Nutritional studies with food vehicles containing seaweed and seaweed bio actives: effects on plasma lipids and glucose

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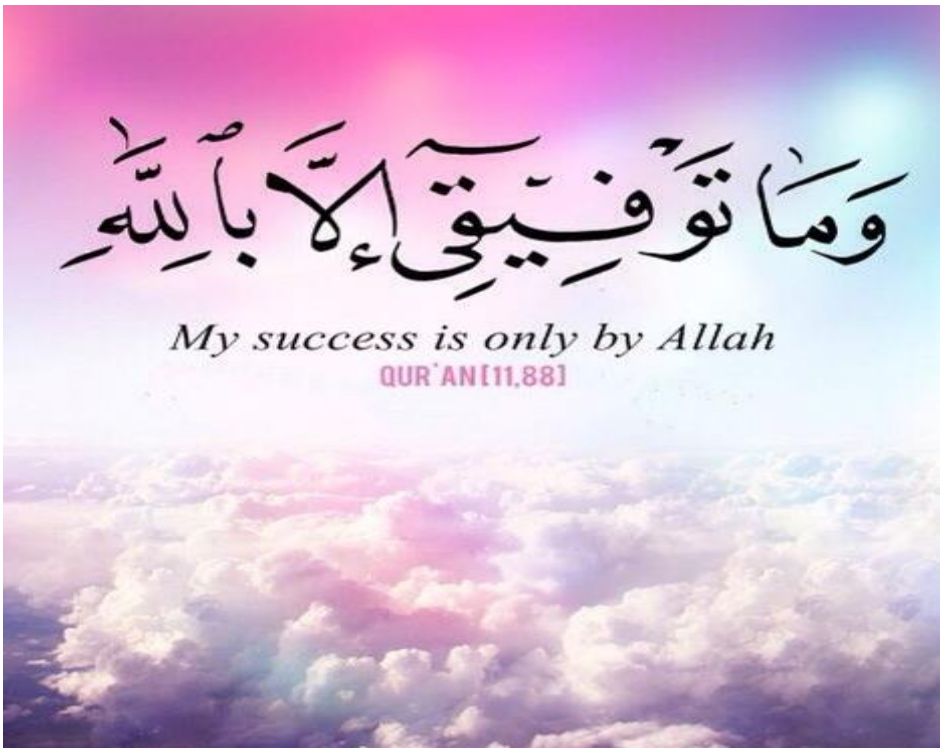
In The Name of Allah, The Most Beneficent, The Most Merciful



Abstract

Evidence would indicate that obesity, as a public health problem, is rising. It is associated with several non-communicable diseases, lower psychosocial wellbeing, and elevated risk of death.

Brown seaweeds and their extract alginates have shown lipase inhibition, attenuating dietary fat digestion, absorption, and could be a possible agent for reducing obesity. Work from our lab has shown significant inhibition of pancreatic lipase action *in vitro* by specific alginate. Importantly, alginate has the potential to be a natural alternative for Orlistat (a lipase inhibitor), an anti-obesity drug which has gastrointestinal (GI) side effects.

Therefore, the focus of this thesis has been on the addition of brown marine non-digestible polysaccharides (alginate) into enriched fat food products such as cheese and pork sausage, maintaining food acceptability and gastrointestinal wellbeing. This aim was achieved through two human intervention studies to assess the acceptability and ease of habitual diet incorporation of novel cheese and pork sausage containing brown seaweed and alginate. Their effect on gastrointestinal wellbeing functions was also examined. In the first study, the participants consumed seaweed, alginate, and control cheese weekly and in the second the participants consumed alginate and control sausages weekly. The results showed no significant changes in macronutrients intake of calories, carbohydrate, fat, and protein and no significant change to the GI wellbeing such as fullness, bloatedness, flatulence, anxiousness, and fitness. However, fibre intake in the alginate sausage week was significantly increased. It was concluded that consuming alginate and seaweed cheese and sausage were acceptable and had no impact on GI wellbeing.

An acute feeding of alginate and control pork sausage meal that investigated the fat and carbohydrate digestion showed that alginate attenuated serum triglycerides concentration in a small sample of healthy participants with no effect on plasma glucose.

The static *in vitro* digestion of enriched alginate foods carried out by the synthetic model gut system, allowed for measurement of glycerol released and alginate quantification. This shows a significant reduction in fat digestion by the seaweed cheese.

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Declaration

The author declares that the work submitted in this PhD thesis is based on research performed at the Institute for Biosciences Newcastle University, has not been submitted for any other award. The work is my own work include the laboratory experiments, data, and result analysis such as collecting the participant's food diaries, sort out and collect the wellbeing questionnaire for each day and week. Reference has been made to the work of others.

Each ethical clearance for the research presented in this thesis has been approved and granted by the Faculty Ethics Committee, Medical school at Newcastle University.

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List of Published Abstracts and Conference Communications

Invitation to speak at ICaBM Away Day16th October 2019, " Studies with food vehicles Containing Seaweed and Seaweed Bioactives " Crown Plaza Hotel.

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Fallatah, Muna. K., M.K, Wilcox, M., Chater, P., Ward, C., & Pearson, J. January 2019. Evaluating the effect of seaweed and alginate enriched cheeses on wellbeing outcomes in a nutritional intervention study (pilot study). ***Endocrine Abstracts (2019).*** The obesity-update-2019 Conference.

Fallatah, Muna. K., Wilcox, M., Chater, P., Ward, C., & Pearson, J. August 2018. Acceptability studies of seaweed and alginate enriched cheeses and their influence on gastrointestinal wellbeing. ***International Journal of Obesity*** Supplements volume 8. The 5th UK Congress on Obesity, Newcastle University.

Poster presented at conference, " Investigation to determine the acceptability of cheese containing seaweed extracts " at the North East Postgraduate Conference 2017, Newcastle.

Poster presented at conference, ‘'Acceptability studies of cheese containing seaweed and alginate " held at the Human Nutrition Research Centre’s, Research Day Conference 2017, Newcastle University.

