

Locations: **United Kingdom**
NU-Food Research Facility
Newcastle upon Tyne, Tyne And Wear, United Kingdom, NE1 7RU
Contact: Duaa Altuwairki, PhD student 07402033360
d.altuwairki2@ncl.ac.uk
Contact: Dr Anthony Watson, PhD

IPDSharing

Plan to Share IPD:

References

Citations:

Links:

Available IPD/Information:	

Appendix L. Study Ethical approval

Newcastle University

Duaa Altuwairki
Institute of Cellular Medicine

Faculty of Medical Sciences
Newcastle University
The Medical School
Framlington Place
Newcastle upon Tyne
NE2 4HH United Kingdom

FACULTY OF MEDICAL SCIENCES: ETHICS COMMITTEE

Dear Duaa,

Title: Assessing the acute Effect of two different cultivars of date fruit on Cognitive function and mood.
Application No: 1408/118/2017
Start date to end date: 01/10/2017 to 20/12/2017

As you are providing expertise and not directly delivering the work overseas Newcastle University will accept the approvals that are already in place at the University of Cape Town.

On behalf of the Faculty of Medical Sciences Ethics Committee, I am writing to confirm that the ethical aspects of your proposal have been considered and your study has been given ethical approval.

The approval is limited to this project: **1408/118/2017**.

During the course of your research project you may find it necessary to revise your protocol. Substantial changes in methodology, or changes that impact on the interface between the researcher and the participants must be considered by the FMS Ethics Committee, prior to implementation.*

At the close of your research project, please report any adverse events that have occurred and the actions that were taken to the FMS Ethics Committee.*

Best wishes,

Yours sincerely

Kimberley Sutherland
On behalf of Faculty Ethics Committee

cc.
Professor Daniel Nettle, Chair of FMS Ethics Committee
Mrs Kay Howes, Research Manager

*Please refer to the latest guidance available on the internal Newcastle web-site.

tel: +44 (0) 191 208 6000
fax: +44 (0) 191 208 6621

www.ncl.ac.uk
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Appendix M. Participant Information Sheet

Effect of date fruit extract consumption on mood and cognitive performance on healthy volunteers

Information Sheet for Participants

Investigator: Duaa Altuwairki

Supervisors:

Pro. Chris Seal

Dr. Anthony Watson

Newcastle University

School of Faculty of Medical Sciences

ICM

Newcastle upon Tyne

NE1 7RU

For further information please contact:

Email: D.altuwairki2@ncl.ac.uk

Telephone: Researcher 07402033360

http:www.ncl.ac.uk/hnrc

Participation Information Sheet Version Duaa Altuwairki (PGR)

04/09/2017

You are being invited to take part in a research study. Before you decide to take part it is important you understand why the research is being conducted, and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this.

What is the purpose of this study?

Date fruit is considered as functional food due to its high nutritional value and possible properties against neurodegenrative diseases and for improving brain functions. There are some animal studies to suggest an important class of compounds like polyphenols, found naturally in date fruit, have beneficial effects upon brain health.

However, the effects of date fruit on humans have not been investigated. This study will investigate the effects of Date fruit on mood and cognitive performance on healthy volunteers.

Why have I been chosen?

We are looking for young volunteers who are non-smokers, not over the age of 35 years, if they are females they should not be pregnant, or seeking to become pregnant or do not use birth control, have no history of, or currently abuse alcohol, have no allergies to any food products, have a BMI belwo 35kg/m^2 and using no dietary supplements, over the counter medicine or recreational drugs, have no history of any metabolic diseases such as type 1 or type 2 diabetes to take part in this study. We will be recruiting 36 volunteers in total from the Newcastle Upon Tyne area.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be asked to sign a consent form on your 'Screening Visit'; you will be given a copy of this to keep.

If you decide to take part and you are a suitable volunteer for the study, we will ask you to sign a full consent form. However, you will be free to withdraw from the study without giving a reason anytime up to the end of your final visit. Shortly after this, all data will be fully annonymised, and therefore, from this point forward it will not be possible to withdraw any data from the study.

What will happen to me if I take part?

If you agree to take part we will ask you to visit **the NU-Food research facility, Newcastle University** on four occasions. The first visit is a screening visit to assess your suitability for the project. We will ask you a series of questions to ensure you`re ok to take part, you will also have the opportunity to discuss the project with the research team. If you are suitable, there will be three further visits. At each visit, you need to come at 10 am to the **NU-Food research facility, Newcastle University**, and you will complete a series of computer programs which will assess your mood and cognitive performance. This will be conducted 4 times per visit. One time at the basilane followed by the treatment of the day and then repeat the computerised assessment at 45 min. 90 min. and 135 min post consumption of the study foods. Each visit will last approximately two hours and 30 minutes. Also your blood glocose will be assessed four times, which will coincided with the cognitive assessments, in each visit using the finger prick method. Finger prick methond is a procedure that helps to assess the blood glucose level with only a drop of blood. The procedure is basically about; Grasping one of your fingers, quickly and gentily puncture with a sterile lancet. Then a drop of your blood that should be formed at the puncture site will be applied to the analysis strip. Then we will provide you with an adhesive sticker to apply on the punctured fingurer.

What else do I have to do?

If you agree to take part, we will ask you to make short visits to NU-Food, Newcastle University, on four occasions. If you are suitable, The first visit is a screening visit to assess your suitability for the project. On the evening before these 4 visits, you will need to fast from 10 pm; this means that you should not eat or drink anything except water until you complete your visit the following morning.

Finally, we will ask you to avoid dark fruit and dark fruit juices the day before the study day.

What will happen to the samples I provide?

Only a few drops of your blood will be collected for the purpose of blood glucose level analysis.

What are the possible disadvantages and risks of taking part?

Taking blood samples may cause minor discomfort and there is a small chance of minor bruising afterwards.

What are the possible benefits of taking part?

Although you will derive no individual benefit, the knowledge gained from this study will help our research into identifying the effects of date fruit on cognitive pefromance.

What will happen if anything goes wrong?

Any complaints you have about this study should be made to Dr Anthony Watson, Newcastle University (Anthony.Watson@ncl.ac.uk or 0191-2087650) and will be fully investigated.

Will my taking part in this study be kept confidential?

Any information which is collected about you during the course of the research will be kept strictly confidential. Your GP will be notified that you are participating in this study.

What will happen to the study results?

We will publish the results of the study in a scientific journal and on the project website. You will not be personally identified in any publications. We will be happy to discuss the overall results with you when the study is completed, and will let you know where you can obtain a copy of the published results if you wish.

Will I be reimbursed for my time?

In recognition of your time commitment, you will be paid an honorarium of £20 in the form of Eldon Square vouchers at the completion of the study.

Contact for further information

If you would like any further information about this study, please do not hesitate to contact **Altuwairki Duaa**

Telephone: 07402033360
Email:D.altuwairki2@ncl.ac.uk

And finally…

Thank you for having taken the time to read this information sheet and for your interest in the study.

Appendix N. Study case report form

Newcastle University

nu-food
Food & Consumer Research Facility

Participant DFCPM1		

Participants initials:

Study code: DFCPM1

<The Effect of Date Fruit on Mood and Cognition in Healthy Adults>

CASE REPORT FORM

Trial Sponsor: NEWCASTLE UNIVERSITY

Final Version Date: 22/08/2017

CRF completion Instructions

When completing the CRF please ensure:

- Black ink should be used.
- Each section is completed fully.
- Any corrections made to any data in the CRF are initialled and dated.
- The consent form is signed, dated and the name of signatory is clearly printed by all parties.
- The date of consent recorded in the CRF is the date the Participant signs the consent form.

Taking Consent

Participant must be eligible and have given consent before entering the study.

For the Participant to give consent they must sign and date two original consent forms after they have completely read the Participant information sheet and have fully understood what the study entails. The Participant must clearly print their name on the consent and the date must be the date the Participant signs the form. No study procedures can occur prior to the Participant signing the consent form.

As well as the Participant signing the consent form, it is necessary for the person explaining the study to the Participant to sign the consent form. By signing the consent form the person explaining the study confirms that they have witnessed the Participant give consent and that the Participant fully understands what the study entails.

Please ensure that two copies of the consent form are signed – one copy to be given to the Participant, one copy to remain at NUFood Research (this copy should be stored in a locked filing cabinet, separate from all participant data).

VISIT 0 - SCREENING

Date: D D M M Y Y Y Y

Participant initials:

Participant number:

PARTICIPANT ELIGIBILITY CHECKLIST - TRIAL ENTRY

Please tick:	YES	NO
Has the Participant given written informed consent?	☐	☐
Is the Participant:		
In good health?	☐	☐
Aged between 18 and 35 years?	☐	☐
Proficient in English equivalent to a native English speaker?	☐	☐
Orientated to person, place and time and has the ability to communicate with study staff?	☐	☐
Motivated to participate in and complete the study as instructed and to attend visit in a well-rested state?	☐	☐
Does the Participant:		
Intend to comply with the study tobacco restriction?	☐	☐
Intend to comply with the study caffeine restriction?	☐	☐
Intend to comply with the study alcohol restriction?	☐	☐
Intend to comply with restriction of dietary/ supplement intake?	☐	☐
Intend to comply with the 12 hour fasting restriction prior to each study session?	☐	☐
Have normal or corrected-to-normal vision?	☐	☐

If NO the Participant is ineligible for the trial. Please only complete pages S1 – S5.

VISIT 0 - SCREENING Date: D D M M Y Y Y Y

Participant initials:
Participant number:

	Please tick: YES	NO
Is the Participant:		
Pregnant or seeking to become pregnant?	☐	☐
Does the Participant:		
Have a history of metabolic diseases like type 1 or type 2 diabetes?	☐	☐
Currently take a pharmaceutical product/medicine (except contraception?)	☐	☐
Have any known allergies or intolerance to any ingredients in the study preparation?	☐	☐
Have any serious disorder that might interfere with the Participation in the test?	☐	☐
Have a Body Mass Index (BMI) above 40 kg/m^2 (severely obese)?	☐	☐
Smoke or consume any tobacco products (even occasionally)?	☐	☐
Currently abuse drugs or alcohol?	☐	☐
Have (or have a history of) head trauma, migraines, gastric problems, learning difficulties, dyslexia, colour blindness or ADHD?	☐	☐
Do you have allergies to **ANY** food product?	☐	☐

If YES the Participant is ineligible for the trial.
At Screening visit, please only complete pages S1 – S5.

VISIT 0 - SCREENING Date: D D M M Y Y Y Y

Participant initials:
Participant number:

DOCUMENTATION OF INFORMED CONSENT

IMPORTANT: Informed consent must be obtained from the Participant BEFORE any trial procedures are started.

Has the Participant's written informed consent been obtained?

Yes ☐
No ☐ (Please tick)

If NO: The Participant is not eligible for the trial

If YES: Keep the site consent form with the Participant's notes

Date of Consent: D D M M Y Y Y Y

Is the Participant eligible for this trial?

Yes - Eligible ☐
No - Screen failure ☐ (Please tick)

If NO: please state main reason:

Fails to meet inclusion / exclusion criteria ☐
Participant has withdrawn consent ☐ (Please tick)

RR's signature

Date: D D M M Y Y Y Y

VISIT 0 - SCREENING

Date: D D M M Y Y Y Y

Participant initials:

Participant number:

If the Participant does not satisfy ALL of the eligibility criteria or has withdrawn consent then please only complete pages S1 – S5. If the Participant satisfies all of the eligibility criteria and has provided appropriate consent please proceed.

VISIT 0 - SCREENING Date: D D M M Y Y Y Y

Participant initials:
Participant number:

PARTICIPANT DEMOGRAPHICS

Date of Birth: D D M M Y Y Y Y

Age: Years_____ Months_____

Sex: male (M) ☐ or female (F) ☐

Race:
- Maori ☐
- Black ☐
- Oriental ☐
- Caucasian ☐
- Other ☐ please specify _____

Does the Participant require glasses/contact lenses to use a computer?

YES ☐
NO ☐
(Please tick)

Which hand does the Participant use to write with? RIGHT ☐ LEFT ☐

Is the participant vegetarian? Yes ☐ No ☐

How many portions of fruit and vegetables does the participant eat in a typical day? [Portion= one piece of fruit, a handful of vegetables or a glass of fresh fruit juice (each additional glass of juice does not count as extra)]

_____ Portion (s).

How many years of full time education has the participant had? _____

What is the highest level of qualification achieved?_____

Height ☐.☐☐ m

Weight ☐☐☐.☐☐ kg

BMI ☐☐.☐ kg/m^2

Blood Pressure

Systolic ☐☐☐

Diastolic ☐☐☐

Heart Rate ☐☐☐ BPM

What was the Participant's blood glucose level?

☐☐ Mmol/L

CONCOMITANT MEDICATION:

Is the Participant receiving any concomitant medications, therapies and/or vitamin supplementation?

Yes ☐

No ☐

(Please tick)

If YES: Please complete the concomitant medication record on page 17.

MEDICAL HISTORY (Within the past 5 years)

Specify Diagnosis	1 = Past 2 = Present	Severity 1 = mild 2 = moderate 3 = severe	Concomitant Treatment 1 = Yes * 2 = No	Details
1.				
2.				
3.				
4.				
5.				
6.				
7.				
8.				
9.				
10.				

* If Yes please complete concomitant medication record on page 17.

Please note that the volunteer may not eligible to participate if taking or intending to take any prescription pharmaceutical product during the study (except for contraception for females and some topically applied therapeutic agents). Please refer to the protocol for the specific guidelines for the study.

Participant initials: ☐☐☐
Cog-track Number: ☐☐☐
Participant Number: ☐☐☐

STUDY DAY 1 Date: D D M M Y Y Y Y ☐☐☐☐☐☐☐☐

Study Day 1

Participant number allocated: ☐☐☐

Have there been any changes to the subject's concomitant medications, therapies and/or vitamin supplementation since their last visit?

No ☐ Yes ☐ (if Yes complete concomitant medication record)

Has the participant experienced any adverse events (illness) since the last visit?

No ☐ Yes ☐ (if Yes complete adverse event record)

Has the participant fasted from 10pm yesterday? (If No re-schedule their study day)

No ☐ Yes ☐

Has participant confirmed they have consumed no caffeine or alcohol since 10pm yesterday? (If No re-schedule their study day)

No ☐ Yes ☐

Baseline

Samples

What were the Participant's blood pressure and heart rate?

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

What was the Participant's blood glucose level?

☐☐ Mmol/L

Mood Assessment (Please tick when completed)

The Profile of Mood States (POMS) ☐

Participant initials: ☐☐☐
Cog-track Number: ☐☐☐
Participant Number: ☐☐☐

STUDY DAY 1 Date: D D M M Y Y Y Y ☐☐☐☐☐☐☐☐

Baseline Cognitive Tasks:

Mood Scales ☐
Digital Vigilance ☐
Stroop ☐
RVIP ☐
Logical Reasoning ☐

Treatment: Treatment taken ☐ Time taken _____

Post dose Blood Glucose, Blood Pressure and Heart Rate (45 minutes post treatment)

Blood Pressure

Systolic ☐☐☐ MM Hg
Diastolic ☐☐☐ MM Hg
Heart Rate ☐☐☐ BPM

What was the Participant's blood glucose level?

☐☐ Mmol/L

Post Dose Cognitive Tasks (45 minutes post treatment):

Mood Scales ☐
Digital Vigilance ☐
Stroop ☐
RVIP ☐
Logical Reasoning ☐

What were the Participant's blood pressure and heart rate?
Blood Pressure

Systolic ☐☐☐ MM Hg
Diastolic ☐☐☐ MM Hg

Participant initials: ☐☐☐
Cog-track Number: ☐☐☐
Participant Number: ☐☐☐

STUDY DAY 1 Date: D D M M Y Y Y Y ☐☐☐☐☐☐☐☐

Heart Rate ☐☐☐ BPM

What was the Participant's blood glucose level?

☐☐ Mmol/L

Post Dose Cognitive Tasks (90 minutes post treatment):

Mood Scales ☐
Digital Vigilance ☐
Stroop ☐
RVIP ☐
Logical Reasoning ☐

What were the Participant's blood pressure and heart rate?

Blood Pressure

Systolic ☐☐☐ MM Hg
Diastolic ☐☐☐ MM Hg
Heart Rate ☐☐☐ BPM

What was the Participant's blood glucose level?

☐☐ **Mmol/L**

Post Dose Cognitive Tasks (135 minutes post treatment):

Mood Scales ☐
Digital Vigilance ☐
Stroop ☐
RVIP ☐
Logical Reasoning ☐

Participant initials: | | |
Cog-track Number: | | |
Participant Number: | | |

STUDY DAY 1 Date: D D M M Y Y Y Y | | | | | | | | |

What were the Participant's blood pressure and heart rate?

Blood Pressure

Systolic | | | MM Hg

Diastolic | | | MM Hg

Heart Rate | | | BPM

What was the Participant's blood glucose level?

| | **Mmol/L**

RR's signature

Date: D D M M Y Y Y Y | | | | | | | | |

Participant initials: | | |
Compass Number: | | |
Participant Number: | | |

STUDY DAY 2 Date: D D M M Y Y Y Y | | | | | | | | |

Study Day 2

Have there been any changes to the subject's concomitant medications, therapies and/or vitamin supplementation since their last visit?

No ☐ Yes ☐ (if Yes complete concomitant medication record)

Has the participant experienced any adverse events (illness) since the last visit?

No ☐ Yes ☐ (if Yes complete adverse event record)

Has the participant fasted from 10pm yesterday? (If No re-schedule their study day)

No ☐ Yes ☐

Has participant confirmed they have consumed no caffeine or alcohol since 10pm yesterday? (If No re-schedule their study day)

No ☐ Yes ☐

Baseline

Samples

What were the Participant's blood pressure and heart rate?