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List of Abbreviations

AB	Alginate Bread
Alg ch	Alginate Cheese
Alg Ps	Alginate Pork Sausage
ANOVA	Analysis of variance
AUC	Area under the curve
BMI	Body mass index
BMR	Basal metabolic rate
CB	Control Bread
$CaCl_2.2H_2O$	Calcium chloride
C ch	Control Cheese
C Ps	Control Pork Sausage
DH_2O	Deionised water
FFA	Free fatty acids
FMC	Food Machinery and chemical Corporation Company
FTO genotype	(the fat mass and obesity associated gene)
GA	The Glycerol Assay
GI	Gastrointestinal tract
HCL	Hydrochloric acid
iAUC	Incremental area under the curve
Kcal	Kilocalorie
KCl	Potassium chloride
KH_2PO_4	Potassium di-hydrogen phosphate
MGS	Model Gut Solution
MG	Model Gut *in vitro*
mg	Milligram
min	Minute
ml	Milliliter
NA	No data available
Na	Sodium
NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate
NaOH	Sodium hydroxide

ns	No Significant Correlation
OD	Optical Density
P	Confidence value
PAS	Periodic Acid Schiff's Assay
PGA	Polyglycolic acid combined with sodium alginate
PGX	PolyGlycopleX
r	Pearsons correlation coefficient
SD	Standard deviation
SI	Small intestine
Sw ch	Seaweed Cheese
T2DM	Type 2 diabetes mellitus
TAGs	Triacylglycerols
VAS	Visual analogue scales
WBGI-Q	Well-being Gastrointestinal Health Questionnaire
WHO	World Health Organization

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Chapter 1: Introduction & Literature Review

1.1 Overview of obesity and nutritional aspects

In human nutrition, diet and food choices are important aspects in maintaining an adequate level of health, well-being and resistance to diseases. Macronutrients that enhance the feeling of fullness (satiety) and reduce dietary consumption could positively influence and maintain body weight (Clark and Slavin, 2013). Therefore, achieving a suitable balance between the energy consumed from a variety of foods, with smart food choices, and the energy released from physical activity is important to maintain sustained health and support weight reduction (Hill *et al.*, 2012).

Currently, there is a surfeit of obesogenic foods and increased consumption of these high calorie foods is a key factor implicated in increasing obesity (Swinburn *et al.*, 2011). The addition of seaweeds to diet foods to reduce body weight has been associated with adequate body weight management and results in a greater intake of seaweed macronutrients such as proteins and dietary fibre, with a low lipid intake (Mohamed *et al.*, 2012). Therefore, it is necessary to provide a safe, suitable dietary candidate derived from natural ingredients able to reduce weight gain and obesity that can be used collaboratively with an adequate level of physical activity together with a reduced intake of energy rich foods (Grove and Lambert, 2010). Additionally, based on the fact that weight loss medications which effectively decrease the desire to eat and reduce lipid hydrolysis, and therefore the quantity of fat absorbed (Guzman *et al.*, 2014), have been associated with an adverse impact on human health and are expensive. There is a need to find a suitable, effective and safe weight-reducing agent with high nutritional properties extracted from marine natural products. Seaweed bio-actives including alginate, fucoidans, fucoxanthin and phlorotannins, have a possible role in protecting against obesity (Yun, 2010; Lange *et al.*, 2015; Wan-Loy and Siew-Moi, 2016).

There is a scarcity of human intervention studies that investigate the acceptability of food products containing seaweed or their extracts such as alginate, and their effects on health indicators. This PhD research took an *in vivo* approach to investigate the acceptability of selected foods products with added seaweed/alginate (cheese and pork sausage) on a healthy subject and their effects on well-being. I also investigated whether ingestion of alginate enriched food in an acute feeding study given as a meal has an effect on the postprandial release of triglyceride (TAG) and blood glucose in a healthy population which could assist as a

potential obesity treatment. Moreover, using an *in vitro* approach, the study looked at the artificial digestion of the incorporated seaweed and alginate food products in a model gut system that has been developed at Newcastle University. Further applications based on the model gut digestion were conducted including measurements of glycerol release as a bio-indicator of fat digestibility as well as the PAS assay which is able to quantify the amounts released from seaweed and alginate throughout the *in vitro* digestion.

1.2 Prevalence of obesity in the world and in the UK

Obesity is a crisis, and the World Health Organization (WHO) stated that throughout the three decades from 1980 to 2008, the obesity rate has increased remarkably by roughly two-fold and that higher than 10% of adults are obese. Additionally, evidence exists that this global increase in obesity tripled within four decades from 1975 until 2016 and is now estimated at 13% of the worldwide adult population in association with a serious increase in childhood and adolescent obesity. It is estimated that more than 340 million cases occurred in 2016 with over 41 million cases in children under five and this is distributed in low, middle and high-income countries (World Health Organisation, 2015).

1.3 Obesity and major comorbidities

1.3.1 Definition

Obesity is a health problem that can be expressed as a metabolic dysfunction resulting in elevated body weight gain due to increased abdominal accumulation of body fat mass leading to many adverse health consequences and lower life expectancy (Haslam and James, 2005).

The relative accumulation of adiposity caused many complications.

1.3.2 Non- communicable diseases and metabolic complications of obesity

In modern medicine, an excess of adipose tissue influences the development of obesity and its related outcomes, mainly central obesity, which is inextricably linked to fatal metabolic markers and various other health complications including: type 2 diabetes, cardiovascular diseases, hypertension, myocardial infarction, asthma, osteoarthritis, reduced life span, non-alcoholic fatty liver disease, several types of cancer, metabolic syndrome and many other disorders such as elevated risk of gastrointestinal reflux disease, renal failure, hyperlipidaemia, hypogonadism.

It has been found that the annual incidence of these various clinical disorders causes about 3.4 million individual deaths (Lindgren *et al.*, 2009; Glandt and Raz, 2011; Catanzaro *et al.*, 2016).

Moreover, obesity has been strongly correlated with many serious parameters of metabolic syndrome that contribute to the progression of this major health burden such as the existence of central obesity, elevated triglyceride levels, increased blood pressure, increased fasting glucose levels and a decreased concentration of high-density lipoprotein. All these metabolic abnormalities commonly occur in a subgroup of obese individuals (Torgerson *et al.*, 2004; Alberti *et al.*, 2006).

1.3.3 Obesity as a major burden

More importantly, obesity and its accompanying health problems are causally linked to a significant health burden in the world. There are statistics to support the concept that obesity and its attributable adverse implications are a huge problem in the world. According to a global systematic economic review, excess adiposity has been associated with 0.7 – 2.8% increase of spending on obese individual's healthcare and with a 30% increased cost compared with spending on the non-obese population. Additionally, in the USA, the financial burden of medical care for the obese and overweight population continues to increase by two-fold each decade. Obesity will contribute to about 16 -18% of the overall American medical care costs by 2030. Likewise, in the UK, in 2007, the UK's Science Foresight Programme stated that increased obesity will contribute to be a greater health-care burden for the National Health Service accounting for up to £ 5.5 billion in 2050(Wang *et al.*, 2011; Economos and Blondin, 2014).

1.4 Causes of obesity

Despite that one of the fundamental factors leading to obesity appears to be consuming a rich caloric diet in conjunction with lower energy expenditure. There are several complex interplaying factors that contribute to excessive adiposity; there is an interaction between hereditary, biological, psychological, financial, environmental, political, and social components, all of which are involved in and result in substantial body weight gain (Wright and Aronne, 2012).