Blood Pressure

Systolic | | | | MM Hg

Diastolic | | | | MM Hg

Heart Rate | | | | BPM

What was the Participant's blood glucose level?

| | | Mmol/L

Mood Assessment (Please tick when completed)

The Profile of Mood States (POMS) ☐

Participant initials: ☐☐☐
Compass Number: ☐☐☐
Participant Number: ☐☐☐

STUDY DAY 2 Date: D D M M Y Y Y Y ☐☐☐☐☐☐☐☐

Baseline Cognitive Tasks:

Mood Scales ☐
Digital Vigilance ☐
Stroop ☐
RVIP ☐
Logical Reasoning ☐

Treatment: Treatment taken ☐ Time taken _____

Post dose Blood Glucose, Blood Pressure and Heart Rate (45 minutes post treatment)

Blood Pressure

Systolic ☐☐☐ MM Hg
Diastolic ☐☐☐ MM Hg
Heart Rate ☐☐☐ BPM

What was the Participant's blood glucose level?

☐☐ Mmol/L

Post Dose Cognitive Tasks (45 minutes post treatment):

Mood Scales ☐
Digital Vigilance ☐
Stroop ☐
RVIP ☐
Logical Reasoning ☐

What were the Participant's blood pressure and heart rate?

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ___ MM Hg

Heart Rate ___ BPM

What was the Participant's blood glucose level?

___ Mmol/L

Post Dose Cognitive Tasks (90 minutes post treatment):

Mood Scales ☐

Digital Vigilance ☐

Stroop ☐

RVIP ☐

Logical Reasoning ☐

What were the Participant's blood pressure and heart rate?

Blood Pressure

Systolic ___ MM Hg

Diastolic ___ MM Hg

Heart Rate ___ BPM

What was the Participant's blood glucose level?

___ **Mmol/L**

Post Dose Cognitive Tasks (135 minutes post treatment):

Mood Scales ☐

Digital Vigilance ☐

Stroop ☐

RVIP ☐

Participant initials: ☐☐☐
Compass Number: ☐☐☐
Participant Number: ☐☐☐

STUDY DAY 2 Date: D D M M Y Y Y Y ☐☐☐☐☐☐☐☐

Logical Reasoning ☐

What were the Participant's blood pressure and heart rate?

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

What was the Participant's blood glucose level?

☐☐ **Mmol/L**

RR's signature _____ Date: D D M M Y Y Y Y ☐☐☐☐☐☐☐☐

Study Day 3: Date: D D M M Y Y Y Y

Participant initials:
Participant number:

Study Day 3

Have there been any changes to the subject's concomitant medications, therapies and/or vitamin supplementation since their last visit?

No ☐ Yes ☐ (if Yes complete concomitant medication record)

Has the participant experienced any adverse events (illness) since the last visit?

No ☐ Yes ☐ (if Yes complete adverse event record)

Has the participant fasted from 10pm yesterday? (If No re-schedule their study day)

No ☐ Yes ☐

Has participant confirmed they have consumed no caffeine or alcohol since 10pm yesterday? (If No re-schedule their study day)

No ☐ Yes ☐

Baseline

Samples

What were the Participant's blood pressure and heart rate?

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

What was the Participant's blood glucose level?

☐☐ Mmol/L

Mood Assessment (Please tick when completed)

The Profile of Mood States (POMS) ☐

Study Day 3: Date: D D M M Y Y Y Y

Participant initials: ☐☐☐
Participant number: ☐☐☐

Baseline Cognitive Tasks:

Mood Scales ☐
Digital Vigilance ☐
Stroop ☐
RVIP ☐
Logical Reasoning ☐

Treatment: Treatment taken ☐ Time taken _____

Post dose Blood Glucose, Blood Pressure and Heart Rate (45 minutes post treatment)

Blood Pressure

Systolic ☐☐☐ MM Hg
Diastolic ☐☐☐ MM Hg
Heart Rate ☐☐☐ BPM

What was the Participant's blood glucose level?

☐☐ Mmol/L

Post Dose Cognitive Tasks (45 minutes post treatment):

Mood Scales ☐
Digital Vigilance ☐
Stroop ☐
RVIP ☐
Logical Reasoning ☐

What were the Participant's blood pressure and heart rate?

Blood Pressure

Systolic ☐☐☐ MM Hg
Diastolic ☐☐☐ MM Hg

Study Day 3: Date: D D M M Y Y Y Y

Participant initials:
Participant number:

Heart Rate ☐☐☐ BPM

What was the Participant's blood glucose level?

☐☐ Mmol/L

Post Dose Cognitive Tasks (90 minutes post treatment):

Mood Scales ☐

Digital Vigilance ☐

Stroop ☐

RVIP ☐

Logical Reasoning ☐

What were the Participant's blood pressure and heart rate?

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

What was the Participant's blood glucose level?

☐☐ **Mmol/L**

Post Dose Cognitive Tasks (135 minutes post treatment):

Mood Scales ☐

Digital Vigilance ☐

Stroop ☐

RVIP ☐

Logical Reasoning ☐

Study Day 3:

Date: D D M M Y Y Y Y

Participant initials:

Participant number:

What were the Participant's blood pressure and heart rate?

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

What was the Participant's blood glucose level?

☐☐ **Mmol/L**

RR's signature

Date: D D M M Y Y Y Y

Study Day 3: Date: D D M M Y Y Y Y

Participant initials:
Participant number:

TRIAL OUTCOME (please tick appropriate box):

Completed trial: ☐

If trial not completed, please complete:

Date of subject withdrawal from the study: D D M M Y Y Y Y

Provide main reason for premature termination (one reason only):

adverse event	☐	withdrawn consent	☐
did not co-operate	☐	refused treatment	☐
administrative reason	☐	lost to follow-up	☐
protocol violation	☐	other	☐

if other please specify: _____

_____ Date: D D M M Y Y Y Y

Investigator's signature

CONCOMITANT MEDICATION RECORD

Date: D D M M Y Y Y Y

Participant initials:
Participant number:

Please complete the following information fully for any concomitant medication, therapies and/or vitamin supplementation:

*** If Indication is due to a new/worsening AE, please complete the AE form (pg 18).**

Concomitant Treatment (please use generic name)	Indication	Single Dose	Total Daily Dose	Units	Frequency (e.g. BID, PRN)	Route code	Start and Stop Dates DD MM YY
1.							Start: / /
							Stop: / /
2.							Start: / /
							Stop: / /
3.							Start: / /
							Stop: / /
4.							Start: / /
							Stop: / /
5.							Start: / /
							Stop: / /
6.							Start: / /
							Stop: / /
7.							Start: / /
							Stop: / /
8.							Start: / /
							Stop: / /
9.							Start: / /
							Stop: / /

ADVERSE EVENTS

Date: D D M M Y Y Y Y

Participant initials:
Participant number:

Adverse Events

Were there any Adverse Events? ☐ 1 NO ☐ 2 YES, please complete all sections below, cross appropriate number.

	if **yes***		Date	Severity	Relation to Study Drug	Action(s) Taken *(several statements are possible)*	Outcome of Event
Adverse Event, specify Please list ONE event per line.	**Serious** 1 No 2 Yes *	**Reason** *(several statements are possible)* 1. Results in Death 2. Life-threatening 5. Hospitalization – new / prolonged 6. Congenital anomaly/birth defect 10. Persistent or significant disability/incapacity 11. Important medical event	**Start / Stop** If ongoing update at next visit dd / mm / yy	1. Mild 2. Moderate 3. Severe	**Possible?** 1. No 2. Yes	1. None 2. Dose of study drug reduced 3. Study drug discontinued and restarted 4. Study drug discontinued permanently 5. Remedial drug therapy, specify on concomitant medication page 6. Other (specify below) 7. Infusion rate of study drug educed 8. Hospitalization required or prolonged	1. Resolved 2. Improved 3. Unchanged 4. Worsened 6. Death 7. Insufficient Follow-up
1.	1 2*	1 2 5 6 10 11	/ / / /	1 2 3	1 2	1 2 3 4 5 6 7 8	1 2 3 4 6 7
2.	1 2*	1 2 5 6 10 11	/ / / /	1 2 3	1 2	1 2 3 4 5 6 7 8	1 2 3 4 6 7
3.	1 2*	1 2 5 6 10 11	/ / / /	1 2 3	1 2	1 2 3 4 5 6 7 8	1 2 3 4 6 7
4.	1 2*	1 2 5 6 10 11	/ / / /	1 2 3	1 2	1 2 3 4 5 6 7 8	1 2 3 4 6 7

Further Details of Adverse Events: _____

D D M M Y Y Y Y

_____ Investigator's Signature Date: _____

Appendix O. Study consent form

nu-food
Food & Consumer Research Facility

Newcastle University

Date fruit cognitive performance and mood 1 (DFCPM1)

CONSENT BY VOLUNTEER TO PARTICIPATE IN A NUTRITIONAL STUDY

Participant ID_____

		Please initial box
1.	I confirm that I have read and understood the information sheet for the above study and have had the opportunity to ask questions.	☐
2.	I understand that my participation is voluntary and that I am free to withdraw at any time.	☐
3.	I understand that I need to give 4 blood samples during the trial	☐
4.	I confirm that I have no allergies or intolerances to any food or drink	☐
5.	I agree to take part in the trial	☐

Name of Volunteer *(Please print)*	Date	Signature
Name of Research Team Member (Please print)	Date	Signature

Appendix P. Study debrief

Newcastlee Universityy

Date fruit study Debrief

Dear Participant,

I would like to thank you for taking part in my trial which is attempting to investigate the acute effects of date fruit on mood and cognitive performance in healthy volunteers. Your participation means a lot to the research team, and this project would not have been possible to conduct without your great contribution.

You have now successfully completed the project. I would like to assure you that any data we collected during the course of the research has been anonymised and kept strictly confidential. This means when the results are published within a scientific journal or onto the project website you will not be able to be personally identified. However, should you wish to know overall results of the study, we will be able to refer you to where you can obtain a copy of the published results once they become available. A member of the research team would be happy to discuss any queries or questions you have at this time.

It was a pleasure to meet you all, and please accept both our gratitude and small reward as a gesture of goodwill for your participation.

Sincerely,

Duaa Altuwairki

A question **Participant number:**

Would you be able to tell us from your own prospective, in which visit do you think you were given the treatment that contained Dates?

1. *First visit*
2. *Second visit*
3. *Third visit*
4. *I don't know*

Thanks

Appendix Q. Multivariant analysis irrespective to treatments

Multivariate Tests

	Value	F	Hypothesis df	Error df	Sig.
Pillai's trace	.038	.525	22.000	594.000	.965
Wilks' lambda	.962	.524[a]	22.000	592.000	.965
Hotelling's trace	.039	.524	22.000	590.000	.965
Roy's largest root	.030	.800[b]	11.000	297.000	.640

Each F tests the multivariate effect of Rep. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

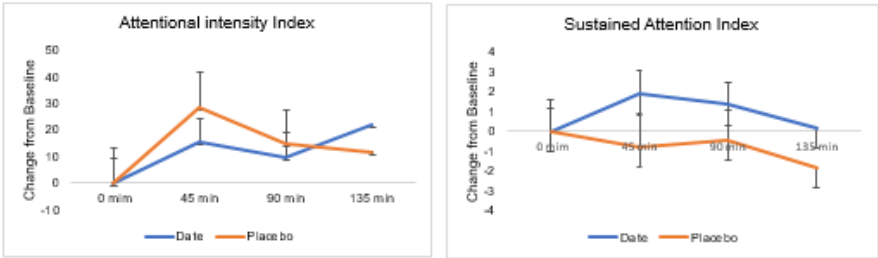
b. The statistic is an upper bound on F that yields a lower bound on the significance level.

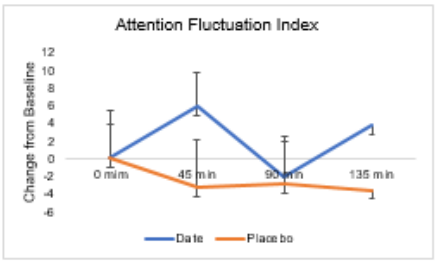
Univariate Tests

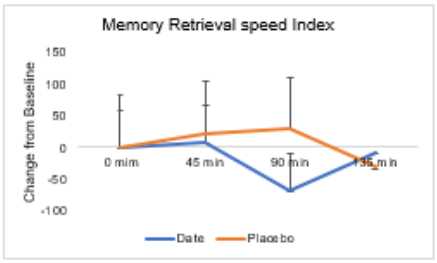
Dependent Variable		Sum of Squares	df	Mean Square	F	Sig.
Attentional intensity	Contrast	3693.813	2	1846.907	.324	.723
	Error	1743049.624	306	5696.241		
Sustained attention	Contrast	135.753	2	67.877	.763	.467
	Error	27228.847	306	88.983		
Attentional fluctuation	Contrast	1266.400	2	633.200	.600	.549
	Error	322847.618	306	1055.058		
Memory speed	Contrast	123801.485	2	61900.742	.260	.772
	Error	72967687.23	306	238456.494		
Cognitive	Contrast	4289.243	2	2144.622	.864	.423
	Error	759819.870	306	2483.071		
working	Contrast	19.864	2	9.932	.028	.973
	Error	110491.427	306	361.083		
Eoisodic	Contrast	2034.931	2	1017.465	.349	.705
	Error	890943.975	306	2911.582		
quality	Contrast	1945.857	2	972.929	.685	.505
	Error	434505.222	306	1419.952		
alert	Contrast	231.330	2	115.665	.563	.570
	Error	62814.220	306	205.275		
content	Contrast	101.341	2	50.671	.345	.709
	Error	44998.492	306	147.054		
calm	Contrast	108.725	2	54.363	.226	.798
	Error	73476.616	306	240.120		

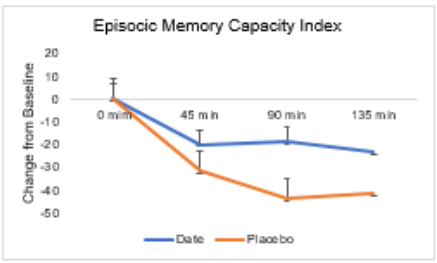
The F tests the effect of Rep. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

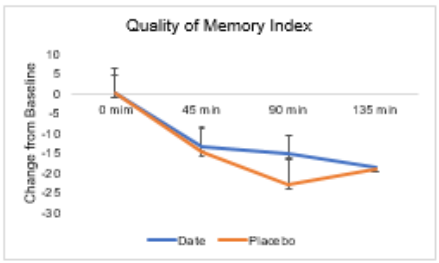
Appendix R. Graphical representation for the Date treatments together vs placebo

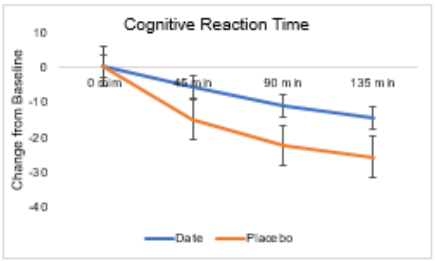


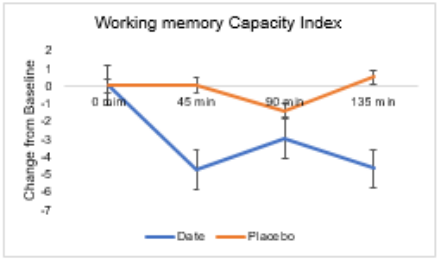


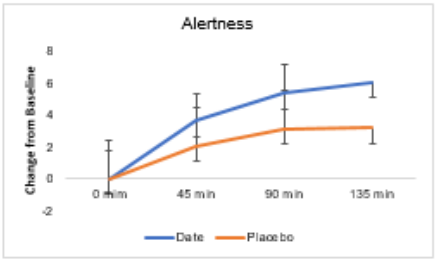


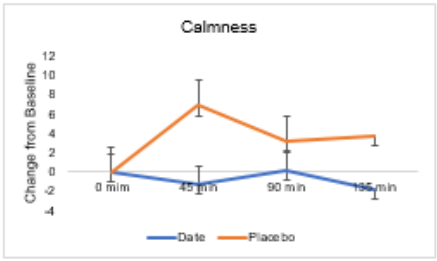




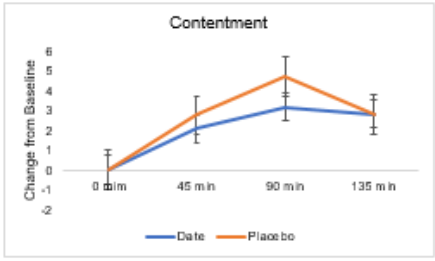








Date treatments data togather aginest placebo



Appendix S. Different approach to calculate the sample size retrospectively

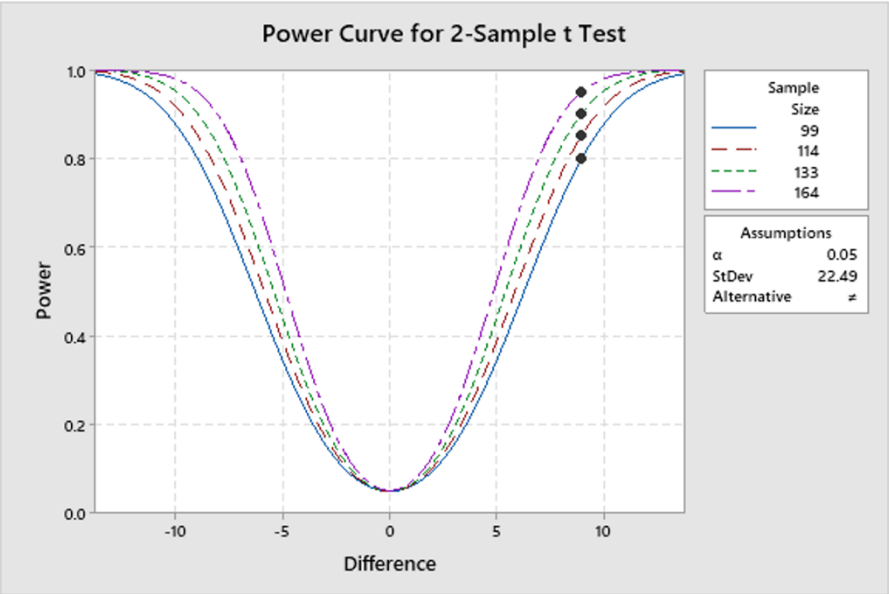
Power and Sample Size

2-Sample t Test
Testing mean 1 = mean 2 (versus ≠)
Calculating power for mean 1 = mean 2 + difference
α = 0.05 Assumed standard deviation = 22.49

Results

Difference	Sample Size	Target Power	Actual Power
9	133	0.90	0.901734
9	164	0.95	0.950845
9	114	0.85	0.852785
9	99	0.80	0.800027

The sample size is for each group.



Appendix T. ANOVA for Cycle 1 of HPLC

Oneway

[DataSet1] C:\Users\b3029524\OneDrive - Newcastle University\HPLC\6 SEEDS ANOVA.sav

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
TPC	Between Groups	27548238.58	5	5509647.716	89.258	.000
	Within Groups	740722.704	12	61726.892		
	Total	28288961.29	17			
GAE	Between Groups	36855.065	5	7371.013	28.789	.000
	Within Groups	3072.481	12	256.040		
	Total	39927.546	17			
CAE	Between Groups	17826.691	5	3565.338	1311.137	.000
	Within Groups	32.631	12	2.719		
	Total	17859.323	17			
EPE	Between Groups	27029471.31	5	5405894.262	85.507	.000
	Within Groups	758656.920	12	63221.410		
	Total	27788128.23	17			

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Date_seed_type	(J) Date_seed_type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
TPC	Barhi	Khassab	3396.04667*	202.85774	.000	2714.6636	4077.4298
		Ajwah	1874.01667*	202.85774	.000	1192.6336	2555.3998
		Majdool	3801.88333*	202.85774	.000	3120.5002	4483.2664
		Sukkari	1983.57333*	202.85774	.000	1302.1902	2664.9564
		Khalas	2693.22333*	202.85774	.000	2011.8402	3374.6064
	Khassab	Barhi	-3396.04667*	202.85774	.000	-4077.4298	-2714.6636
		Ajwah	-1522.03000*	202.85774	.000	-2203.4131	-840.6469
		Majdool	405.83667	202.85774	.395	-275.5464	1087.2198
		Sukkari	-1412.47333*	202.85774	.000	-2093.8564	-731.0902
		Khalas	-702.82333*	202.85774	.042	-1384.2064	-21.4402
	Ajwah	Barhi	-1874.01667*	202.85774	.000	-2555.3998	-1192.6336
		Khassab	1522.03000*	202.85774	.000	840.6469	2203.4131
		Majdool	1927.86667*	202.85774	.000	1246.4836	2609.2498
		Sukkari	109.55667	202.85774	.993	-571.8264	790.9398
		Khalas	819.20667*	202.85774	.016	137.8236	1500.5898
	Majdool	Barhi	-3801.88333*	202.85774	.000	-4483.2664	-3120.5002
		Khassab	-405.83667	202.85774	.395	-1087.2198	275.5464
		Ajwah	-1927.86667*	202.85774	.000	-2609.2498	-1246.4836
		Sukkari	-1818.31000*	202.85774	.000	-2499.6931	-1136.9269
		Khalas	-1108.66000*	202.85774	.002	-1790.0431	-427.2769
	Sukkari	Barhi	-1983.57333*	202.85774	.000	-2664.9564	-1302.1902
		Khassab	1412.47333*	202.85774	.000	731.0902	2093.8564
		Ajwah	-109.55667	202.85774	.993	-790.9398	571.8264
		Majdool	1818.31000*	202.85774	.000	1136.9269	2499.6931
		Khalas	709.65000*	202.85774	.040	28.2669	1391.0331
	Khalas	Barhi	-2693.22333*	202.85774	.000	-3374.6064	-2011.8402
		Khassab	702.82333*	202.85774	.042	21.4402	1384.2064
		Ajwah	-819.20667*	202.85774	.016	-1500.5898	-137.8236
		Majdool	1108.66000*	202.85774	.002	427.2769	1790.0431
		Sukkari	-709.65000*	202.85774	.040	-1391.0331	-28.2669

CAE	Barhi	Khassab	84.44333*	1.34642	.000	79.9208	88.9659
		Ajwah	84.44333*	1.34642	.000	79.9208	88.9659
		Majdool	84.44333*	1.34642	.000	79.9208	88.9659
		Sukkari	84.44333*	1.34642	.000	79.9208	88.9659
		Khalas	84.44333*	1.34642	.000	79.9208	88.9659
	Khassab	Barhi	-84.44333*	1.34642	.000	-88.9659	-79.9208
		Ajwah	.00000	1.34642	1.000	-4.5225	4.5225
		Majdool	.00000	1.34642	1.000	-4.5225	4.5225
		Sukkari	.00000	1.34642	1.000	-4.5225	4.5225
		Khalas	.00000	1.34642	1.000	-4.5225	4.5225
	Ajwah	Barhi	-84.44333*	1.34642	.000	-88.9659	-79.9208
		Khassab	.00000	1.34642	1.000	-4.5225	4.5225
		Majdool	.00000	1.34642	1.000	-4.5225	4.5225
		Sukkari	.00000	1.34642	1.000	-4.5225	4.5225
		Khalas	.00000	1.34642	1.000	-4.5225	4.5225
	Majdool	Barhi	-84.44333*	1.34642	.000	-88.9659	-79.9208
		Khassab	.00000	1.34642	1.000	-4.5225	4.5225
		Ajwah	.00000	1.34642	1.000	-4.5225	4.5225
		Sukkari	.00000	1.34642	1.000	-4.5225	4.5225
		Khalas	.00000	1.34642	1.000	-4.5225	4.5225
	Sukkari	Barhi	-84.44333*	1.34642	.000	-88.9659	-79.9208
		Khassab	.00000	1.34642	1.000	-4.5225	4.5225
		Ajwah	.00000	1.34642	1.000	-4.5225	4.5225
		Majdool	.00000	1.34642	1.000	-4.5225	4.5225
		Khalas	.00000	1.34642	1.000	-4.5225	4.5225
	Khalas	Barhi	-84.44333*	1.34642	.000	-88.9659	-79.9208
		Khassab	.00000	1.34642	1.000	-4.5225	4.5225
		Ajwah	.00000	1.34642	1.000	-4.5225	4.5225
		Majdool	.00000	1.34642	1.000	-4.5225	4.5225
		Sukkari	.00000	1.34642	1.000	-4.5225	4.5225

GAE	Barhi	Khassab	-50.69667*	13.06497	.021	-94.5809	-6.8125
		Ajwah	19.84000	13.06497	.660	-24.0442	63.7242
		Majdool	8.64667	13.06497	.983	-35.2375	52.5309
		Sukkari	23.05333	13.06497	.520	-20.8309	66.9375
		Khalas	-102.09667*	13.06497	.000	-145.9809	-58.2125
	Khassab	Barhi	50.69667*	13.06497	.021	6.8125	94.5809
		Ajwah	70.53667*	13.06497	.002	26.6525	114.4209
		Majdool	59.34333*	13.06497	.007	15.4591	103.2275
		Sukkari	73.75000*	13.06497	.001	29.8658	117.6342
		Khalas	-51.40000*	13.06497	.019	-95.2842	-7.5158
	Ajwah	Barhi	-19.84000	13.06497	.660	-63.7242	24.0442
		Khassab	-70.53667*	13.06497	.002	-114.4209	-26.6525
		Majdool	-11.19333	13.06497	.950	-55.0775	32.6909
		Sukkari	3.21333	13.06497	1.000	-40.6709	47.0975
		Khalas	-121.93667*	13.06497	.000	-165.8209	-78.0525
	Majdool	Barhi	-8.64667	13.06497	.983	-52.5309	35.2375
		Khassab	-59.34333*	13.06497	.007	-103.2275	-15.4591
		Ajwah	11.19333	13.06497	.950	-32.6909	55.0775
		Sukkari	14.40667	13.06497	.871	-29.4775	58.2909
		Khalas	-110.74333*	13.06497	.000	-154.6275	-66.8591
	Sukkari	Barhi	-23.05333	13.06497	.520	-66.9375	20.8309
		Khassab	-73.75000*	13.06497	.001	-117.6342	-29.8658
		Ajwah	-3.21333	13.06497	1.000	-47.0975	40.6709
		Majdool	-14.40667	13.06497	.871	-58.2909	29.4775
		Khalas	-125.15000*	13.06497	.000	-169.0342	-81.2658
	Khalas	Barhi	102.09667*	13.06497	.000	58.2125	145.9809
		Khassab	51.40000*	13.06497	.019	7.5158	95.2842
		Ajwah	121.93667*	13.06497	.000	78.0525	165.8209
		Majdool	110.74333*	13.06497	.000	66.8591	154.6275
		Sukkari	125.15000*	13.06497	.000	81.2658	169.0342

		Sukkari	.00000	1.34042	1.000	-4.3225	4.3225
EPE	Barhi	Khassab	3362.31000*	205.29882	.000	2672.7275	4051.8925
		Ajwah	1769.73333*	205.29882	.000	1080.1508	2459.3158
		Majdool	3708.79333*	205.29882	.000	3019.2108	4398.3758
		Sukkari	1876.07333*	205.29882	.000	1186.4908	2565.6558
		Khalas	2710.87333*	205.29882	.000	2021.2908	3400.4558
	Khassab	Barhi	-3362.31000*	205.29882	.000	-4051.8925	-2672.7275
		Ajwah	-1592.57667*	205.29882	.000	-2282.1592	-902.9942
		Majdool	346.48333	205.29882	.563	-343.0992	1036.0658
		Sukkari	-1486.23667*	205.29882	.000	-2175.8192	-796.6542
		Khalas	-651.43667	205.29882	.068	-1341.0192	38.1458
	Ajwah	Barhi	-1769.73333*	205.29882	.000	-2459.3158	-1080.1508
		Khassab	1592.57667*	205.29882	.000	902.9942	2282.1592
		Majdool	1939.06000*	205.29882	.000	1249.4775	2628.6425
		Sukkari	106.34000	205.29882	.994	-583.2425	795.9225
		Khalas	941.14000*	205.29882	.006	251.5575	1630.7225
	Majdool	Barhi	-3708.79333*	205.29882	.000	-4398.3758	-3019.2108
		Khassab	-346.48333	205.29882	.563	-1036.0658	343.0992
		Ajwah	-1939.06000*	205.29882	.000	-2628.6425	-1249.4775
		Sukkari	-1832.72000*	205.29882	.000	-2522.3025	-1143.1375
		Khalas	-997.92000*	205.29882	.004	-1687.5025	-308.3375
	Sukkari	Barhi	-1876.07333*	205.29882	.000	-2565.6558	-1186.4908
		Khassab	1486.23667*	205.29882	.000	796.6542	2175.8192
		Ajwah	-106.34000	205.29882	.994	-795.9225	583.2425
		Majdool	1832.72000*	205.29882	.000	1143.1375	2522.3025
		Khalas	834.80000*	205.29882	.015	145.2175	1524.3825
	Khalas	Barhi	-2710.87333*	205.29882	.000	-3400.4558	-2021.2908
		Khassab	651.43667	205.29882	.068	-38.1458	1341.0192
		Ajwah	-941.14000*	205.29882	.006	-1630.7225	-251.5575
		Majdool	997.92000*	205.29882	.004	308.3375	1687.5025
		Sukkari	-834.80000*	205.29882	.015	-1524.3825	-145.2175

*. The mean difference is significant at the 0.05 level.

Appendix U. One-way ANOVA of cycle 3 of HPLC

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
GAE	Between Groups	37.951	1	37.951	.733	.440
	Within Groups	207.225	4	51.806		
	Total	245.176	5			
CAE	Between Groups	5868.754	1	5868.754	1768.576	.000
	Within Groups	13.273	4	3.318		
	Total	5882.027	5			
EPE	Between Groups	4803035.905	1	4803035.905	362.274	.000
	Within Groups	53032.110	4	13258.028		
	Total	4856068.015	5			
TPC	Between Groups	99360586.49	1	99360586.49	2164.258	.000
	Within Groups	183639.059	4	45909.765		
	Total	99544225.55	5			
CC	Between Groups	1990264.339	1	1990264.339	1785.486	.000
	Within Groups	4458.762	4	1114.691		
	Total	1994723.102	5			

```
ONEWAY TPC CC BY COFFEE_TYPE
  /MISSING ANALYSIS
  /POSTHOC=TUKEY ALPHA(0.05).
```

Oneway

Appendix V. Latin square randomisation sheet

Participant ID	**Visit 1**	**Visit 2**	**Visit 3**
AD1	A	B	C
AD2	A	C	B
AD3	B	A	C
AD4	B	C	A
AD5	C	A	B
AD6	C	B	A
AD7	A	B	C
AD8	A	C	B
AD9	B	A	C
AD10	B	C	A
AD11	C	A	B
AD12	C	B	A
AD13	A	B	C

Participant ID	Visit 1	Visit 2	Visit 3
AD14	A	C	B
AD15	B	A	C
AD16	B	C	A
AD17	C	A	B
AD18	C	B	A
AD19	A	B	C
AD20	A	C	B
AD21	B	A	C
AD22	B	C	A
AD23	C	A	B
AD24	C	B	A
AD25	A	B	C
AD26	A	C	B

Participant ID	Visit 1	Visit 2	Visit 3
AD27	B	A	C
AD28	B	C	A
AD29	C	A	B
AD30	C	B	A
AD31	A	B	C
AD32	A	C	B
AD33	B	A	C
AD34	B	C	A
AD35	C	A	B
AD36	C	B	A
AD37	A	B	C
AD38	A	C	B
AD39	B	A	C

Participant ID	Visit 1	Visit 2	Visit 3
AD40	B	C	A
AD41	C	A	B
AD42	C	B	A
AD43	A	B	C
AD44	A	C	B
AD45	B	A	C
AD46	B	C	A
AD47	C	A	B
AD48	C	B	A
AD49	A	B	C
AD50	A	C	B
AD51	B	A	C
AD52	B	C	A

Participant ID	**Visit 1**	**Visit 2**	**Visit 3**
AD53	C	A	B
AD54	C	B	A
AD55	A	B	C
AD56	A	C	B
AD57	B	A	C
AD58	B	C	A
AD59	C	A	B
AD60	C	B	A

Treatments codes (to be filled by the Third party)			
Treatments coding	A	B	C
Treatments decoding			

Appendix W. Clinical trial protocol registration form

ClinicalTrials.gov PRS
Protocol Registration and Results System

ClinicalTrials.gov Protocol Registration and Results System (PRS) Receipt
Release Date: February 28, 2020

ClinicalTrials.gov ID: NCT04009564

Study Identification

Unique Protocol ID:	DSCPM
Brief Title:	Effect of Date Seeds Coffee on Mood and Cognitive Performance
Official Title:	Investigating the Acute Effects of Mood and Cognitive Performance Following the Administration of a Coffee Made of Date Seeds on Healthy Young Volunteers.
Secondary IDs:	

Study Status

Record Verification:	July 2019
Overall Status:	Recruiting
Study Start:	June 20, 2019 [Actual]
Primary Completion:	March 30, 2020 [Anticipated]
Study Completion:	March 30, 2020 [Anticipated]

Sponsor/Collaborators

Sponsor:	Newcastle University
Responsible Party:	Sponsor
Collaborators:	

Oversight

U.S. FDA-regulated Drug:	No
U.S. FDA-regulated Device:	No
U.S. FDA IND/IDE:	No
Human Subjects Review:	Board Status: Approved Approval Number: 1646/9711/2019 Board Name: FMS Ethics committee Board Affiliation: Newcastle university Phone: 0191 208 5633 Email: kimberley.sutherland@newcastle.ac.uk Address: Faculty Support Assistant Research & Innovation Office

Faculty of Medical Sciences

Newcastle University
Newcastle upon Tyne
NE2 4HH

Data Monitoring:

Study Description

Brief Summary: Limited utilizations of date seeds have previously been explored, and so previously wastage has often been the normality. However, research now indicates that several fruit seeds contain higher concentrations of beneficial total phytochemicals within their seeds in comparison to the flesh. As well as high nutritional values of date seeds for fibre, protein and micronutrients, this increased phytochemical content has been proven to be true for date seeds, with mainly phenolic acids (24.6 g k GAE) 3 and total flavonoids (3.67 g k RE). With the seeds presently being used to produce new coffee products, it raises questions on whether consumption of date seeds can alter mood and cognitive behaviour and therefore research into investigate the acute effect of date seeds coffee on mood and cognitive function on healthy young volunteers. However, to the best of the research team knowledge, this is the first human trial to investigate these effects.

Detailed Description: Introduction

Seeds of the date palm (P. dactylifera) are a very rich source of bioactive compounds, thus constituting strong candidates for functional food additives and nutraceuticals. Many promising results were observed when the effect of date flesh and seeds consumption have been studied in animals, for their role as either a protective1 against neurodegenerative 2,4,5 diseases, or as a cure for it 2. Most of the observed effects were attributed to the antioxidant and anti-inflammatory properties in dates flesh and seeds fruit due to it high content of phenolic compounds 3.

Rational For years, date seeds were considered a waste, having no other uses except for feeding animals. However, as has already been demonstrated in other fruits and corresponding seeds, total phytochemical content of the date seeds was higher than in the edible flesh9. Date seeds have also been shown to have an excellent nutritional quality due to high amounts of fibre (676–742 g/kg) 3 depending on variety, considerable amounts of minerals, vitamins, lipids and protein. Additionally, date seeds were shown to be rich in antioxidants containing mainly phenolic acids (24.6 g k Gallic Acid equivalent) 3 and total flavonoids (3.67 g k Rutin Equivalent).

Nowadays, and on sight of the aforementioned findings, date seeds are used to make a new coffee product. This ‘’coffee alternative’’ is commercially available and is becoming more popular.

Although, many studies have demonstrated that date seeds possess high antioxidant activities, due to their high content of flavonoids and phenolic compounds, no human trial has investigated the effect of the consumption of date seeds on humans and especially on mood and cognitive behaviour. Therefore, this study, for the first time, to the best of the research team knowledge will investigate the acute effect of date seeds coffee on mood and cognitive function on healthy young volunteers.

Aim The aim of this study is to investigate the acute effects of a coffee made of date seeds on mood and cognitive performance.