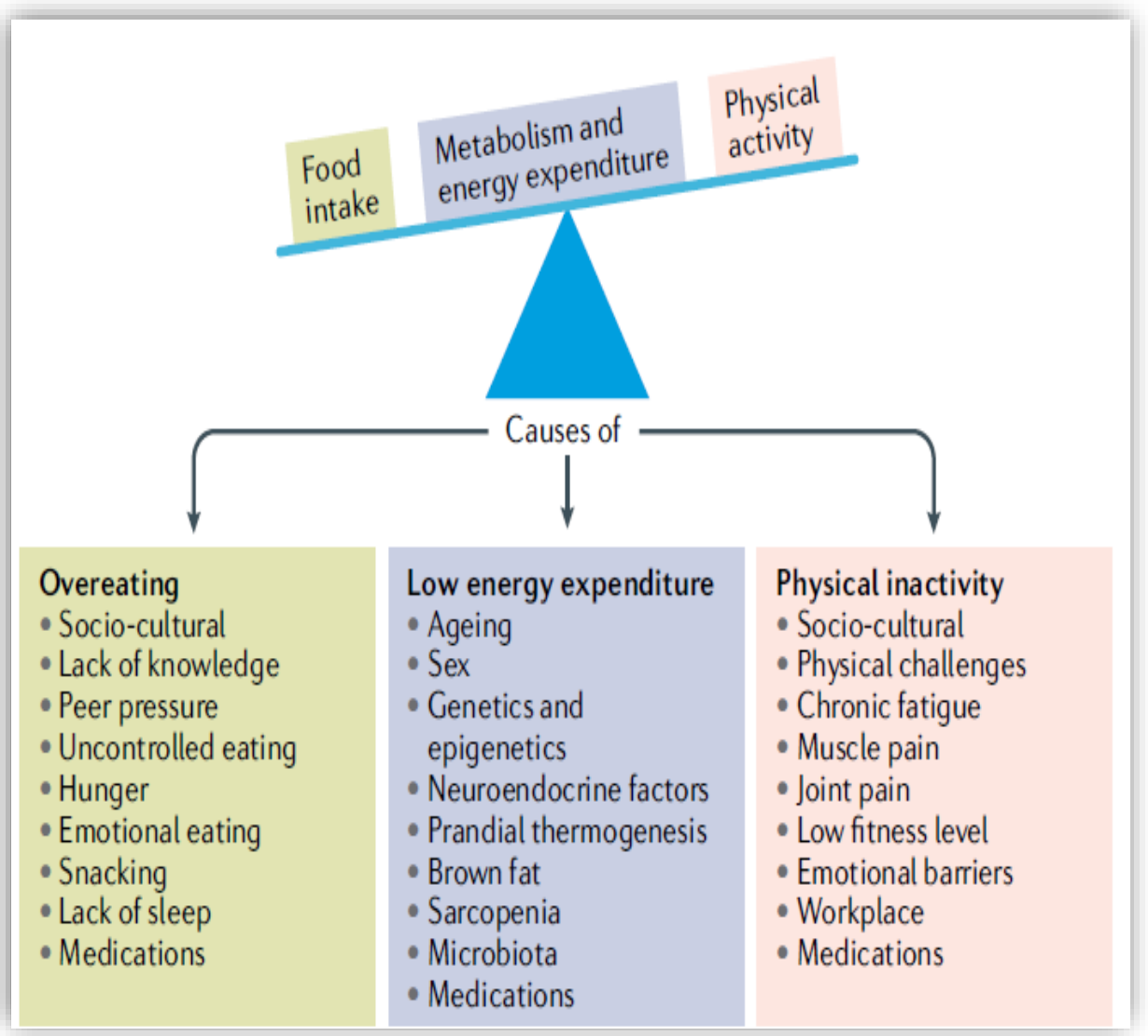


Figure 1.1 Factors that are responsible for obesity when the energy balance is altered. This figure is adapted from Blüher, 2019.

As can be seen in **Figure 1.1**, several factors are responsible for an increased body weight. This results from interactions between increased energy consumption and decreased energy expenditure in association with low physical activity level. All these factors are considered to contribute to this phenomenon including;

1.4.1 Genetics

There is evidence reported that some genes have an impact on the distribution of adipose tissue (Lindgren *et al.*, 2009) and genotype has been determined to be strongly associated with the occurrence of obesity. However, several publications have stated that there is little evidence to show that epigenetic changes are related to an increased risk of obesity and that hereditary phenotypic changes and the risk of obesity are not linked to genetics reviewed in Willyard 2014)(Willyard, 2014).

In a systematic review and meta-analysis of eight studies which used a large number of obese subjects (9563 adults) who were carrying the FTO (the fat mass and obesity associated gene) genotype to examine the influence of this genotype on body weight reduction relationship to food intake, weight loss medication and physical exercise trials. Randomised controlled trials found that there was no association between carrying this genotype and losing body weight generated by lifestyle and behavioural interventions (Livingstone *et al.*, 2016).

1.4.2 Insulin resistance

In diabetes mellitus and obesity pathology, one such factor is that adipose tissue acts as an endocrine organ which is responsible for the release of hormones that modulate metabolism and appetite activities (Kershaw and Flier, 2004). Leptin, adipose-derived protein, is available in an increased rate within obese individuals (Friedman, 2002), which significantly contribute to insulin resistance (Lazar, 2005).

1.4.3 Dietary environment and sedentary behaviour

An increase in the frequency of consumption of a rich calorific diet with lower energy expenditure, undoubtedly, is a leading cause of increased obesity. Many factors have been attributed to increasing obesity; an elevated consumption of high-energy foods which are normally abundantly available is a causative factor for obesity (Rolls *et al.*, 2006). An elevated consumption of dietary macronutrients especially a calorific rich, sugary, fatty diet especially high in saturated fat and containing low levels of fruits and vegetables, in conjunction with lower energy expenditure due to a less active life style has a significant influence on increasing this obesity epidemic (Huebbe *et al.*, 2017).

1.5 Treatment of Obesity

1.5.1 Dietary approaches

Controlling obesity can be achieved by diet changes and physical activity. On a review of the results from long-term diet-induced weight loss studies and evaluation of which type of dietary pattern and anti-obesity intervention were used, it was revealed that the success of dieting as a long-term treatment of obesity was just 15% (Ayyad and Andersen, 2000). Also, a dietary intervention study with overweight-obese postmenopausal women, where they were told to increase their consumption of fruits and vegetables, demonstrated a significant body weight reduction but not as much as a group advised to reduce intake of high fat foods (Lapointe *et al.*, 2010). Thus, the consumption of diets low in calories from fat which are particularly designed to enhance the satiety level, can be considered important candidates to reduce body weight and to reduce nutrient uptake (Pelkman *et al.*, 2007). Many studies have demonstrated that a weight reduction of around 5 – 10 % resulted in enhanced metabolic parameters and lower the risk of T2DM (type 2 diabetes mellitus) (Deibert *et al.*, 2007). Additionally, in one study of the impact on obese premenopausal and postmenopausal women on reducing body weight by lowering fat content and using meal replacement drinks with modulation of their physical activity level showed that there was a significantly lowered risk of metabolic syndrome in the postmenopausal group from 16-42% compared with no significant reduction in the risk of metabolic syndrome in the premenopausal group (Deibert *et al.*, 2007).

1.5.2 Bariatric surgery

Bariatric surgery that decreases the stomach's magnitude including gastric bands, gastric bypass, and intra gastric balloons has achieved a desired level as a weight loss intervention. A meta-analysis of 136 research studies involving 22,000 patients stated that a considerable body weight reduction had been maintained by 61% for all types of bariatric surgery, providing a range of benefits related to diabetes, sleep apnoea, elevated blood pressure and blood lipids levels (Buchwald *et al.*, 2004). However, both medication and surgery for obesity treatment are expensive so their use is limited (Grove and Lambert, 2010). Furthermore, in the UK, all types of bariatric surgery are mainly prescribed for patients across the range of 35 to 40 BMI, as well as for people with a BMI higher than 40 with the consideration of their morbid condition (Chater *et al.*, 2015a). Thus, this type of medication is limited.

1.5.3 Pharmacologic Treatment of Obesity (Orlistat)

Many anti-obesity medications have been effectively shown to reduce the risk of developing obesity and promote weight loss in obese individuals. However, some of these obesity medications have been withdrawn from patient use related to the fact that these drugs caused detrimental side effects. One of the most effective therapeutic weight loss candidates is Orlistat, (Xenical, Hoffman-La Roche). This medication serves as a tool for the treatment of obesity in the form of pancreatic and gastric lipase inhibition. As it has been reviewed, Orlistat has shown a lowering of plasma triglyceride and cholesterol, a reduction in hypertension, and an enhanced glucose tolerance (Ballinger and Peikin, 2002). Interestingly, in a study conducted on 322 patients with obesity and type 2 diabetes that investigated the effects of taking Orlistat (120 mg) three times a day on bodyweight reduction over one year, it was found that patients with Orlistat medication had a significantly reduced body weight of more than 10% of their original body weight compared to the placebo group (Hollander *et al.*, 1998).

In addition, in a 4-year double-blind, randomized study conducted on obese individuals who were taking Xenical (Orlistat) and adhering to a modulated lifestyle pattern. It was found that there was a significant decrease in the incidence of T2DM and an increased body weight reduction in this group of patients compared with the placebo group (Torgerson *et al.*, 2004). Orlistat is the only licensed agent used as an anti-obesity drug in the UK (Wan-Loy and Siew-Moi, 2016). However, the use of Orlistat has been associated with adverse side effects in the form of gastrointestinal discomfort, bloating, faecal urgency and increased defecation (Glandt and Raz, 2011) as well as an elevated formation of gallstones, insufficient amounts of fat soluble vitamins including A, E, D, and beta-carotene. This is seen especially when this medication is used with a reduced fatty diet (Ballinger and Peikin, 2002). Moreover, in the UK, several types of other obesity medication such as phenylpropanolamine, amphetamine, methamphetamine, and fenfluramine, are not prescribed owing to their side effects (Haddock *et al.*, 2002).

1.6 Dietary fibre in obesity management

1.6.1 The importance of dietary fibre

An adequate intake of dietary fibre has long been related to health improvement. There are many essential nutrients known to be effective in tackling obesity and its related disorders such as protein and fibre (Rebello *et al.*, 2014). Increased dietary fibre intake may play a role in influencing body health and the maintenance of gastrointestinal tract well-being, as well as aid the digestion of foods. Dietary fibres are characterised as a portion of carbohydrate impervious to be digested and absorbed by the alimentary enzymes in the stomach and small intestine, and can be partially or totally fermented in the colon (Mišurcová *et al.*, 2010).

In addition, viscous dietary fibre such as alginate, with gelling formation can interact with the stomach and intestine, thereby slowing gastric emptying and obstructing macronutrients absorption, which is relevant to appetite regulation, enhancing satiety, and promoting body weight reduction (Wanders *et al.*, 2011; El Khoury *et al.*, 2015). Also, fibre promotes bile acid secretion, and increasing stool volume; fibres react with bile acids preventing them from being absorbed and assisting their passage through the colon (Naumann *et al.*, 2019). These physiological functions contribute to slowing glucose accumulation in blood, and a decreased level of low-density lipoprotein cholesterol (Clark and Slavin, 2013).

Dietary fibres can act as functional foods and, the usage of functional food products to control appetite or to increase satiety could represent a mechanism to reduce the quantity of a meal consumed and reduce food intake between the main meals. This is an effective approach in lowering the total macronutrient intake (Hunter *et al.*, 2019). Importantly, although several fibres such as alginate, β-glucan, resistant starch, guar gum and PolyGlycopleX (PGX), have reported an effective reduction in the blood glucose levels following a meal in considerable dietary researches, the usage of theses fibres in food manufacture is still limited (Cassidy *et al.*, 2018).

1.6.2 Overview of Marine Fibre (Brown algae)

Seaweed polysaccharides are perceived as an excellent source of soluble and insoluble dietary fibres. The various species of brown algae provide an abundance of biological activity, due to the presence of polysaccharide, pigments, and polyphenols. Algal polysaccharides are regarded as functional bioactive components in foods owing to their role in health promotion and disease prevention. Chemically, brown algae are characterized by the presence of a considerable number of polysaccharides such as laminarin, alginate, agar, mannitol, cellulose, carrageenans and fucoidan(Mohamed *et al.*, 2012; Lee and Jeon, 2013; Chater *et al.*, 2015b; Roohinejad *et al.*, 2017). **Table 1.1** shows the dietary fibre components of seaweeds compared to some terrestrial foodstuffs. The key components of these seaweeds are alginates, carrageenans, and agar depending on the class of seaweed (MacArtain *et al.*, 2007).

Many of these polysaccharides are not digested in the human gastrointestinal tract due to a lack of degradation enzymes. They are, therefore, classified as dietary fibres with the total fibre content estimated to be between 29.3 g and 62.3g per 100g (Jiménez-Escrig and Sánchez-Muniz, 2000; Dawczynski *et al.*, 2007; Gómez-Ordóñez *et al.*, 2010). Interestingly, the sum of marine dietary fibre components is significantly greater than the dietary fibre of fruits and vegetables and is associated with better outcomes in reducing serum cholesterol levels and reducing the risk of heart disease as well as blood pressure (Fleurence, 1999; Jiménez-Escrig and Sánchez-Muniz, 2000; Lordan *et al.*, 2011; Hamed *et al.*, 2015).

Importantly, the daily dietary reference value of non-starch polysaccharides is 24 g per day (Garrow, 1991) and based on this amount of fibre, seaweeds are able to contribute up to 12.5% of an adult daily fibre requirement in an 8g portion size of seaweed. The newer recommendation has increased this value to 30 g per day.

Food type	Total fibre	Soluble fibre	Insoluble fibre	Carbohydrates
Seaweed (g/100g wet weight) *				
Ascophyllum nodosum	8.8	7.5	1.3	13.1
Laminaria digitata	6.2	5.4	0.8	9.9
Himanthalia elongata	9.8	7.7	2.1	15.0
Undaria pinnatifida	3.4	2.9	0.5	4.6
Porphyra umbilicalis	3.8	3.0	1.0	5.4
Palmaria palmata	5.4	3.0	2.3	10.6
Ulva sp.	3.8	2.1	1.7	4.1
Enteromorpha sp.	4.9	2.9	2.1	7.8
Whole food (g/100 g weight) †				
Brown rice	3.8			81.3
Prunes	2.4			19.7
Porridge	0.8			9.0
Lentils green/brown	8.9			48.8
Cabbage	2.9			4.1
Carrots	2.6			7.9
Apples	2.0			11.8
Bananas	3.1			23.2

Table 1.1 The Fibre content of seaweeds compared to whole foods. Adapted from MacArtain *et al*., 2007.