Conditions

Conditions: Health Status

Keywords:

Study Design

Study Type: Interventional

Primary Purpose: Basic Science

Study Phase: N/A

Interventional Study Model: Crossover Assignment

Number of Arms: 3

Masking: Double (Participant, Investigator)

Allocation: Randomized

Enrollment: 46 [Anticipated]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Date seeds filtered coffee Each participant will consume this arm in a visit has been allocated by Latin Square randomisation order: 45 g of date seeds coffee in 280 ml of boiled water, coffee flavour and brown food colouring (made using a filter coffee machine). it will be served in a paper cup with lid	Dietary Supplement: Date coffee 45 g of date seeds coffee in 280 ml of water
Experimental: Normal filtered coffee Each participant will consume this arm in a visit has been allocated by Latin Square randomisation order: 6 g of coffee in 280 ml of boiled water (made using a filter coffee machine) it will be served in a paper cup with lid	Dietary Supplement: Normal coffee 6 g of normal coffee in 280ml of water
Placebo Comparator: Placebo Each participant will consume this arm in a visit has been allocated by Latin Square randomisation order: 280 of boiled water, coffee flavour and brown food colouring it will be served in a paper cup with lid	Dietary Supplement: Placebo water, food colouring and coffee flavouring

Outcome Measures

Primary Outcome Measure:

1. The average of the change in 9 cognitive indexes including: Attentional Intensity, Sustained Attention, Attentional Fluctuation, Memory Retrieval Speed, Cognitive Reaction Time, Working Memory Capacity, Episodic Memory Capacity, Quality of Memory
   Cog-track an online set of nine cognitive tests (www.wesnes.com).

   [Time Frame: Change from baseline at 45 minutes post dose and at 90 minutes post dose for each visit]

Secondary Outcome Measure:

2. Change in Caffeine consumption using caffeine consumption questionnaire
   A paper based questionnaire developed by Erika Bühler et al., 2014. At the screening visit and on each study day participants will be asked to complete the table in order to build a picture of their individual caffeine habits

[Time Frame: Change form Baseline for each visit only]

3. Caffeine research Visual Analogue Scales
   A caffeine research Visual Analogue Scales which comprises of 7 scales (relaxed, alert, jittery, tired, tense, headache and overall mood).

   [Time Frame: Change from baseline 45 minutes post dose and at 90 minutes post dose.]

4. Change in Mood using Bond Lader Visual Analogue Scales
   This widely used set of 16 100 mm VASs yields three extensively validated factor scores: alertness, calmness and contentment.

   [Time Frame: Change from baseline 45 minutes post dose and at 90 minutes post dose.]

Eligibility

Minimum Age:	18 Years
Maximum Age:	35 Years
Sex:	All
Gender Based:	No
Accepts Healthy Volunteers:	Yes
Criteria:	Inclusion Criteria:

Healthy young volunteers age between 18 to 35

Exclusion Criteria:

Participants will be ineligible to participate in the study if any of the following apply:

1. Have a BMI above 35kg/m2
2. Smokers or tobacco product consumers which includes electronic cigarettes
3. Are taking any illicit or prescribed drugs.
4. Have a history of, or currently, abuse alcohol
5. Have a history of dyslexia, ADHD, learning difficulties or colour blindness,
6. Females who are pregnant, lactating or seeking to become pregnant, or are at risk of pregnancy as they to do not use birth control measures
7. Have allergies to any food product.

Contacts/Locations

Central Contact Person:	
Central Contact Backup:	
Study Officials:	
Locations:	**United Kingdom**

NU-Food Research Facility
[Recruiting]
Newcastle upon Tyne, Tyne And Wear, United Kingdom, NE1 7RU
Contact: Duaa Altuwairki, PhD student 07402033360
d.altuwairki2@ncl.ac.uk
Contact: Dr Anthony Watson, PhD

NU-Food Research Facility
[Recruiting]

	Newcastle upon Tyne, Tyne And Wear, United Kingdom, NE1 7RU Contact: Duaa Altuwairki, PhD student 07402033360 d.altuwairki2@ncl.ac.uk Contact: Dr Anthony Watson, PhD

IPDSharing

Plan to Share IPD:	Undecided

References

Citations:	
Links:	
Available IPD/Information:	

Appendix X. The Caffeine consumption questionnaire

We would like to know how much caffeine you consume

Indicate in the table, how much you drank yesterday.
Put a line in the box for every cup/glass.
If you didn't drink the item, leave the box empty.

Portion sizes

Small cup 150 mL	Large cup 250 mL	Small glass 150 mL	Large glass 250 mL	Energy drink 250 mL can	Energy drink 500 mL can	Energy Shot 60 mL can	Espresso cup 60 mL	Chocolate bar 20g
☕	☕	🥛	🥛	▯	▯	▮	☕	▦

For example, like this:

	Coffee		Cola, fizzy soft drink		Chocolate
	☕	☕	🥛	🥛	▦
Breakfast	II			I	I

	Coffee		Decaffeinated coffee		Espresso	Black-, green, white, mate tea		Cocoa drink		Iced tea, drinks with tea extract		Cola, mixed cola beverages (but not orangeade and lemonade)		Energy drink		Energy shot	Alcopops with energy drink, cola or coffee		Chocolate
	☕	☕	☕	☕	☕	☕	☕	☕	☕	🥛	🥛	🥛	🥛	▯	▯	▮	🥛	🥛	▦
Breakfast																			
Between breakfast and lunch																			
lunch																			
Between lunch and dinner																			
Dinner																			
After dinner																			

Name: _____ Age: _____ Gender: ☐ female ☐ male
Height: _____ Weight: _____ Date: _____

Abb. 1: Foodstuffs with portion sizes and caffeine content incorporated in the survery tool

Appendix Y. The participant's information sheet

Newcastle University

Effect of date fruit extract consumption on mood and cognitive performance on healthy volunteers

Information Sheet for Participants

Investigator: Duaa Altuwairki

Supervisors:

Dr. Kirsten Brandt

Dr. Anthony Watson

Newcastle University

School of Faculty of Medical Sciences

ICM

Newcastle upon Tyne

NE1 7RU

For further information please contact:

Email: D.altuwairki2@ncl.ac.uk

Telephone: Researcher 07402033360

http:www.ncl.ac.uk/hnrc

Participation Information Sheet Version Duaa Altuwairki (PGR)

13/11/2018

Do you like drinking coffee?

If yes, then you are being invited to take part in a research study. Before you decide to take part it is important you understand why the research is being conducted, and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this.

What is the purpose of this study?

Seeds of the date palm are a very rich source of bioactive compounds, thus constituting strong candidates for functional food additives and nutraceuticals. For years, date seeds were considered a waste, having no other uses except for feeding animals. However, as has already been demonstrated in other fruits and corresponding seeds, total phytochemical content of the date seeds was higher than in the edible flesh. Date seeds have also been shown to have an excellent nutritional quality due to high amounts of fibre and considerable amounts of minerals, vitamins, lipids and protein. Additionally, date seeds were shown to be rich in antioxidants containing mainly phenolic acids and total flavonoids.

Nowadays, date seeds are used to make a new coffee product. This "coffee alternative" is commercially available and has become increasingly popular.

Although, many studies have demonstrated that date seeds possess high antioxidant activities due to their high content of flavonoids and phenolic compounds, which may have a positive effect on health, no human trial has

investigated the effect of the consumption of date seeds on humans, especially on mood and cognitive behaviour. Therefore, this study, for the first time to the best of our knowledge, will investigate the acute effect of date seeds coffee on mood and cognitive function on healthy young volunteers.

Why have I been chosen?

We are looking for young volunteers who are non-smokers (which includes electronic cigarettes), not addicted to coffee, not over the age of 35 years, if they are females they should not be pregnant, or seeking to become pregnant, have no history of, or currently abuse alcohol, have no allergies to any food products, have a BMI below 35kg/m^2 and using no dietary supplements, over the counter medicine or recreational drugs and have no history of any metabolic diseases such as type 1 or type 2 diabetes to take part in this study. We will be recruiting 44 volunteers in total from the Newcastle Upon Tyne area.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be asked to sign a consent form on your 'Screening Visit'; you will be given a copy of this to keep.

If you decide to take part and you are a suitable volunteer for the study, we will ask you to sign a full consent form. However, you will be free to withdraw from the study without giving a reason anytime up to the end of your final visit. Shortly after this, all data will be fully annonymised, and therefore, from this point forward it will not be possible to withdraw any data from the study.

What will happen to me if I take part?

If you agree to take part we will ask you to visit **the NU-Food research facility, Newcastle University** on four occasions. The first visit is a screening visit to assess your suitability for the project. We will ask you a series of questions to ensure you're ok to take part, you will also have the opportunity to discuss the project with the research team. If you are suitable, there will be three further visits. At each visit, you need to come at 9:30 am to the **NU-Food research facility, Newcastle University**, and you will complete a series of computer programs which will assess your mood and cognitive performance. This will be conducted 3 times per visit. One time at the base line followed by the treatment of the day and then you will repeat the computerised assessment at 45 min and 90 min post consumption of the study foods. Each visit will last approximately three hours.

What else do I have to do?

If you agree to take part, we will ask you to make short visits to NU-Food, Newcastle University, on four occasions. If you are suitable, The first visit is a screening visit to assess your suitability for the project. On the evening before these 3 visits, you will need to fast from 10 pm; this means that you should not eat or drink anything except water until you complete your visit the following morning.

Finally, we will ask you to avoid dark fruits and dark fruit juices the day before the study day and to limit your coffee intake.

What will happen to the samples I provide?

No biological samples will be collected.

What are the possible disadvantages and risks of taking part?

Ingestion of food products may cause allergies, however, all study products will be everyday foods/ food supplements/ food ingredients with Generally Regarded As Safe (GRAS) approval. Any allergies identified from the risk assessment and volunteer screening interviews will lead to that participant's exclusion from the trial on safety grounds. In the event of the occurrence of a participant's unexpected allergic reaction to trial foodstuff, the immediate cessation of that foodstuff will be overseen by the lead researcher.

What are the possible benefits of taking part?

Although you will derive no individual benefit, the knowledge gained from this study will help our research into identifying the effects of date seeds coffee on cognitive pefromance.

What will happen if anything goes wrong?

Any complaints you have about this study should be made to Dr Kirsten brandt, Newcastle university (kirsten.brandt@newcastle.ac.uk) or

Dr Anthony Watson, Newcastle University (Anthony.Watson@ncl.ac.uk) and will be fully investigated.

Will my taking part in this study be kept confidential?

Any information which is collected about you during the course of the research will be kept strictly confidential.

What will happen to the study results?

We will publish the results of the study in a scientific journal and on the project website. You will not be personally identified in any publications. We will be happy to discuss the overall results with you when the study is completed, and will let you know where you can obtain a copy of the published results if you wish.

Will I be reimbursed for my time?

In recognition of your time commitment, you will be paid an honorarium of £40 in the form of Eldon Square vouchers at the completion of the study. And you will be provided with a free lunch after each visit.

Contact for further information

If you would like any further information about this study, please do not hesitate to contact the researcher on either of the following contacting methods:

Altuwairki Duaa

Telephone: 07402033360

Email:D.altuwairki2@ncl.ac.uk

And finally…

Thank you for taking the time to read this information sheet, and for your interest in the study

"This study was approved by the Faculty of Medical Sciences Research Ethics Committee, part of Newcastle University's Research Ethics Committee. This committee contains members who are internal to the Faculty, as well as one external member. This study was reviewed by members of the committee, who must provide impartial advice and avoid significant conflicts of interests."

Appendix Z. The participant's consent form

nu-food
Food & Consumer Research Facility

Newcastle University

The effect of date seeds coffee on cognitive performance and mood (DSCPM)

CONSENT BY VOLUNTEER TO PARTICIPATE IN A NUTRITIONAL STUDY

Participant ID_____

		Please initial box
1.	I confirm that I have read and understood the information sheet for the above study and have had the opportunity to ask questions.	☐
2.	I understand that my participation is voluntary and that I am free to withdraw at any time.	☐
3.	I confirm that I have no allergies or intolerances to any food or drink	☐
4.	I agree to take part in the trial	☐

Name of Volunteer *(Please print)*	Date	Signature
Name of Research Team Member (Please print)	Date	Signature

Appendix AA. The ethical approval

07 February 2019

Duaa Altuwairki
Institute of Cellular Medicine

Faculty of Medical Sciences
Newcastle University
Medical School
Framlington Place
Newcastle upon Tyne
NE2 4HH

FACULTY OF MEDICAL SCIENCES: ETHICS COMMITTEE

Dear Duaa

Title: Assessing the acute effect of date seeds coffee on cognitive function and mood on healthy young volunteers
Application No: 1646/9711/2019
Start date to end date: 21/01/2019 to 28/06/2019

On behalf of the Faculty of Medical Sciences Ethics Committee, I am writing to confirm that the ethical aspects of your proposal have been considered and your study has been given ethical approval.

The approval is limited to this project: **1646/9711/2018**. If you wish for a further approval to extend this project, please submit a re-application to the FMS Ethics Committee and this will be considered.

During the course of your research project you may find it necessary to revise your protocol. Substantial changes in methodology, or changes that impact on the interface between the researcher and the participants must be considered by the FMS Ethics Committee, prior to implementation.*

At the close of your research project, please report any adverse events that have occurred and the actions that were taken to the FMS Ethics Committee.*

Best wishes,

Yours sincerely

M. Holbrough

Marjorie Holbrough
On behalf of Faculty Ethics Committee

cc.
Professor Daniel Nettle, Chair of FMS Ethics Committee
Mrs Kay Howes, Research Manager

*Please refer to the latest guidance available on the internal Newcastle web-site.

Appendix BB. The extended ethical approval

08 July 2019

Duaa Altuwairki
Institute of Cellular Medicine

Faculty of Medical Sciences
Newcastle University
Medical School
Framlington Place
Newcastle upon Tyne
NE2 4HH

FACULTY OF MEDICAL SCIENCES: ETHICS COMMITTEE

Dear Duaa

Title: Assessing the acute effect of date seeds coffee on cognitive function and mood on healthy young volunteers
Application No: 1646_1/9711/2019 (Amendment)
Start date to end date: 21/01/2019 to 31/10/2019

On behalf of the Faculty of Medical Sciences Ethics Committee, I am writing to confirm that the ethical aspects of your proposal have been considered and your study has been given ethical approval.

The approval is limited to this project: **1646_1/9711/2019 (Amendment)**. If you wish for a further approval to extend this project, please submit a re-application to the FMS Ethics Committee and this will be considered.

During the course of your research project you may find it necessary to revise your protocol. Substantial changes in methodology, or changes that impact on the interface between the researcher and the participants must be considered by the FMS Ethics Committee, prior to implementation.*

At the close of your research project, please report any adverse events that have occurred and the actions that were taken to the FMS Ethics Committee.*

Best wishes,

Yours sincerely

M. Holbrough

Marjorie Holbrough
On behalf of Faculty Ethics Committee

cc.
Professor Daniel Nettle, Chair of FMS Ethics Committee
Mrs Kay Howes, Research Manager

*Please refer to the latest guidance available on the internal Newcastle web-site.

Appendix CC. The study debrief

Date seeds coffee study Debrief

Dear Participant,

I would like to thank you for taking part in my trial which is attempting to investigate the acute effects of date seeds coffee on mood and cognitive performance in healthy volunteers. Your participation means a lot to the research team, and this project would not have been possible to conduct without your great contribution.

You have now successfully completed the project. I would like to assure you that any data we collected during the course of the research has been anonymised and kept strictly confidential. This means when the results are published within a scientific journal or onto the project website you will not be able to be personally identified. However, should you wish to know overall results of the study, we will be able to refer you to where you can obtain a copy of the published results once they become available. A member of the research team would be happy to discuss any queries or questions you have at this time.

It was a pleasure to meet you all, and please accept both our gratitude and small reward as a gesture of goodwill for your participation.

Sincerely,

Duaa Altuwairki

A question **Participant number:**

Would you be able to tell us from your own prospective, in which visit do you think you were given the treatment that contained Date seeds coffee?

1. *First visit*
2. *Second visit*
3. *Third visit*
4. *I don't know*

Thanks

Appendix DD. The case report form

Participant DSCPM

Participants initials:

nu-food
Food & Consumer Research Facility

Study code: DSCPM

<The Effect of Date seed coffee on Mood and Cognition in Healthy Adults>

CASE REPORT FORM

Trial Sponsor: NEWCASTLE UNIVERSITY

1

CRF completion Instructions

When completing the CRF please ensure:

- Black ink should be used.
- Each section is completed fully.
- Any corrections made to any data in the CRF are initialled and dated.
- The consent form is signed, dated and the name of signatory is clearly printed by all parties.
- The date of consent recorded in the CRF is the date the Participant signs the consent form.

Taking Consent

Participant must be eligible and have given consent before entering the study.

For the Participant to give consent they must sign and date two original consent forms after they have completely read the Participant information sheet and have fully understood what the study entails. The Participant must clearly print their name on the consent and the date must be the date the Participant signs the form. No study procedures can occur prior to the Participant signing the consent form.

As well as the Participant signing the consent form, it is necessary for the person explaining the study to the Participant to sign the consent form. By signing the consent form the person explaining the study confirms that they have witnessed the Participant give consent and that the Participant fully understands what the study entails.

Please ensure that two copies of the consent form are signed – one copy to be given to the Participant, one copy to remain at NUFood Research (this copy should be stored in a locked filing cabinet, separate from all participant data).

VISIT 0 - SCREENING

Date: D D M M Y Y Y Y

Participant initials:

Participant number:

PARTICIPANT ELIGIBILITY CHECKLIST - TRIAL ENTRY

Please tick:	YES	NO
Has the Participant given written informed consent?	☐	☐
Is the Participant:		
In good health?	☐	☐
Aged between 18 and 35 years?	☐	☐
Proficient in English equivalent to a native English speaker?	☐	☐
Orientated to person, place and time and has the ability to communicate with study staff?	☐	☐
Motivated to participate in and complete the study as instructed and to attend visit in a well-rested state?	☐	☐
Does the Participant:		
Intend to comply with the study tobacco restriction?	☐	☐
Intend to comply with the study caffeine restriction?	☐	☐
Intend to comply with the study alcohol restriction?	☐	☐
Intend to comply with restriction of dietary/ supplement intake?	☐	☐
Intend to comply with the 12 hour fasting restriction prior to each study session?	☐	☐
Have normal or corrected-to-normal vision?	☐	☐

If NO the Participant is ineligible for the trial. Please only complete pages S1 – S5.