*Values for seaweeds from the Institute de Phytonutrition Beausoleil France. †Values for whole foods from McCance *et al*., 1993.

1.7 Seaweed (nutritional aspects & health benefits)

Marine algae can be classified into four categories: red algae called Rhodophycae, brown algae (Phyophyceae), green algae (Chlorophyceae), and a group of blue-green algae (Cyanophyceae). Seaweeds are rich in natural bioactive compounds with several useful properties for industry and health-enhancing activities. These involve bioactives such as several algal polysaccharides (for example alginate and fucoidan), and polyphenols such as phlorotannins, which are not available in terrestrial plants. In addition, seaweed contains marine protein such as phycobiliproteins, carotenoids like fucoxanthin, pigments, phytochemicals, antioxidants and some long-chain polyunsaturated fatty acids such as eicosapentaenoic acid and omega-3 fatty acids. Interestingly, seaweeds contain a minimal amount of lipid. Seaweed has been classified as an efficacious resource for diets enriched in fibre and antioxidants (MacArtain *et al.*, 2007; Kadam and Prabhasankar, 2010; Lordan *et al.*, 2011; Lange *et al.*, 2015; Wan-Loy and Siew-Moi, 2016; Cherry *et al.*, 2019).

As well as the above, seaweeds have numerous micronutrients including essential amino acids, a large content of minerals such as Ca, Mg, Na, P and K, and micro-mineral levels of Zn and Mn. They are also a great source of iodine and vitamins (A, B1, B2, B3, B6, B12, C, D, E, pantothenic acid and folic acid). They also contain a wide range of soluble dietary fibres (edible fibre) (Hall *et al.*, 2012; Wijesinghe and Jeon, 2012; Awang *et al.*, 2014; Roohinejad *et al.*, 2017).

Regarding marine based food intake, in the adult population, there is a growing interest in maintaining a healthy diet and improving healthcare, therefore the consumption of marine-based foods is increasing. Marine bioactive substances and marine extracted nutrients have been widely utilized in the food industry and as a functional food component (Kadam and Prabhasankar, 2010; Lordan *et al.*, 2011; Roohinejad *et al.*, 2017). For example, there is a growing interest in European Countries to produce novel functional food products given red macroalgae is found to be an effective nutritional source of dietary proteins (Fleurence, 1999).

Several epidemiological studies have been carried out with marine microalgae in the form of bio-active ingredients, whole seaweed diet and foods supplemented with seaweed derivatives. They illustrated a significant relationship between the dietary consumption of seaweeds, health promotion and chronic disease prevention. For instance, a reduction was seen in type 2 diabetes, the plasma levels of cholesterol and triacylglycerols, cardiovascular disease, hyperlipidaemia, and cancer. In addition, they also showed anti-pancreatic lipase activity, with the potential use

as an obesity management. They also showed they are effective in protecting against oxidants, bacteria and viruses(Kadam and Prabhasankar, 2010; Awang *et al.*, 2014; Brown *et al.*, 2014b; Lange *et al.*, 2015; Sharifuddin *et al.*, 2015).

Furthermore, numerous epidemiological studies have evaluated the relationship between Western and South-east Asian dietary patterns. They demonstrated that the consumption of seaweed rich diets reduced the incidence rate of adverse health effects including cancers, coronary heart disease and increased lipoedema (Kim *et al.*, 2009; Iso, 2011).

Moreover, this finding is in line with the fact that, over the past centuries, many East Asian countries such as Japan, China, and Korea, have been consuming seaweed as an essential food ingredient known as marine vegetables. The Japanese have the greatest worldwide consumption of seaweed which is estimated to be 10-15% of their habitual diets and has been correlated with a lower incidence of heart disease, thyroid disease, and cancers. However, in Western societies, the major use of seaweed is in the form of raw algal storage polysaccharides such as alginate, agar and carrageenan, which are commercially utilized as tools to increase the thickness of foods and as a stabilizing agent in food industry applications (Brownlee *et al.*, 2005; Lordan *et al.*, 2011; Hall *et al.*, 2012; Lee and Jeon, 2013; Griffin, 2015).

As marine algae efficacy has been recognised, they have vast possibilities for utilisation in food products such as bakery, pasta, cheese, frozen meat products and sausages. They have also demonstrated a preservative property in terms of increased shelf-life. However, the employment of seaweed as an addition to food products has potential obstacles due to sensory alteration of these products and the potential risk of pollution as a result of heavy metals or by manufacturing waste (Brownlee *et al.*, 2012; Roohinejad *et al.*, 2017).

Figure 1.2 illustrates the beneficial effects of seaweed and seaweed bio-active extracts combined within food products.

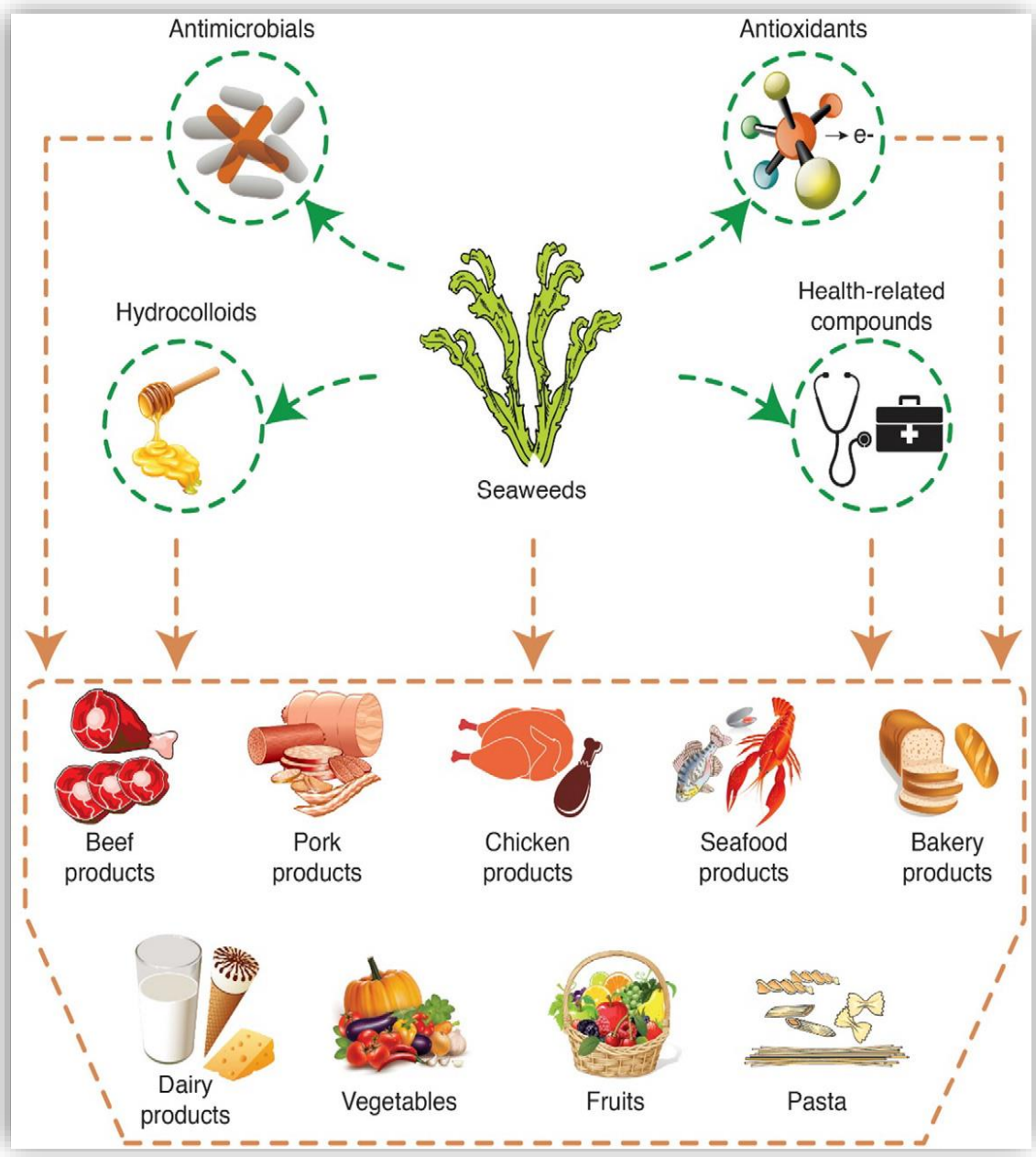


Figure 1.2 The beneficial effects of foods containing seaweeds in food manufacture applications. Adopted from Roohinejad *et al.*, 2017.

1.8 Alginate

Alginate is a linear biopolymer, indigestible by human enzymes and referred to a polysaccharide dietary fibre. It is extracted from brown seaweed *Phaeophyceae* for commercial purposes and includes: *Laminaria Hyperborea, Laminaria Digitata, Laminaria Japonica, Ascophyllum Nodosum, Macrocystis Pyrifera, Eclonia Maxima, Lessonia Nigrescense, Durvillea Antarcitica and Sargassum Spp.* In Northern Europe, Ascophyllum and Laminaria algal polysaccharides from marine brown algae are the main raw material for the alginate industry. In addition, some alginates have been derived from some species of bacteria such as *Azotobacter vinelandii*, and *Pseudomonas*. Alginate consists of a wide-range of varied block structures of (1,4)-linked β-D-mannuronate (M) and α-L-guluronate (G) residues (**Figure 3**) that are almost identical to those block structures present in certain types of seaweeds (Lee and Mooney, 2012; Chater, 2014b; Nalamothu *et al.*, 2014).

Further to this, alginates as a natural marine derived material and the bacterial alginate have been extensively utilized for several biomedical applications throughout the decades in hydrogel formation such as wound healing, delivery of low molecular weight drugs and in the form of a gel in cell transplantation in tissue engineering, due to it being non-toxic. Alginate has a unique ability to form two types of hydrogels depending on the pH level used and this has led to numerous differences in its physicochemical features. Alginates can be freeze-dried and compressed into the form of tablets. Thus, alginates are used in the biomedical field as well as the food industry. Alginates function as organoleptic agents, thickeners, emulsifiers and stabilizers of the food's texture with a viscosity enhancement. Moreover, alginate derivatives, particularly sodium alginate (E 401, defines sodium alginate authorised as a food additives in the European Union) (Younes *et al.*, 2017), is the most used and recognised approved food preservative. It forms a protective edible covering in many foodstuff applications, providing a barrier against browning and microbial compounds. It improves foods texture, crispness and reduces the vitamin C wastage several fresh fruits, vegetables and meats products, and is effective in extending the product's shelf life (Tønnesen and Karlsen, 2002; Brownlee *et al.*, 2005; Lee and Mooney, 2012; Nalamothu *et al.*, 2014; Wan-Loy and Siew-Moi, 2016).

1.8.1 Chemical Structure of Alginates

Alginates are "viscous dietary fibres" (Brownlee *et al.*, 2005) and are the main structural polysaccharides derived from brown seaweeds. They are in the cell walls in the form of mixed cationic salts. Generally, alginates are gelling polysaccharides present as salts such as sodium (E 401), potassium (E 402), ammonium (E 403) and calcium alginates (E 404) derived from alginic acid (E 400)(Draget and Taylor, 2011; Sellimi *et al.*, 2015)(OMRI for the USDA National Organic Program, 2015).

Algal alginates can be considered as glucose-like polymers consisting of 1-4 linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues. **Figure 1.3** shows the position of both M and G acids in alginate's structure and provides three diverse blocks of alginate including: M polysaccharide blocks, G polysaccharide blocks, and alternating M and G blocks.

Consequently, various alginate derivatives are different in M and G content and the length of each block unit. For these reasons, more than 200 diverse alginates at present are used in industry (Skaugrud *et al.*, 1999; Tønnesen and Karlsen, 2002; Brownlee *et al.*, 2005; Dettmar *et al.*, 2011; Georg Jensen *et al.*, 2012b; Georg *et al.*, 2012; Lee and Mooney, 2012).

As described by (Ertesvåg and Valla, 1998), there is a clear structural variation among all the block species, for example, MG blocks are most likely to be a flexible polysaccharide chain with higher dissolvable properties at lower pH than both the M block and G block. Additionally, they have the ability to be soluble in water and to form viscous gels (Seal and Mathers, 2001a). There are various factors that have an effects on the chemical synthesis of alginate in seaweeds, such as the types of seaweed, the geographic position, the differences of the alginate location in the same algae (for example, whether it is extracted from leaf or stem), seasonal changes and the condition of the saltwater (Dettmar *et al.*, 2011; Mohamed *et al.*, 2012).