VISIT 0 - SCREENING

Date: D D M M Y Y Y Y

Participant initials:

Participant number:

	Please tick: YES	NO
Is the Participant:		
Pregnant or seeking to become pregnant?	☐	☐
Does the Participant:		
Have a history of metabolic diseases like type 1 or type 2 diabetes?	☐	☐
Currently take a pharmaceutical product/medicine (except contraception?)	☐	☐
Have any known allergies or intolerance to any ingredients in the study preparation?	☐	☐
Have any serious disorder that might interfere with the Participation in the test?	☐	☐
Have a Body Mass Index (BMI) above 40 kg/m^2 (severely obese)?	☐	☐
Smoke or consume any tobacco products (even occasionally)?	☐	☐
Currently abuse drugs or alcohol?	☐	☐
Have (or have a history of) head trauma, migraines, gastric problems, learning difficulties, dyslexia, colour blindness or ADHD?	☐	☐
Do you have allergies to **ANY** food product?	☐	☐

If YES the Participant is ineligible for the trial.
At Screening visit, please only complete pages S1 – S5.

DOCUMENTATION OF INFORMED CONSENT

VISIT 0 - SCREENING

Date: D D M M Y Y Y Y

Participant initials:

Participant number:

IMPORTANT: Informed consent must be obtained from the Participant BEFORE any trial procedures are started.

Has the Participant's written informed consent been obtained?

Yes ☐

No ☐

(Please tick)

If NO: The Participant is not eligible for the trial

If YES: Keep the site consent form with the Participant's notes

Date of Consent: D D M M Y Y Y Y

Is the Participant eligible for this trial?

Yes - Eligible ☐

No - Screen failure ☐

(Please tick)

If NO: please state main reason:

Fails to meet inclusion / exclusion criteria ☐

Participant has withdrawn consent ☐

(Please tick)

RR's signature

Date: D D M M Y Y Y Y

If the Participant does not satisfy ALL of the eligibility criteria or has withdrawn consent then please only complete pages S1 – S5. If the Participant satisfies all of the eligibility criteria and has provided appropriate consent please proceed.

VISIT 0 - SCREENING

Date: D D M M Y Y Y Y

Participant initials:

Participant number:

PARTICIPANT DEMOGRAPHICS

Date of Birth: D D M M Y Y Y Y

Age: Years_____ Months_____

Sex: male (M) ☐ or female (F) ☐

Race:
- Maori ☐
- Black ☐
- Oriental ☐
- Caucasian ☐
- Other ☐ please specify _____

Does the Participant require glasses/contact lenses to use a computer?

YES ☐

NO ☐

(Please tick)

Which hand does the Participant use to write with? RIGHT ☐ LEFT ☐

Is the participant vegetarian? Yes ☐ No ☐

How many portions of fruit and vegetables does the participant eat in a typical day? [Portion= one piece of fruit, a handful of vegetables or a glass of fresh fruit juice (each additional glass of juice does not count as extra)]

_____ Portion (s).

VISIT 0 - SCREENING Date: D D M M Y Y Y Y

Participant initials: ☐☐☐
Participant number: ☐☐☐

How many years of full time education has the participant had? _____

What is the highest level of qualification achieved?_____

Height ☐.☐☐ m

Weight ☐☐☐.☐☐ kg

BMI ☐☐.☐ kg/m^2

Blood Pressure

Systolic ☐☐☐

Diastolic ☐☐☐

Heart Rate ☐☐☐ BPM

CONCOMITANT MEDICATION:

VISIT 0 - SCREENING Date: D D M M Y Y Y Y

Participant initials:
Participant number:

Is the Participant receiving any concomitant medications, therapies and/or vitamin supplementation?

Yes ☐
No ☐ (Please tick)

If YES: Please complete the concomitant medication record on page 17.

MEDICAL HISTORY (Within the past 5 years)

Specify Diagnosis	1 = Past 2 = Present	Severity 1 = mild 2 = moderate 3 = severe	Concomitant Treatment 1 = Yes * 2 = No	Details
1.				
2.				
3.				
4.				
5.				
6.				
7.				
8.				
9.				
10.				

* If Yes please complete concomitant medication record on page 17.

Please note that the volunteer may not eligible to participate if taking or intending to take any prescription pharmaceutical product during the study (except for contraception for females and some topically applied therapeutic agents). Please refer to the protocol for the specific guidelines for the study.

Participant initials: | | | |
Cog-track Number: | | | |
Participant Number: | | | |

STUDY DAY 1 Date: D D M M Y Y Y Y | | | | | | | | |

Study Day 1

Participant number allocated: | | | |

Have there been any changes to the subject's concomitant medications, therapies and/or vitamin supplementation since their last visit?

No ☐ Yes ☐ (if Yes complete concomitant medication record)

Has the participant experienced any adverse events (illness) since the last visit?

No ☐ Yes ☐ (if Yes complete adverse event record)

Has the participant fasted from 10pm yesterday? (If No re-schedule their study day)

No ☐ Yes ☐

Has participant confirmed they have consumed no caffeine since 10pm yesterday? (If No re-schedule their study day)

No ☐ Yes ☐

Has participant confirmed they have consumed no alcohol since last 24 hours? (If No re-schedule their study day)

No ☐ Yes ☐

Baseline

Mood Assessment (Please tick when completed)

Caffeine consumption Questionnaire ☐

Baseline Cognitive Tasks:

Bond lader Mood Scales ☐

Simple Reaction Time ☐

Digit Vigilance ☐

Choice Reaction Time ☐

Numeric Working Memory ☐

Spatial Working Memory ☐

Participant initials: ☐☐☐
Cog-track Number: ☐☐☐
Participant Number: ☐☐☐

STUDY DAY 1 Date: D D M M Y Y Y Y ☐☐☐☐☐☐☐☐

Immediate Word Recall ☐
Delayed Word Recall ☐
Word Recognition ☐
Pattern Separation ☐
Caffeine research Visual Analogue Scales (VASs) ☐

Treatment: Treatment taken ☐ Time taken _____

Post Dose Cognitive Tasks (45 minutes post treatment):

Bond lader Mood Scales ☐
Simple Reaction Time ☐
Digit Vigilance ☐
Choice Reaction Time ☐
Numeric Working Memory ☐
Spatial Working Memory ☐
Immediate Word Recall ☐
Delayed Word Recall ☐
Word Recognition ☐
Pattern Separation ☐
Caffeine research Visual Analogue Scales (VASs) ☐

Post Dose Cognitive Tasks (90 minutes post treatment):

Bond lader Mood Scales ☐
Simple Reaction Time ☐
Digit Vigilance ☐

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Participant initials:
Cog-track Number:
Participant Number:

STUDY DAY 1 Date: D D M M Y Y Y Y

Choice Reaction Time ☐

Numeric Working Memory ☐

Spatial Working Memory ☐

Immediate Word Recall ☐

Delayed Word Recall ☐

Word Recognition ☐

Pattern Separation ☐

Caffeine research Visual Analogue Scales (VASs) ☐

RR's signature

Date: D D M M Y Y Y Y

Participant initials: | | | |
Compass Number: | | | |
Participant Number: | | | |

STUDY DAY 2 Date: D D M M Y Y Y Y | | | | | | | | |

Study Day 2

Have there been any changes to the subject's concomitant medications, therapies and/or vitamin supplementation since their last visit?

No ☐ Yes ☐ (if Yes complete concomitant medication record)

Has the participant experienced any adverse events (illness) since the last visit?

No ☐ Yes ☐ (if Yes complete adverse event record)

Has the participant fasted from 10pm yesterday? (If No re-schedule their study day)

No ☐ Yes ☐

Has participant confirmed they have consumed no caffeine or alcohol since 10pm yesterday? (If No re-schedule their study day)

No ☐ Yes ☐

Baseline

Mood Assessment (Please tick when completed)

Caffeine consumption Questionnaire ☐

Baseline Cognitive Tasks:

Bond lader Mood Scales ☐

Simple Reaction Time ☐

Digit Vigilance ☐

Choice Reaction Time ☐

Numeric Working Memory ☐

Participant initials: ☐☐☐
Compass Number: ☐☐☐
Participant Number: ☐☐☐

STUDY DAY 2 Date: D D M M Y Y Y Y ☐☐☐☐☐☐☐☐

Spatial Working Memory ☐

Immediate Word Recall ☐

Delayed Word Recall ☐

Word Recognition ☐

Pattern Separation ☐

Caffeine research Visual Analogue Scales (VASs) ☐

Treatment: Treatment taken ☐ Time taken _____

Post Dose Cognitive Tasks (45 minutes post treatment):

Bond lader Mood Scales ☐

Simple Reaction Time ☐

Digit Vigilance ☐

Choice Reaction Time ☐

Numeric Working Memory ☐

Spatial Working Memory ☐

Immediate Word Recall ☐

Delayed Word Recall ☐

Word Recognition ☐

Participant initials: ☐☐☐
Compass Number: ☐☐☐
Participant Number: ☐☐☐

STUDY DAY 2 Date: D D M M Y Y Y Y ☐☐☐☐☐☐☐☐

Pattern Separation ☐

Caffeine research Visual Analogue Scales (VASs) ☐

Post Dose Cognitive Tasks (90 minutes post treatment):

Bond lader Mood Scales ☐

Simple Reaction Time ☐

Digit Vigilance ☐

Choice Reaction Time ☐

Numeric Working Memory ☐

Spatial Working Memory ☐

Immediate Word Recall ☐

Delayed Word Recall ☐

Word Recognition ☐

Pattern Separation ☐

Caffeine research Visual Analogue Scales (VASs) ☐

D D M M Y Y Y Y

Date: ☐☐☐☐☐☐☐☐

RR's signature

Study Day 3: Date: D D M M Y Y Y Y

Participant initials:
Participant number:

Study Day 3

Have there been any changes to the subject's concomitant medications, therapies and/or vitamin supplementation since their last visit?

No ☐ Yes ☐ (if Yes complete concomitant medication record)

Has the participant experienced any adverse events (illness) since the last visit?

No ☐ Yes ☐ (if Yes complete adverse event record)

Has the participant fasted from 10pm yesterday? (If No re-schedule their study day)

No ☐ Yes ☐

Has participant confirmed they have consumed no caffeine or alcohol since 10pm yesterday? (If No re-schedule their study day)

No ☐ Yes ☐

Baseline

Mood Assessment (Please tick when completed)

Caffeine consumption Questionnaire ☐

Baseline Cognitive Tasks:

Bond lader Mood Scales ☐

Simple Reaction Time ☐

Digit Vigilance ☐

Choice Reaction Time ☐

Numeric Working Memory ☐

Spatial Working Memory ☐

Immediate Word Recall ☐

Delayed Word Recall ☐

Word Recognition ☐

Pattern Separation ☐

Study Day 3: Date: D D M M Y Y Y Y

Participant initials:
Participant number:

Caffeine research Visual Analogue Scales (VASs) ☐

Treatment: Treatment taken ☐ Time taken _____

Post Dose Cognitive Tasks (45 minutes post treatment):

Bond lader Mood Scales ☐
Simple Reaction Time ☐
Digit Vigilance ☐
Choice Reaction Time ☐
Numeric Working Memory ☐
Spatial Working Memory ☐
Immediate Word Recall ☐
Delayed Word Recall ☐
Word Recognition ☐
Pattern Separation ☐
Caffeine research Visual Analogue Scales (VASs) ☐

Post Dose Cognitive Tasks (90 minutes post treatment):

Bond lader Mood Scales ☐
Simple Reaction Time ☐
Digit Vigilance ☐
Choice Reaction Time ☐
Numeric Working Memory ☐
Spatial Working Memory ☐
Immediate Word Recall ☐
Delayed Word Recall ☐

Study Day 3:

Date: D D M M Y Y Y Y

Participant initials:

Participant number:

Word Recognition ☐

Pattern Separation ☐

Caffeine research Visual Analogue Scales (VASs) ☐

D D M M Y Y Y Y

Date:

RR's signature

Study Day 3: Date: D D M M Y Y Y Y

Participant initials:
Participant number:

TRIAL OUTCOME (please tick appropriate box):

Completed trial: ☐

If trial not completed, please complete:

Date of subject withdrawal from the study: D D M M Y Y Y Y

Provide main reason for premature termination (one reason only):

adverse event	☐	withdrawn consent	☐
did not co-operate	☐	refused treatment	☐
administrative reason	☐	lost to follow-up	☐
protocol violation	☐	other	☐

if other please specify: _____

_____ Date: D D M M Y Y Y Y

Investigator's signature

CONCOMITANT MEDICATION RECORD

Date: D D M M Y Y Y Y

Participant initials:
Participant number:

Please complete the following information fully for any concomitant medication, therapies and/or vitamin supplementation:

*** If Indication is due to a new/worsening AE, please complete the AE form (pg 18).**

Concomitant Treatment (please use generic name)	Indication	Single Dose	Total Daily Dose	Units	Frequency (e.g. BID, PRN)	Route code	Start and Stop Dates DD MM YY
1.							Start: / /
							Stop: / /
2.							Start: / /
							Stop: / /
3.							Start: / /
							Stop: / /
4.							Start: / /
							Stop: / /
5.							Start: / /
							Stop: / /
6.							Start: / /
							Stop: / /
7.							Start: / /
							Stop: / /
8.							Start: / /
							Stop: / /
9.							Start: / /
							Stop: / /

ADVERSE EVENTS

Date: D D M M Y Y Y Y

Participant initials:
Participant number:

Adverse Events

Were there any Adverse Events? ☐ 1 NO ☐ 2 YES, please complete all sections below, cross appropriate number.

	if **yes***		**Date**	**Severity**	**Relation to Study Drug**	**Action(s) Taken** *(several statements are possible)*	**Outcome of Event**
Adverse Event, specify Please list ONE event per line.	**Serious** 1 No 2 Yes *	**Reason** *(several statements are possible)* 1. Results in Death 2. Life-threatening 5. Hospitalization – new / prolonged 6. Congenital anomaly/birth defect 10. Persistent or significant disability/incapacity 11. Important medical event	**Start / Stop** If ongoing update at next visit dd / mm / yy	1. Mild 2. Moderate 3. Severe	**Possible?** 1. No 2. Yes	1. None 2. Dose of study drug reduced 3. Study drug discontinued and restarted 4. Study drug discontinued permanently 5. Remedial drug therapy, specify on concomitant medication page 6. Other (specify below) 7. Infusion rate of study drug educed 8. Hospitalization required or prolonged	1. Resolved 2. Improved 3. Unchanged 4. Worsened 6. Death 7. Insufficient Follow-up
1.	1 2* ☐☐	1 2 5 6 10 11 ☐☐☐☐☐☐	/ / / /	1 2 3 ☐☐☐	1 2 ☐☐	1 2 3 4 5 6 7 8 ☐☐☐☐☐☐☐☐	1 2 3 4 6 7 ☐☐☐☐☐☐
2.	1 2* ☐☐	1 2 5 6 10 11 ☐☐☐☐☐☐	/ / / /	1 2 3 ☐☐☐	1 2 ☐☐	1 2 3 4 5 6 7 8 ☐☐☐☐☐☐☐☐	1 2 3 4 6 7 ☐☐☐☐☐☐
3.	1 2* ☐☐	1 2 5 6 10 11 ☐☐☐☐☐☐	/ / / /	1 2 3 ☐☐☐	1 2 ☐☐	1 2 3 4 5 6 7 8 ☐☐☐☐☐☐☐☐	1 2 3 4 6 7 ☐☐☐☐☐☐
4.	1 2* ☐☐	1 2 5 6 10 11 ☐☐☐☐☐☐	/ / / /	1 2 3 ☐☐☐	1 2 ☐☐	1 2 3 4 5 6 7 8 ☐☐☐☐☐☐☐☐	1 2 3 4 6 7 ☐☐☐☐☐☐

Further Details of Adverse Events: _____

D D M M Y Y Y Y

_____ Investigator's Signature Date: ☐☐☐☐☐☐☐☐

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Appendix EE. The calculations of core measures from Cogtrack tasks.

COMPOSITES	VARIABLE NAME	CALCULATION
Attentional Intensity Index	POW_ATTM	SRTM+CRTM+VIGRT
Sustained Attention Index	CONT_ATT	((crtacc-50) * 2 + (vigacc *.45 - vigfa) * 100/45)/2
Attentional Fluctuation Index	POW_CV	SRTCV+CRTCV+VIGCV
Memory Retrieval Speed Index	SPEEDMEMM	SPMRTM+NWMRTM+DRECRTM+DPICRTM
Cognitive Reaction Time	COGRTM	CRTM-SRTM
Working Memory Capacity Index	QL_WORK	SPMOACC+SPMNACC+NWMOACC+NWMNACC-200/2
Episodic Memory Capacity Index	QL_EPIS	(DRECOACC+DRECNACC–100)+(DPICOACC+DPICNACC–100)+((IRCL–IRCLERR)*100/15)+((DRCL–DRCLERR)*100/15)

COMPOSITES	VARIABLE NAME	CALCULATION
Quality of Memory Index	QL_MEM	(NWMOACC+NWMNACC–100)+(SPMOACC+SPMNACC–100)+(DRECOACC+DRECNACC–100)+(DPICOACC+DPICNACC–100)+((IRCL–IRCLERR)*100/15)+((DRCL–DRCLERR)*100/15)/6

Appendix FF. The calculation of Bond-Lader VASs of mood scale

Derived factor	Equation	Value
Self-rated Alertness	((100-VAS_1)+(100-VAS_3)+VAS_4+(100-VAS_5)+VAS_6+VAS_9+(100-VAS_11)+VAS_12+(100-VAS_15))/9	0 to 100
Self-rated Contentment	((100-VAS_7)+VAS_8+(100-VAS_13)+VAS_14+VAS_16)/5	0 to 100
Self-rated Calmness	((100-VAS_2)+VAS_10)/2	0 to 100

The calculation of Bond-Lader VASs of mood scale, whereas: VAS1 Alert-drowsy, VAS2 calm-excited, VAS3 strong-feeble, VAS4 muzzy-clear headed, VAS5 well-coordinated-clumsy, VAS6 lethargic-energetic, VAS7 contented-discontented, VAS8 troubled -tranquil, VAS9 mentally slow-quick witted, VAS10 tense-relaxed, VAS11

attentive-dreamy, VAS12 incompetent-proficient, VAS13 happy-sad, VAS14 antagonistic-friendly, VAS15 interested-board, VAS16 withdrawn-sociable.