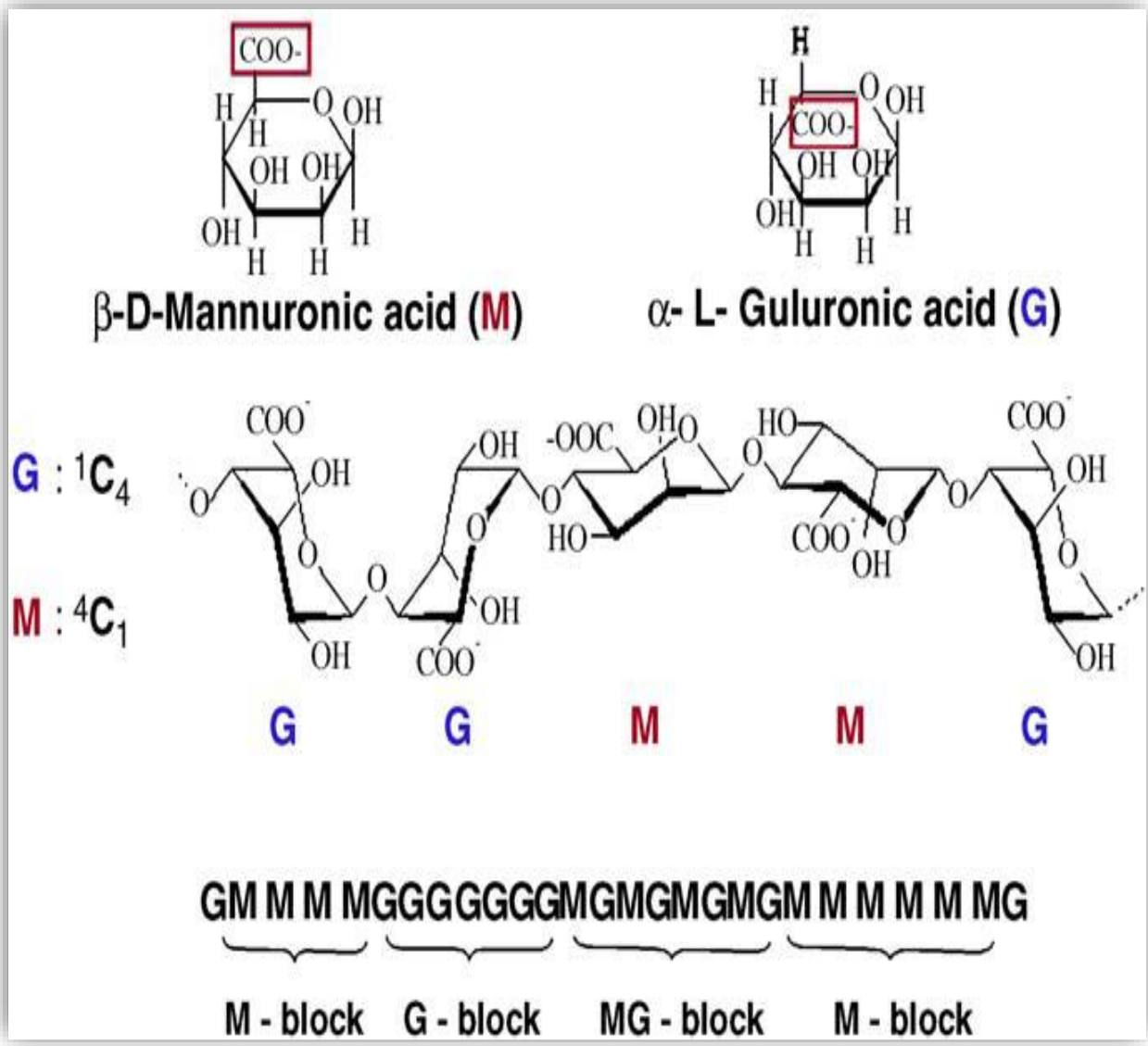


Figure 1.3 The structure of alginate. Alginate molecules are linear block copolymers β-D-mannuronic acid and α-L-guluronic acid, with a variation in the composition and sequential arrangements. Taken from (de Vos *et al.*, 2006).

1.8.2 Food industry and functional products of alginate

Alginate is a powerful agent that has been enormously utilised in numerous industrial applications such as a gelling agent and film formation, water-binding, stabilizer vehicle and viscosity agent, as well as in the food product industry. Alginate has been successfully involved in food industry applications as a result of its chemical structure as a soluble polymer in cold water with the ability to create thermostable gels. For instance, alginate is used as a thickening agent in the production of beverages and ice-creams (Ertesvåg and Valla, 1998; Seal and Mathers, 2001b; Hall *et al.*, 2012). In the food industrial sectors, there are a considerable number of safe to eat polymers such as alginate, pectin, Xanthan gum, and gelatine, which have been mainly used as a wrapping, filling agent, and an ingredient of protective coatings. In a study of food manufacturing which emphasized the importance of alginate in capsules distributed in a cheddar cheese matrix to delivery folic acid, it was found that the integration of alginate and pectin polymers as edible polymers in these capsules resulted in a higher retained level of folic acid in the cheddar cheese (Madziva *et al.*, 2006). **Table 1.2** shows the major uses of alginate in the food industry and PGA indicates Polyglycolic acid combined with sodium alginate and FMC is the Food Machinery and chemical Corporation Company.

Application of alginate	% of total alginate food applications	Notes on application
Premium beer foam stabilizer	21.2	PGA usage allows better head retention, and protects against foam-negative contaminants
Restructured foods	19.6	Use in reformation of food materials (e.g. onion rings, pimento pieces in olives). Endows food product with thermostability and desired consistency.
Bakery products	14.9	Improves shelf life and moisture retention in bread and cake mixes. Allows cold solubility in instant flan preparations.
Fruit preserves	6.5	Commonly used as gelling, thickening, and stabilizing agents in jams, marmalades, and fruit sauces.
Ice cream	3.8	Allows correct viscosity of ice cream, while avoiding crystallisation and shrinkage.
Other	15.1	Desserts (e.g., mousses, instant puddings, ripple syrups). Emulsions and sauces (e.g., low-fat mayonnaise, tomato ketchup).
Further uses of PGA	18.9	PGA is acid stable and resists loss of viscosity. Has unique suspension and foaming properties. Wide range of applications including: • Sorbet • Ice cream • Noodles/pasta Soft drinks • Dressings/condiments • Milk drinks

Compiled by FMC (the food machinery and Chemical Corporation. All applications use alginate, unless otherwise stated.

Table 1.2 Common uses of alginate in food products. Adapted from (Brownlee *et al.*, 2005). PGA indicates Polyglycolic acid combined with sodium alginate and FMC is the Food Machinery and chemical Corporation Company.

1.8.3 The Biological Effects of Alginate on

1.8.3.1 Food intake, Satiety and Body weight

The inclusion of alginic acid and its salts are authorised in a widespread range of food products and beverages. Most notably, they have an influence on appetite, and it is therefore an important agent to enhance the sensation of satiety and reduce the sensation of hunger, reduce the amount of consumed foods, reduce the quantity of fat absorbed and promote a favourable glycaemic response (Hall *et al.*, 2012; Younes *et al.*, 2017).

Several human intervention studies have demonstrated that a higher intake of fibre including alginate in foods or as a supplement was associated with a reduction in both adipose tissue and dietary energy consumed. Also, sodium alginate has been shown to reduce the dietary calories consumed and to be an enhancer of satiety(Georg *et al.*, 2012; Brown *et al.*, 2014a).

Alginates have the ability to form an ionic gel in the presence of gastric acid which can positively impact on several health issues. These include reducing the stomach emptying rate, which will slow intestinal food intake, and improving the post prandial glycaemic response as stated by (Brownlee *et al.*, 2005).