Appendix GG. Descriptive analysis for caffeine consumption questionnaire

Crosstabs

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Visit * Caffiene_average_day	196	100.0%	0	0.0%	196	100.0%

Visit * Caffiene_average_day Crosstabulation

			Caffiene_average_day												
			.00	1.00	1.25	1.33	1.50	1.67	1.75	2.00	2.50	2.67	3.00	4.00	5.00	Total
Visit	Screening	Count	8	14	0	3	6	1	0	11	1	0	4	0	1	49
		% within Visit	16.3%	28.6%	0.0%	6.1%	12.2%	2.0%	0.0%	22.4%	2.0%	0.0%	8.2%	0.0%	2.0%	100.0%
		% within Caffiene_average_day	25.0%	18.2%	0.0%	60.0%	46.2%	50.0%	0.0%	25.6%	50.0%	0.0%	30.8%	0.0%	25.0%	25.0%
		% of Total	4.1%	7.1%	0.0%	1.5%	3.1%	0.5%	0.0%	5.6%	0.5%	0.0%	2.0%	0.0%	0.5%	25.0%
	Visit 1	Count	6	23	1	0	2	0	0	10	1	0	4	1	1	49
		% within Visit	12.2%	46.9%	2.0%	0.0%	4.1%	0.0%	0.0%	20.4%	2.0%	0.0%	8.2%	2.0%	2.0%	100.0%
		% within Caffiene_average_day	18.8%	29.9%	100.0%	0.0%	15.4%	0.0%	0.0%	23.3%	50.0%	0.0%	30.8%	50.0%	25.0%	25.0%
		% of Total	3.1%	11.7%	0.5%	0.0%	1.0%	0.0%	0.0%	5.1%	0.5%	0.0%	2.0%	0.5%	0.5%	25.0%
	Visit 2	Count	9	18	0	1	4	0	0	10	0	1	4	0	2	49
		% within Visit	18.4%	36.7%	0.0%	2.0%	8.2%	0.0%	0.0%	20.4%	0.0%	2.0%	8.2%	0.0%	4.1%	100.0%
		% within Caffiene_average_day	28.1%	23.4%	0.0%	20.0%	30.8%	0.0%	0.0%	23.3%	0.0%	100.0%	30.8%	0.0%	50.0%	25.0%
		% of Total	4.6%	9.2%	0.0%	0.5%	2.0%	0.0%	0.0%	5.1%	0.0%	0.5%	2.0%	0.0%	1.0%	25.0%
	Visit 3	Count	9	22	0	1	1	1	1	12	0	0	1	1	0	49
		% within Visit	18.4%	44.9%	0.0%	2.0%	2.0%	2.0%	2.0%	24.5%	0.0%	0.0%	2.0%	2.0%	0.0%	100.0%
		% within Caffiene_average_day	28.1%	28.6%	0.0%	20.0%	7.7%	50.0%	100.0%	27.9%	0.0%	0.0%	7.7%	50.0%	0.0%	25.0%
		% of Total	4.6%	11.2%	0.0%	0.5%	0.5%	0.5%	0.5%	6.1%	0.0%	0.0%	0.5%	0.5%	0.0%	25.0%
Total		Count	32	77	1	5	13	2	1	43	2	1	13	2	4	196
		% within Visit	16.3%	39.3%	0.5%	2.6%	6.6%	1.0%	0.5%	21.9%	1.0%	0.5%	6.6%	1.0%	2.0%	100.0%
		% within Caffiene_average_day	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
		% of Total	16.3%	39.3%	0.5%	2.6%	6.6%	1.0%	0.5%	21.9%	1.0%	0.5%	6.6%	1.0%	2.0%	100.0%

Appendix HH. Missing data from three participants in the date seeds and cognitive function trial.

Participant with ID (AD52) had the following missing data, from the baseline of study day 2:

DPICOACC	Pattern Separation - Original Stimuli - Accuracy
DPICNACC	Pattern Separation - New Stimuli - Accuracy
DPICORT	Pattern Separation - Original Stimuli - Average Speed
DPICNR	Pattern Separation - New Stimuli - Average Speed
DPICRT	Pattern Separation - Average Speed
DPICORTM	Picture Recognition Original Stimuli - Speed: Median
DPICNRTM	Picture Recognition New Stimuli - Speed: Median
DPICRTM	Picture Recognition - Speed: Median
DPICSD	Pattern Separation - Standard Deviation

Participant with ID (AD54) had missing data, from the baseline of study day 1; the missing data was in the same measures as the participant with ID (AD52).

Participant with ID (AD56) had the following missing data, from the baseline of study day 2:

NWMOACC	Numeric Working Memory Original Stimuli - Accuracy
NWMNACC	Numeric Working Memory New Stimuli - Accuracy
NWMORT	Numeric Working Memory Original Stimuli - Average Speed
NWMNRT	Numeric Working Memory New Stimuli
NWMRT	Numeric Working Memory - Average Speed
NWMORTM	Numeric Working Memory Original Stimuli - Speed: Median
NWMNRTM	Numeric Working Memory New Stimuli - Speed: Median
NWMRTM	Numeric Working Memory - Speed: Median
NWMSD	Numeric Working Memory - Standard Deviation
DRCL	Delayed Word Recall - Number of words correctly recalled
DRCLACC	Delayed Word Recall - Percentage of words correctly recalled
DRCLERR	Delayed Word Recall - Number of words incorrectly recalled
DRECOACC	Word Recognition - Original Stimuli - Accuracy
DRECNACC	Word Recognition - New Stimuli - Accuracy
DRECORT	Word Recognition - Original Stimuli - Average Speed

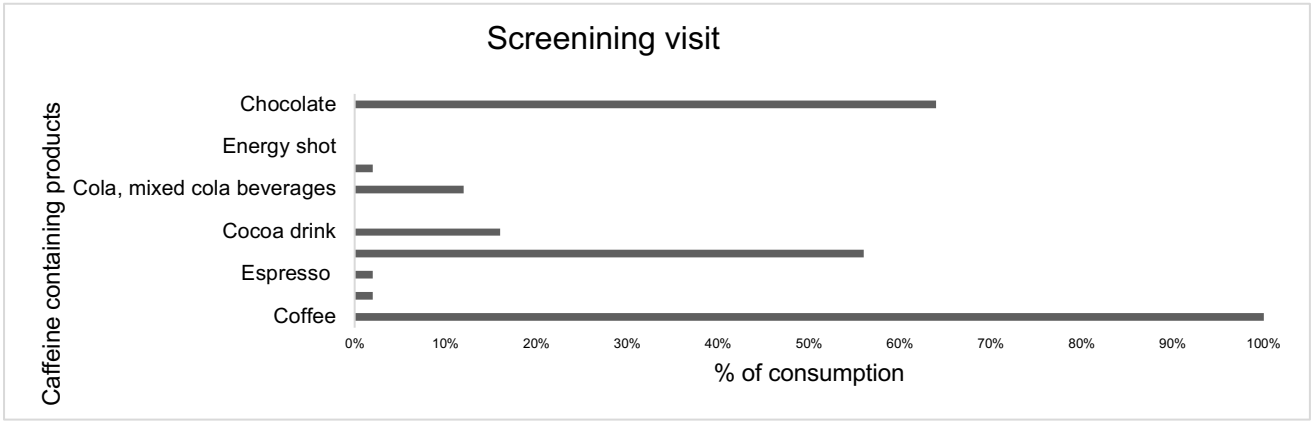
DRECNRT	Word Recognition - New Stimuli - Average Speed
DRECRT	Word Recognition - Average Speed
DRECORTM	Word Recognition - Original Stimuli - Speed: Median
DRECNRTM	Word Recognition - New Stimuli - Speed: Median
DRECRTM	Word Recognition - Speed: Median
DRECSD	Word Recognition - Standard Deviation
DRECCV	Word Recognition - Coefficient of Variance
DPICOACC	Pattern Separation - Original Stimuli - Accuracy
DPICNACC	Pattern Separation - New Stimuli - Accuracy
DPICORT	Pattern Separation - Original Stimuli - Average Speed
DPICNRT	Pattern Separation - New Stimuli - Average Speed
DPICRT	Pattern Separation - Average Speed
DPICORTM	Picture Recognition Original Stimuli - Speed: Median
DPICNRTM	Picture Recognition New Stimuli - Speed: Median
DPICRTM	Picture Recognition - Speed: Median

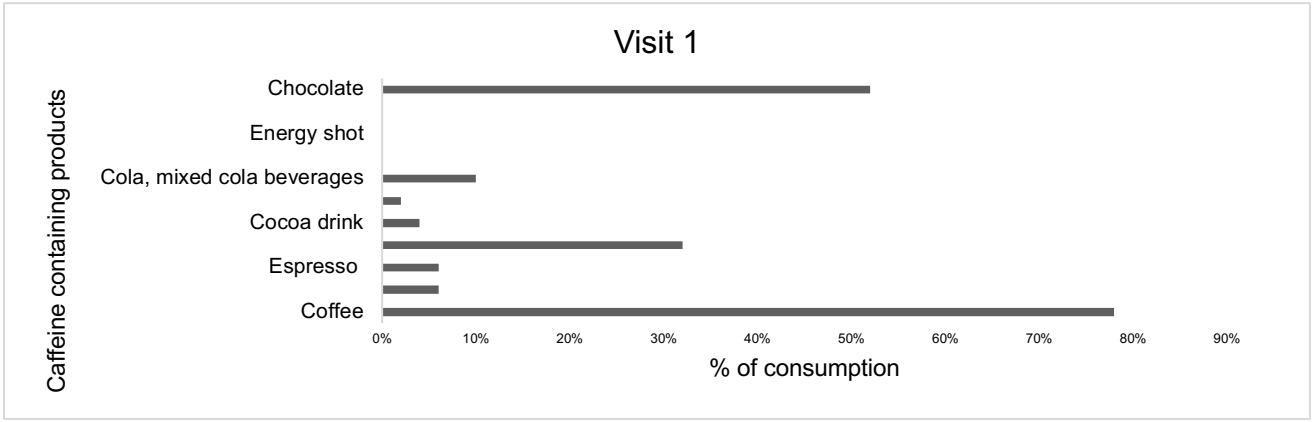
DPICSD Pattern Separation - Standard Deviation

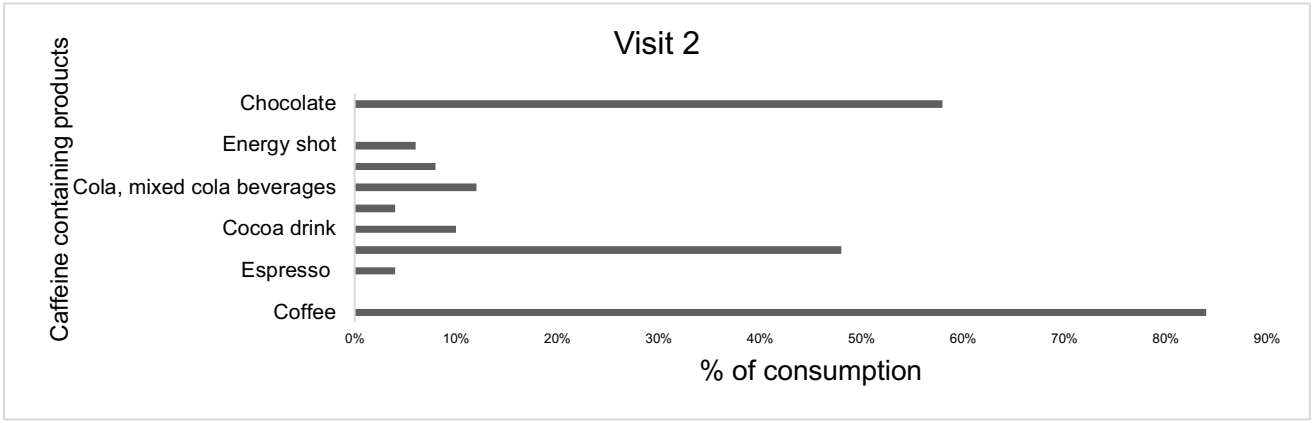
Appendix II. The initial recipe created to make the date-containing treatments and the placebo

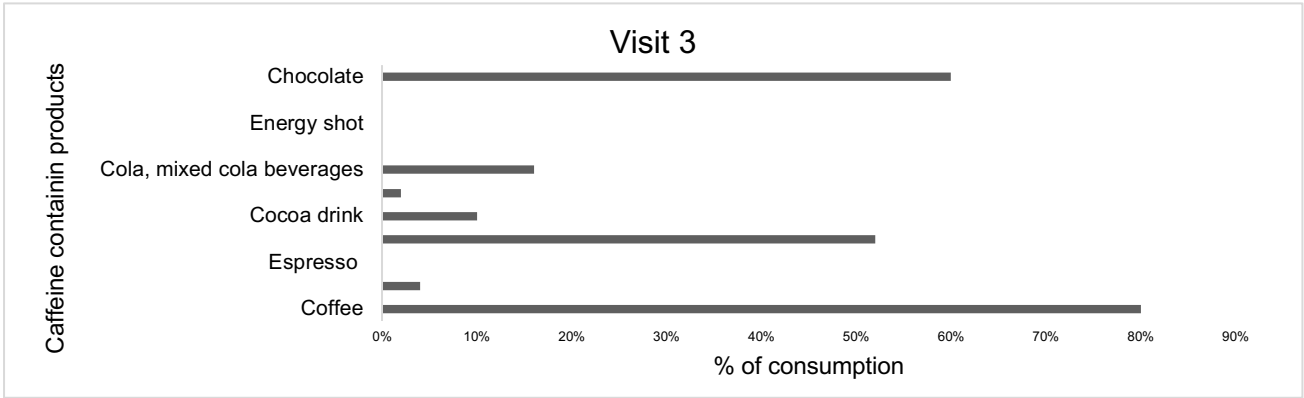
Ingredients	Barhi treatment	Khassab treatment	Placebo
Date powder	48 g	34.5 g	0 g
Water	75 ml	90 ml	0 ml
0 % fat yoghurt	150 g	150 g	150 g
Glucose	0 g	4.94 g	16.78 g
Fructose	0 g	6.48 g	15.84 g
Soluble fibres	0 g	0 g	1.04 g
Insoluble fibres	2.46 g	0 g	6.54 g
Strawberry flavour	5 drops	5 drops	5 drops
Food colouring Red	5 drops	5 drops	5 drops
Food colouring Orange	0	3 drops	3 drops

Appendix JJ. Percentage of caffeine-containing products









Appendix KK. HACCP plan for treatments making for chapter 2

The purpose of this HACCP plan:

Although a risk assessment form for the trial was made and submitted to the ethical committee prior to the start of the experiment, a HACCP plan was made due to the need of a clear plan for controlling any critical points, to facilitate the conduction of the trial and to minimise human errors. The plan aimed to incorporate the HACCP principles in a way that would help the researcher and the third party who was in charge of making the treatments, to avoid any confusion or dispersion about ‘’what shall we do’’ when random circumstances happen. For example, giving the wrong treatment to the wrong participant due to mismatching between the randomisation sheet and a participants’ codes, or by pouring the wrong treatment into the wrong labelled plate.

The HACCP system consisted of the following seven principles:

PRINCIPLE 1: Conduct a hazard analysis.

PRINCIPLE 2: Determine the Critical Control Points (CCPs).

PRINCIPLE 3: Establish critical limit(s).

PRINCIPLE 4: Establish a system to monitor control of the CCP.

PRINCIPLE 5: Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control.

PRINCIPLE 6: Establish procedures for verification to confirm that the HACCP system is working effectively.

PRINCIPLE 7: Establish documentation concerning all procedures and records appropriate to these principles and their application.

These principles have been obtained from the Food standard Agency website: (Agency)

The HACCP plan is detailed below.

HACCP plan for production of the treatments and placebo.

PROCESS STEP	HAZARD	PREVENTION PROCEDURES	CRITICAL POINTS	CORRECTIVE ACTION & RESPONSIBILITY
DELIVERY Date freeze-dried powder delivered to the NU- Food facility inspected and stored.	Impurities, damages or holes in date packages	Check all date bags for visual damages	Major visual damage to the date packages	Discard any damaged packages
STORAGE Date unloaded, inspected and weighted (into single dose) packages. Each bag air vacuumed, sealed and labelled. All packages stored in the walk-in freezer.	Impurities, damages or holes in date packages	Check all date bags for visual damages	Major visual damage to the date packages	Discard any damaged packages

PROCESS STEP	HAZARD	PREVENTION PROCEDURES	CRITICAL POINTS	CORRECTIVE ACTION & RESPONSIBILITY
<u>TREATMENT PREPERTION</u> All treatments prepared as described in the recipe. Preserved in the fridge.	Expiration in yoghurt production date	Check all yoghurt production dates	Passed the production date	Discard, and purchase new yoghurt
<u>RANDOMIZATION</u> All treatments plates should be labelled twice by a third party, on the edge of the plate and on the foil cover. All plates should be labelled according to the “Latin square” randomisation sheet.	Giving the wrong treatment to the wrong participant due to mismatching between randomisation sheet and participants’ codes	Check the Latin square randomisation sheet. Check the label on the plate Check the label on the foil cover	Uncertainty about treatments’ type, labelling	Discard the treatment Inform the researcher and record the incident
<u>SERVING TREATMENT</u> All treatments should be served within the study day.	A delay in participants’ arrival exceeding the maximum 2 hours	-	-	Discard the treatment, make a new one and Inform the researcher and record the incident

General instructions for making the treatments

All treatments must be made by a third party throughout the trial duration.

- All treatments should be made by 8:00 am in the morning prior to the researcher and the participants' arrival.
- All participants should arrive at the NU-Food facilities at 9 am to begin their baseline assessments. Treatments should be consumed between 9:20 to 9:40 am, maximum 10 am.
- It is the responsibility of the third party to label the treatment plates with the treatments' code, however, treatment codes MUST BE KEPT ANONYMOUS from the researcher, or any person involved in serving the treatments.
- It is the responsibility of the third party to label the treatment plates with the participants' ID according to the randomisation sheet allocated by Latin square.
- All dry ingredients for each treatment are weighted, labelled and preserved in plastic bags, which include the following: Khassab bags and Barhi bags. The air in these bags has been vacuumed out using an air vacuum machine, they are sealed and kept in the walk-in freezer until the trial starts.
- Other liquid materials, such as food colouring and mineral water, can be found on one of the kitchen shelves, clearly labelled with the study name.
- Plastic bowls, foil rolls and marker pens can also be found in a box which has the trial name on, this is in the pilot kitchen.
- In case any participant is late for the start time of the trial, the treatment can still be served, however, if the participant is not able to come on that particular day the treatment should be discarded, and the third party should be notified.

Treatment execution instructions

The instructions to make the treatments are straight forward and as follows:

- Weigh all dry ingredients, excluding date powder (as they have been weighted and packed in bags), and measure the water needed depending on the recipe of each treatment: Khassab, Barhi and Placebo.

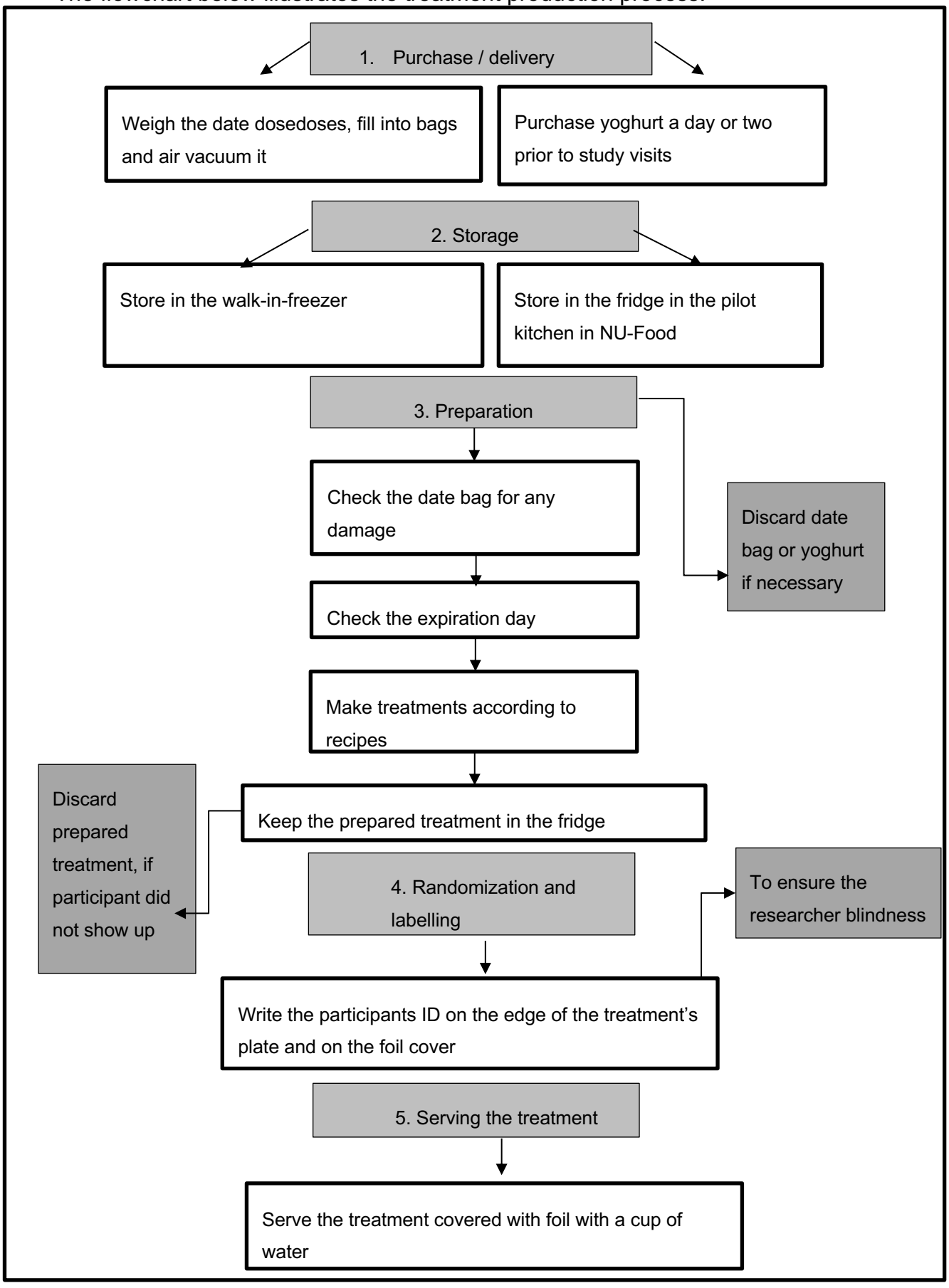
- Put in the type of date powder that you are making (Khassab or Barhi) into the plastic bowl, pour in room temperature water, mix well, add the food colouring, and all of the other dry ingredients, then mix well.
- Once the mixture is homogenised, add the yoghurt and mix well.
- Participants' ID and the treatment code should be clearly written on the treatment plate using the marker pen.
- It is the responsibility of the researcher to make sure that the Latin square sheet and the pre-labelled treatment plates are corresponding (please see HACCP plan for more information).

Latin square randomisation sheet

This randomisation sheet is made by the researcher (see Appendix V) and it's ok to be seen and checked by the researcher, however the identity of the treatments and encoding information must be kept anonymised from the researcher until the trial is completed and the data is analysed.

Making the treatments

The flowchart below illustrates the treatment production process.



Appendix LL. HACCP plan for treatments making for chapter 4

The purpose of this HACCP plan:

Although a risk assessment form for the trial has been made and submitted to the ethical committee prior to the start of this experiment, this has been made due to the need of a clear plan for controlling any critical points being needed, to facilitate the conduction of the trial and to minimise human errors. This plan is aiming to incorporate the HACCP principles in a way that can help the researcher and the third party who is in charge of making the treatment to avoid any confusion or dispersion about ‘’what shall we do’’ when random circumstances happen. For example, giving the wrong treatment to the wrong participant due to mismatching between randomisation sheet and a participants’ codes or by pouring the wrong treatment into the wrong labelled cup.

The HACCP system consists of the following seven principles:

- PRINCIPLE 1: Conduct a hazard analysis.
- PRINCIPLE 2: Determine the Critical Control Points (CCPs).
- PRINCIPLE 3: Establish critical limit(s).
- PRINCIPLE 4: Establish a system to monitor control of the CCP.
- PRINCIPLE 5: Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control.
- PRINCIPLE 6: Establish procedures for verification to confirm that the HACCP system is working effectively.
- PRINCIPLE 7: Establish documentation concerning all procedures and records appropriate to these principles and their application.

These principles have been obtained from the Food standard Agency website: https://www.food.gov.uk/business-guidance/safe-catering

Coffee processing Steps and HACCP plan in accordance.

PROCESS STEP	HAZARD	PREVENTATIVE PROCEDURES	CRITICAL CONTROL POINTS	CORRECTIVE ACTION & RESPONSIBILITY
1 **Delivery** Coffee delivered to the NU- Food facility inspected and stored	Impurities, damages or holes in the coffee package	Check all coffee bags for visual damages	Major visual damage to the coffee packages	Discard any damaged packages
2 **Storage** Coffee unloaded, inspected and weighted (into single dose) packages. Each bag air vacuumed, sealed and labelled. All packages stored in the walk-in freezer.	Impurities, damages or holes in the coffee package	Check all coffee bags for visual damages	Major visual damage to the coffee packages	Discard any damaged packages

PROCESS STEP	HAZARD	PREVENTATIVE PROCEDURES	CRITICAL CONTROL POINTS	CORRECTIVE ACTION & RESPONSIBILITY
3 **<u>Coffee preparation</u>** All coffee prepared as described in the recipe Preserved in pre-labelled thermos.	Looseness of the thermoses' lids	Close the thermos' lids tightly. Check coffee temperature consistently	Major drop in the coffee temperature by ≤ 5°C	Discard the treatment, make a new one and Inform the researcher, ensure to record the incident
4 **<u>Randomisation</u>** All three thermos flasks labelled by a third party All cups should be labelled according to "Latin square'' randomisation sheet	Giving the wrong treatment to the wrong participant due to mismatching between randomisation sheet and participants' codes Pouring the wrong treatment in the wrong labelled cup	Check the Latin square randomisation sheet Check thermos label Check cups label	Uncertainty about treatments' type, thermos' labelling or cup labelling	Discard the treatment and pour a new one in a new correct cup Inform the researcher and record the incident

PROCESS STEP	HAZARD	PREVENTATIVE PROCEDURES	CRITICAL CONTROL POINTS	CORRECTIVE ACTION & RESPONSIBILITY
5 **<u>Serving the coffee</u>** All treatments should be served within the acceptable range of treatments' temperature	A delay in participants' arrival exceeding the maximum 2 hours	Checking the thermos temperature	Major drop in the coffee temperature by ≤ 5°C	Discard the treatment, make a new one and Inform the researcher and record the incident If the third party is no longer available to make a new batch, reschedule the participant

General instructions for making the treatments

- All treatments must be made by a third party throughout the trial duration.
- All treatments should be made by 8:00 am in the morning prior to the researcher and the participants' arrivals.
- All participants should be arrived in NU-Food at 9 am to begin with their baseline assessments. Treatments should be consumed between 9:20 to 9:40 am, maximum 10 am.
- It is the responsibility of the third party to label the treatments thermoses with the treatments' code, however, treatments' codes MUST BE KEPT ANONYMS from the researcher, or any person involve in serving the treatments.
- It is the responsibility of the third party to label the treatments cups with both: the participants' ID and the treatment code, according to the randomisation sheet allocated by Latin square.
- All dry ingredients of each treatment are weighted, labelled and preserved in plastic bags, which include the following: date seeds drink bags, regular coffee bags, decaffeinated coffee bags. The air in these bags has been vacuumed out using an air vacuumed machine, sealed and kept in the walk-in freezer until the trial starts.
- Other liquid materials such as food colouring and mineral water can be found in the ladled bag with the trial name.
- Cups, lids, filters of the chine and marker pens can also be found in a box which has the trial name on, this is in the pilot kitchen.
- Date coffee and regular coffee will be made using the filter coffee machine in the pilot kitchen (there are three of them).
- Water should be added to the coffee machine, this should be room temperature water (no need to boil it).
- The coffee temperatures must be recorded consistently as following: promptly after making the coffee, promptly after pouring the coffee into the thermos and promptly after pouring the coffee into the participants' cup.
- In case any participant was late for the start time of the trial, the coffee temperature must be examined before serving it. If the temperature has dropped by (≤ 6°C) the coffee should be discarded, and a new coffee should

be made if there is time to do so (please see the flowchart within this document).

Treatment execution instructions

- The instruction to make these treatments are straight forward and as follows:
- Turn on the coffee machine and open its lid.
- Put the filter in place.
- Pour some boiled water into the thermoses to warm it up for a few minutes then get rid of the water and cover the thermoses properly until it's used for the coffee.
- Put in the type of coffee that you are making (regular or date seeds drink) to the machine, pour the room temperature water into the designated area in the machine, close the lid and press the start button.
- When the machine is finished, pour the coffee into the matching pre-labelled thermos, record the temperature, add the needed amount of the food colouring drops as illustrated in the table of ingredients and then close the thermos lid tightly.
- Repeat step 5 for the second type of coffee.
- For making the placebo treatment, boiled water is needed and should be poured promptly into the pre-labelled thermos for the placebo. Followed by adding the food colouring drops as illustrated in the table of ingredients then the thermos lid must be closed tightly.
- Participant's ID and the treatment code should be clearly written on the coffee cups using the marker pen.
- It is the responsibility of the researcher to make sure that the Latin square sheet and the pre-labelled coffee cups are corresponding (please see HACCP plan for more information).

Treatment temperature

The treatments' temperature should be as in the table below, and the temperature of every prepared cup of treatment should be measure and recorded.

Treatment temperature guidelines.

The temperature of the coffee promptly after making it	The temperature of the coffee when serving it	Acceptable range	Unacceptable range
65ºC	61ºC	60-56ºC	55ºC and less

The thermos should preserve the coffee temperature for a maximum duration of one hour, which is more than sufficient for the participant to finish the baseline assessments. In case of any delay caused by a late arrival of the participants, please refer to the HACCP plan.

Latin square randomisation sheet

This randomisation sheet is made by the researcher (see Appendix V) and it's ok to be seen and checked by the researcher, however the identity of the treatments and encoding information must be kept anonymised from the researcher until the trial is completed and the data is analysed.

Temperature recording sheet

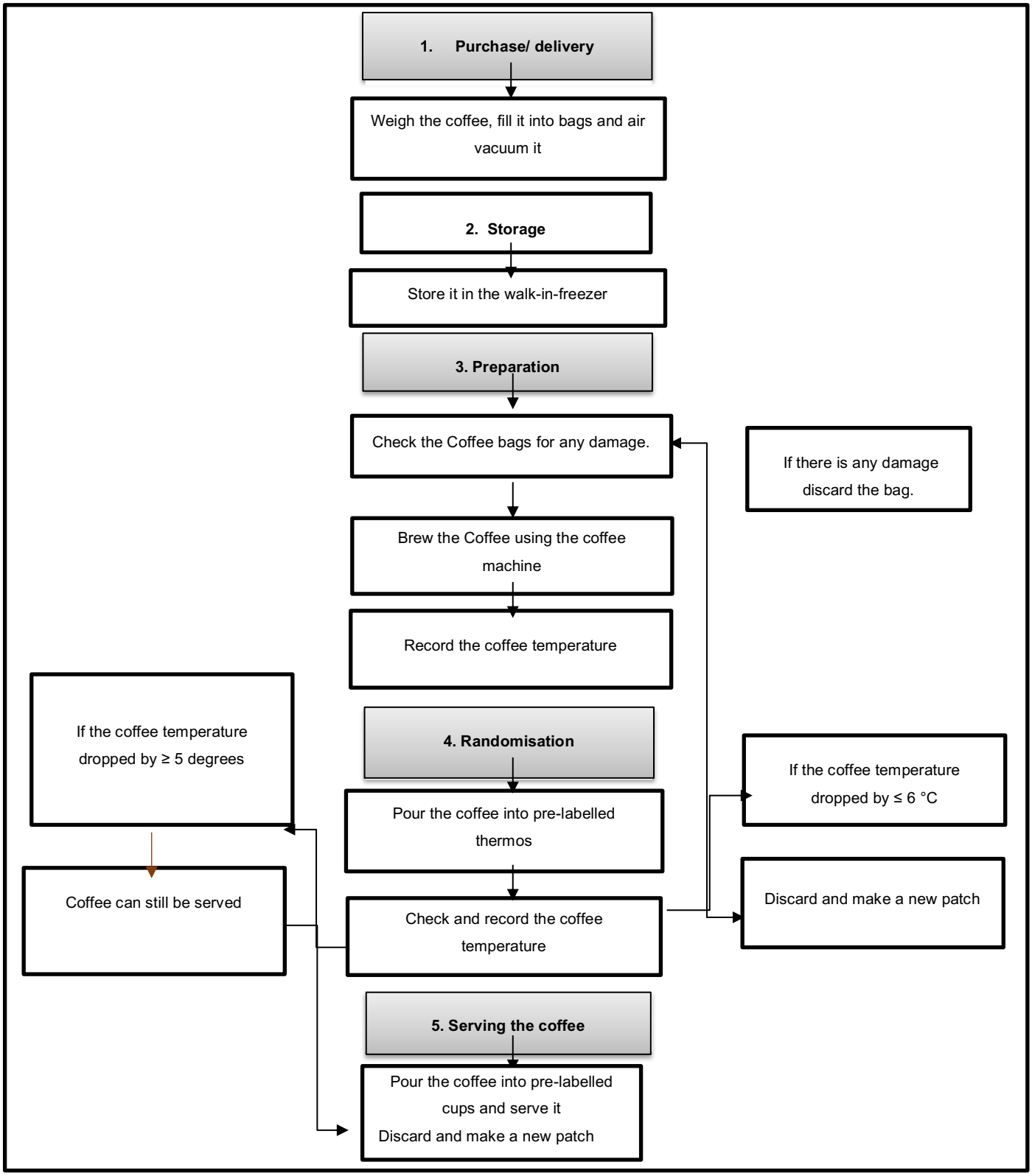
The temperature of each cup of treatment should be recorded in the table below.

Temperature recording sheet.

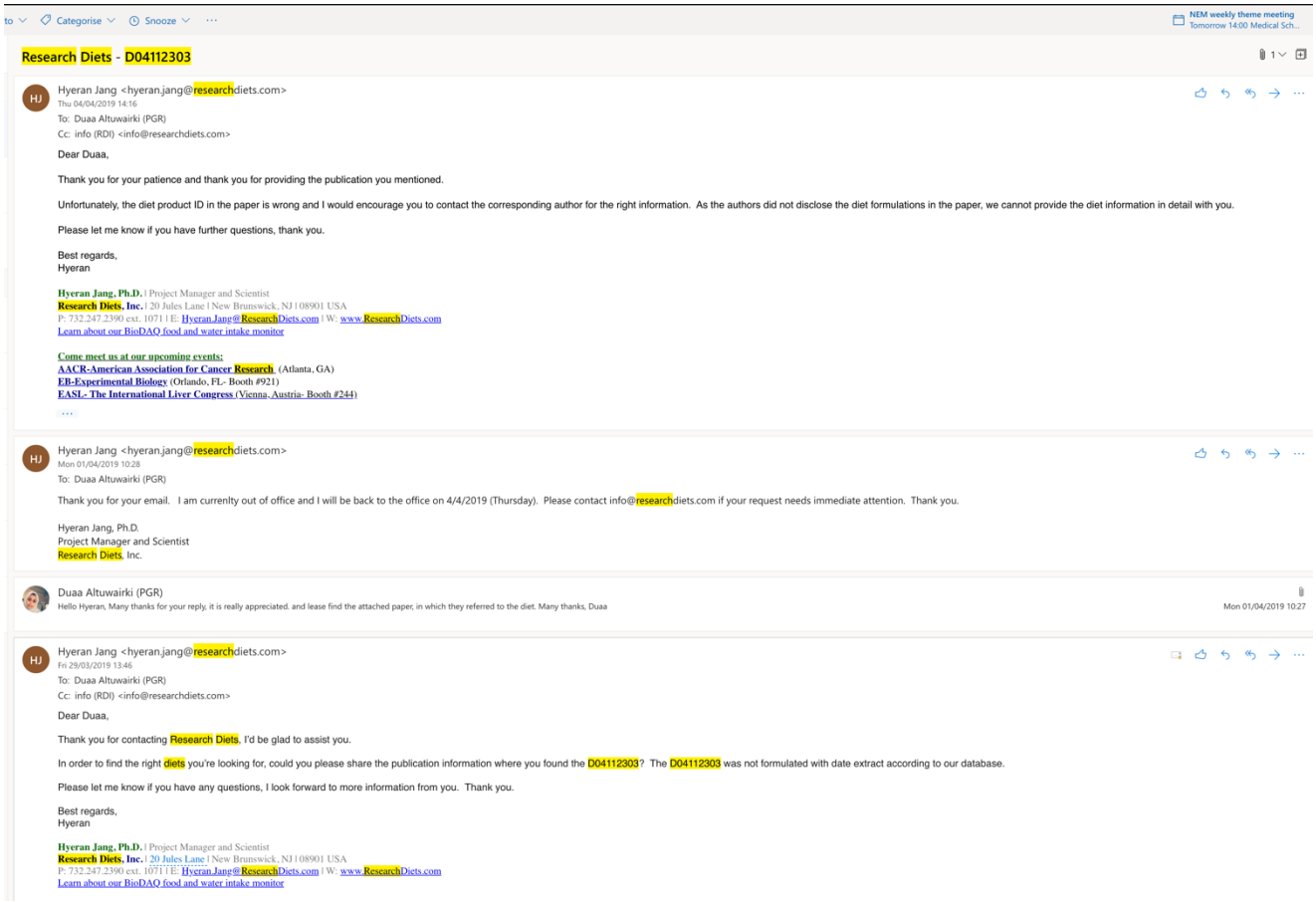
Date of the visit	Temperature when it is made	Temperature when it is served	Emergency cases (details)

Making the treatment

The flowchart below illustrates the treatment production process.


1. Purchase/ delivery
Weigh the coffee, fill it into bags and air vacuum it
2. Storage
Store it in the walk-in-freezer
3. Preparation
Check the Coffee bags for any damage.
If there is any damage discard the bag.
Brew the Coffee using the coffee machine
Record the coffee temperature
If the coffee temperature dropped by ≥ 5 degrees
4. Randomisation
If the coffee temperature dropped by ≤ 6 °C
Pour the coffee into pre-labelled thermos
Coffee can still be served
Check and record the coffee temperature
Discard and make a new patch
5. Serving the coffee
Pour the coffee into pre-labelled cups and serve it
Discard and make a new patch

Appendix MM. The reply email from the Research Diet Inc. NJ, USA



to Categorise Snooze ···

NEM weekly theme meeting
Tomorrow 14:00 Medical Sch...

Research Diets - D04112303

1

HJ Hyeran Jang <hyeran.jang@researchdiets.com>
Thu 04/04/2019 14:16
To: Duaa Altuwairki (PGR)
Cc: info (RDI) <info@researchdiets.com>

Dear Duaa,

Thank you for your patience and thank you for providing the publication you mentioned.

Unfortunately, the diet product ID in the paper is wrong and I would encourage you to contact the corresponding author for the right information. As the authors did not disclose the diet formulations in the paper, we cannot provide the diet information in detail with you.

Please let me know if you have further questions, thank you.

Best regards,
Hyeran

Hyeran Jang, Ph.D. | Project Manager and Scientist
Research Diets, Inc. | 20 Jules Lane | New Brunswick, NJ 08901 USA
P: 732.247.2390 ext. 1071 | E: Hyeran.Jang@ResearchDiets.com | W: www.ResearchDiets.com
Learn about our BioDAQ food and water intake monitor

Come meet us at our upcoming events:
AACR-American Association for Cancer Research (Atlanta, GA)
EB-Experimental Biology (Orlando, FL- Booth #921)
EASL- The International Liver Congress (Vienna, Austria- Booth #244)

HJ Hyeran Jang <hyeran.jang@researchdiets.com>
Mon 01/04/2019 10:28
To: Duaa Altuwairki (PGR)

Thank you for your email. I am currenlty out of office and I will be back to the office on 4/4/2019 (Thursday). Please contact info@researchdiets.com if your request needs immediate attention. Thank you.

Hyeran Jang, Ph.D.
Project Manager and Scientist
Research Diets, Inc.

Duaa Altuwairki (PGR)
Hello Hyeran, Many thanks for your reply, it is really appreciated. and lease find the attached paper, in which they referred to the diet. Many thanks, Duaa
Mon 01/04/2019 10:27

HJ Hyeran Jang <hyeran.jang@researchdiets.com>
Fri 29/03/2019 13:46
To: Duaa Altuwairki (PGR)
Cc: info (RDI) <info@researchdiets.com>

Dear Duaa,

Thank you for contacting Research Diets. I'd be glad to assist you.

In order to find the right diets you're looking for, could you please share the publication information where you found the D04112303? The D04112303 was not formulated with date extract according to our database.

Please let me know if you have any questions, I look forward to more information from you. Thank you.

Best regards,
Hyeran

Hyeran Jang, Ph.D. | Project Manager and Scientist
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Appendix NN. Correlation matrix analysis for the mood and cognitive scores for indices and blood glucose levels.

Correlations

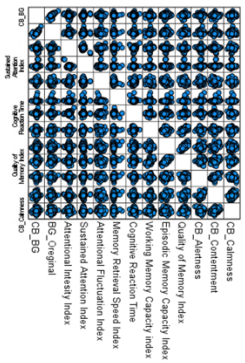
```
[DataSet1] H:\DUAA\first cognition trial\trial no. 1\latest data sheets\21-5-2018 after salah\new restructured data 7-6-2018\Analysis with Ant\Full Data after Ant 12-3-2019.sav
```

Correlations

		CB_BG	Attentional Intesity Index	Sustained Attention Index	Attentional Fluctuation Index	Memory Retrieval Speed Index	Cognitive Reaction Time	Working Memory Capacity index	Episodic Memory Capacity Index	Quality of Memory Index	CB_Alertness	CB_Contentment	CB_Calmness
CB_BG	Pearson Correlation	1	-.052	.181**	.053	.039	-.008	.026	.057	.072	.011	.035	.066
	Sig. (2-tailed)		.358	.001	.345	.489	.888	.644	.312	.204	.847	.537	.248
	N	315	315	315	315	315	315	315	315	315	309	309	309
Attentional Intesity Index	Pearson Correlation	-.052	1	-.391**	.199**	.116*	.096	.041	-.077	-.045	-.078	-.033	-.014
	Sig. (2-tailed)	.358		.000	.000	.040	.089	.464	.173	.426	.170	.565	.811
	N	315	315	315	315	315	315	315	315	315	309	309	309
Sustained Attention Index	Pearson Correlation	.181**	-.391**	1	-.021	.074	.043	-.023	-.016	-.021	.074	.118*	.038
	Sig. (2-tailed)	.001	.000		.713	.187	.448	.682	.775	.716	.194	.039	.503
	N	315	315	315	315	315	315	315	315	315	309	309	309
Attentional Fluctuation Index	Pearson Correlation	.053	.199**	-.021	1	-.164**	.124*	-.071	-.119*	-.120*	.095	-.083	-.046
	Sig. (2-tailed)	.345	.000	.713		.003	.028	.211	.035	.033	.095	.147	.418
	N	315	315	315	315	315	315	315	315	315	309	309	309
Memory Retrieval Speed Index	Pearson Correlation	.039	.116*	.074	-.164**	1	-.130*	-.012	.183**	.061	.133*	.238**	.056
	Sig. (2-tailed)	.489	.040	.187	.003		.021	.829	.001	.280	.020	.000	.329
	N	315	315	315	315	315	315	315	315	315	309	309	309
Cognitive Reaction Time	Pearson Correlation	-.008	.096	.043	.124*	-.130*	1	.038	.012	.097	.017	-.037	-.022
	Sig. (2-tailed)	.888	.089	.448	.028	.021		.502	.826	.085	.772	.511	.703
	N	315	315	315	315	315	315	315	315	315	309	309	309
Working Memory Capacity index	Pearson Correlation	.026	.041	-.023	-.071	-.012	.038	1	.048	.536**	-.101	-.021	.057
	Sig. (2-tailed)	.644	.464	.682	.211	.829	.502		.391	.000	.077	.710	.318
	N	315	315	315	315	315	315	315	315	315	309	309	309
Episodic Memory Capacity Index	Pearson Correlation	.057	-.077	-.016	-.119*	.183**	.012	.048	1	.734**	.146*	.274**	.053
	Sig. (2-tailed)	.312	.173	.775	.035	.001	.826	.391		.000	.010	.000	.353
	N	315	315	315	315	315	315	315	315	315	309	309	309
Quality of Memory Index	Pearson Correlation	.072	-.045	-.021	-.120*	.061	.097	.536**	.734**	1	.038	.180**	.102
	Sig. (2-tailed)	.204	.426	.716	.033	.280	.085	.000	.000		.510	.001	.074
	N	315	315	315	315	315	315	315	315	315	309	309	309
CB_Alertness	Pearson Correlation	.011	-.078	.074	.095	.133*	.017	-.101	.146*	.038	1	.549**	-.063
	Sig. (2-tailed)	.847	.170	.194	.095	.020	.772	.077	.010	.510		.000	.271
	N	309	309	309	309	309	309	309	309	309	309	309	309
CB_Contentment	Pearson Correlation	.035	-.033	.118*	-.083	.238**	-.037	-.021	.274**	.180**	.549**	1	.323**
	Sig. (2-tailed)	.537	.565	.039	.147	.000	.511	.710	.000	.001	.000		.000
	N	309	309	309	309	309	309	309	309	309	309	309	309
CB_Calmness	Pearson Correlation	.066	-.014	.038	-.046	.056	-.022	.057	.053	.102	-.063	.323**	1
	Sig. (2-tailed)	.248	.811	.503	.418	.329	.703	.318	.353	.074	.271	.000	
	N	309	309	309	309	309	309	309	309	309	309	309	309

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).




```
GRAPH
  /SCATTERPLOT(MATRIX)=CB_BG Sustained_Attention_Index
  /MISSING=LISTWISE.
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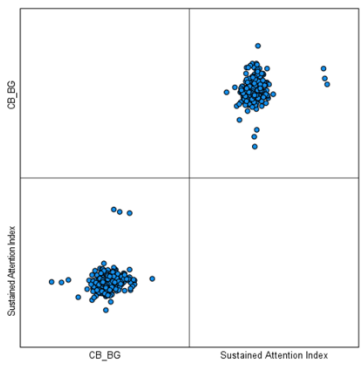
Graph



Appendix OO. Correlation matrix analysis for the outcomes of the individual tasks of cognitive and blood glucose levels.

Correlations

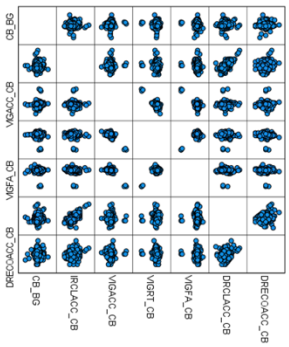
Correlations

		CB_BG	IRCLACC_CB	VIGACC_CB	VIGRT_CB	VIGFA_CB	DRCLACC_CB	DRECOACC_CB
CB_BG	Pearson Correlation	1	.013	.117*	-.034	-.063	.049	.054
	Sig. (2-tailed)		.818	.037	.542	.265	.383	.340
	N	315	315	315	315	315	315	315
IRCLACC_CB	Pearson Correlation	.013	1	.040	-.043	-.074	.683**	.196**
	Sig. (2-tailed)	.818		.478	.450	.189	.000	.000
	N	315	315	315	315	315	315	315
VIGACC_CB	Pearson Correlation	.117*	.040	1	-.650**	-.604**	.007	.044
	Sig. (2-tailed)	.037	.478		.000	.000	.902	.438
	N	315	315	315	315	315	315	315
VIGRT_CB	Pearson Correlation	-.034	-.043	-.650**	1	.549**	-.017	-.099
	Sig. (2-tailed)	.542	.450	.000		.000	.763	.078
	N	315	315	315	315	315	315	315
VIGFA_CB	Pearson Correlation	-.063	-.074	-.604**	.549**	1	-.002	-.040
	Sig. (2-tailed)	.265	.189	.000	.000		.971	.478
	N	315	315	315	315	315	315	315
DRCLACC_CB	Pearson Correlation	.049	.683**	.007	-.017	-.002	1	.233**
	Sig. (2-tailed)	.383	.000	.902	.763	.971		.000
	N	315	315	315	315	315	315	315
DRECOACC_CB	Pearson Correlation	.054	.196**	.044	-.099	-.040	.233**	1
	Sig. (2-tailed)	.340	.000	.438	.078	.478	.000	
	N	315	315	315	315	315	315	315

*. Correlation is significant at the 0.05 level (2-tailed).

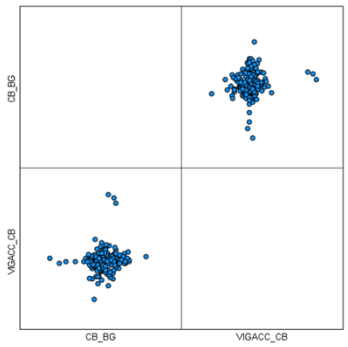
**. Correlation is significant at the 0.01 level (2-tailed).

Graph

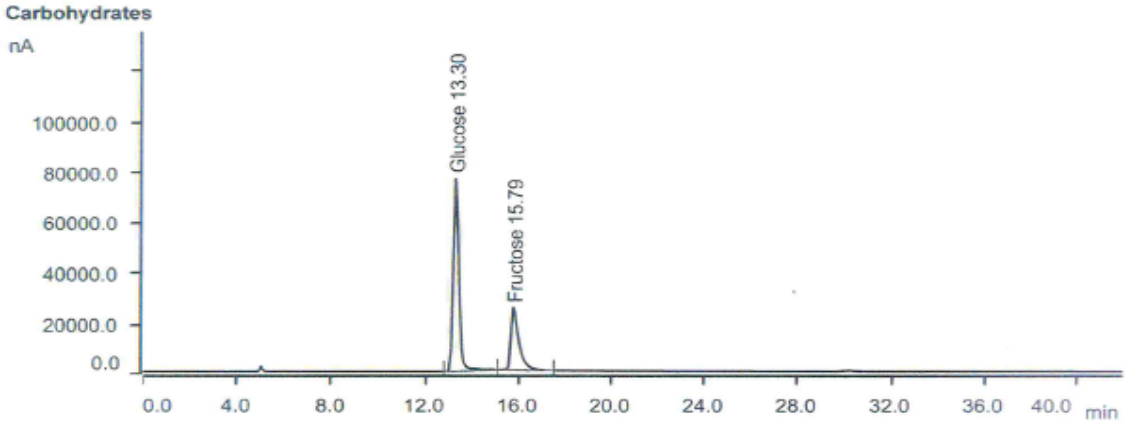



```
GRAPH
  /SCATTERPLOT(MATRIX)=CB_BG VIGACC_CB
  /MISSING=LISTWISE.
```

Graph



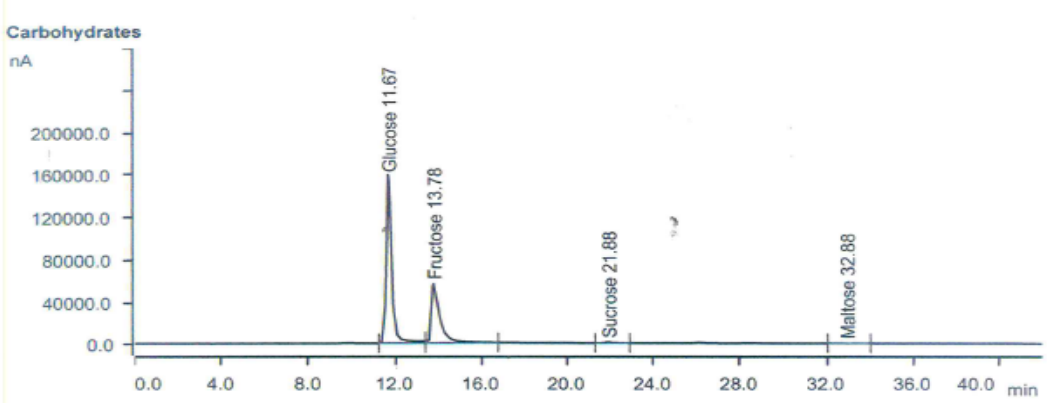
Appendix PP. Sugar quantification and identification chromatogram conducted by Huson & Hardwick Laboratories



Peak number	Component name	Retention time min	Area (nA) x min	Height nA	Concentration ppm	Calculated value %	LOD ppb
1	Glucose	13.297	21608.0856	76431.865	612.226	34.241	65.475
2	Fructose	15.790	9902.5849	25034.541	483.483	27.040	157.863

61.281%

Chromatogram for Barhi



Peak number	Component name	Retention time min	Area (nA) x min	Height nA	Concentration ppm	Calculated value %	LOD ppb
1	Glucose	11.675	45871.7137	157990.277	1753.237	38.497	91.065
2	Fructose	13.783	26035.0326	55684.356	1654.393	36.327	243.808
3	Sucrose	21.884	337.3276	616.411	72.879	1.600	970.226
4	Maltose	32.880	54.1618	58.575	78.750	1.729	11032.675

48.155%

Chromatogram for Khassab

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