A recent investigation performed in the rat showed that the short duration (4 hours) of sodium alginate-based diet resulted in gel formation in the rat's stomach, increased gastric distention, extended emptying time and produced a reduction in macronutrients consumption. The longer experimental duration (6 hours) was associated with lower food intake, reduced adiposity, and lower plasma glucose levels. This suggests that sodium alginate is an effective agent for obesity and metabolic syndrome in human (Guo *et al.*, 2019).

Furthermore, alginate has been shown to decrease the amount of ingested foods and the feeling of hunger after consumption of alginate rich foods. This is believed to be due to the increased viscosity and gel formation in the stomach in the presence of low pH (Dettmar *et al.*, 2011; Houghton *et al.*, 2015).

In a pilot study examining the efficacy of consuming a sodium alginate–based beverage on healthy males, a noticeable reduction in the postprandial glucose response was demonstrated. Therefore ,this alginate beverage could be regarded as an effective regulator of appetite and a potential intervention for overweight and obese populations (Paxman *et al.*, 2008a). Evidence

from human intervention trials has demonstrated that the supplementation of a diet with alginate has the potential of being used to treat obesity.

However, most of them have been implemented in short-term interventions which do not contribute to a greater assessment of the potential health benefits from alginate intake on appetite and energy consumed (Lange *et al.*, 2015; Wan-Loy and Siew-Moi, 2016).

By reviewing the current literature, **Table1.3** adapted from Lange *et al* (2015), presents the potential for alginate in weight loss management in studies with overweight and obese human subjects demonstrating the role of seaweed fibre (alginate) in body weight reduction.

Marine algae derivatives (bioactive components) in the form of alginates, phlorotannins, fucoxanthin and fucoidans have the potential for utilisation as food supplements or in functional diets and have shown associated beneficial effects which support their use as a treatment of obesity (Wan-Loy and Siew-Moi, 2016). **Table 1.3** illustrates the mechanism of action of these bioactive potential ingredients from marine algae.

Authors	Subjects	Substance	Study design	Study duration	Dependent variables	Results	Major limitations
(Pelkman *et al.*, 2007)	Overweight and obese women (*n* = 29)	Alginate	Within-subjects, double-blind, placebo-controlled crossover trial. 3 conditions: 1.0 g/drink, 2.8 g/drink or no alginate (control). 2 test drinks daily for 7 days. 1-week washout.	5 weeks	Energy intake on days 1 and 7	Decrease in energy intake for both active formulations compared to control group.	Short study duration, only 1 dependent variable assessed
(Paxman *et al.*, 2008c)	Healthy men and women (*n* = 68)	Sodium alginate from brown algae	Randomized, controlled two-way crossover study. Preprandial 1.5 g sodium alginate beverage or control	4 weeks	Energy intake	Decrease in daily energy intake in sodium alginate group.	Short study duration, only 1 dependent variable assessed

			drink for 7 days. 2-week washout phase. Crossover				
(Paxman *et al.*, 2008b)	Healthy men (*n* = 14)	Sodium alginate from brown algae	Crossover study. 1.5 g sodium alginate beverage or control drink 3 hours after set breakfast before test lunch. 7 days washout. Crossover	1-day measurement	Pre- and postprandial plasma levels of glucose, triacylglycerol and cholesterol	Decrease in glucose and cholesterol uptake in participants of sodium alginate group with higher body mass index.	Small sample size, only 1 time point measured
(Odunsi *et al.*, 2010)	Overweight and obese male and female subjects (*n* = 48)	Alginate (brown seaweed *Laminaria digitata*)	Randomized, parallel, placebo-controlled, allocation-concealed study. 3 capsules of CM3 alginate (lyophilized sodium–alginate active complex) or	10 days	Gastric emptying, gastric volume, satiation, calorie intake, gut hormones	No group effect of treatment on any dependent variable.	

			control capsules per day before meals for 7 days. 6 capsules on days 8–10.				
(Georg Jensen *et al.*, 2011)	Obese subjects (*n* = 24)	Alginate	Randomized, controlled intervention study. 3% alginate 500 mL drink or placebo 500 mL drink. 3 times preload drink per day before meals as an adjuvant to a calorie-restricted diet.	2 weeks	Weight loss	Weight loss in both groups due to calorie restriction but not enhanced by alginate supplementation.	Short study duration, small sample size
(Georg Jensen *et al.*, 2012b)	Normal-weight men (*n* = 10) and women (*n* = 10)	Alginate	Randomized placebo-controlled, double-blind, four-way crossover trial. 4 conditions: low	1 day each on four treatments	Energy intake, satiety feelings, hunger feelings	Low volume-alginate drink reduced energy intake, high volume alginate drink	Short study duration, small sample size.

			volume alginate (9.9 g) 330 mL drink, high volume alginate (15 g) 500 mL drink, 330 mL control drink or 500 mL control drink.			increased satiety and reduced hunger.	
(Georg Jensen *et al.*, 2012a)	Obese men and women (*n* = 96)	Alginate	Randomized, double-blind, placebo-controlled study. 3% alginate 500 mL drink or placebo 500 mL drink. 3 times preload drink per day before meals as an adjuvant to a calorie-restricted diet.	12 weeks	Weight loss and metabolic risk markers (plasma glucose, insulin, C-reactive protein, ghrelin, HOMA-IR (homeostatic model assessment – insulin resistance and	Weight loss in alginate group greater than in placebo group mainly due to reduced body fat. No change in metabolic risk markers.	

					lipid metabolism)		
(Hall *et al.*, 2012)	Healthy overweight men (*n* = 12)	*Ascophyllum nodosum*	Single blind crossover study. 100 g of bread containing *Ascophyllum nodosum* (4%) or no seaweed	1-day measurement	Energy intake, plasma glucose and cholesterol	Decrease in energy intake at meal 4 hours following administration of *Ascophyllum nodosum* bread. No changes in plasma glucose and cholesterol levels.	Measurement at one time point only Small study group and short duration
(El Khoury *et al.*, 2014)	Healthy men (n = 24)	Alginate	Randomized crossover study. 325 mL drinks of chocolate milk, 1.25% alginate chocolate milk, 2.5% alginate chocolate milk or 2.5% alginate	1-day measurement	Energy intake, appetite, plasma glucose and insulin.	No group differences in energy intake. 2.5% alginate chocolate milk reduced peak glucose levels compared to 1.25% alginate chocolate milk and chocolate milk alone. Insulin	Small study group and short duration.

			solution 2 hours before an ad libitum meal.			peaks reduced after 2.5% alginate chocolate milk compared to chocolate milk. Alginate chocolate milk reduced pre-meal appetite. 2.5% alginate chocolate milk reduced appetite compared to chocolate milk alone.	

Table 1.3 The effects of seaweed fibre on body weight, overweightness and obesity in human. Adapted from Lange et al., 2015.

1.8.3.2 Gastrointestinal health, Plasma cholesterol & Insulinemic Responses

Marine algae derivatives (bioactive components) in the form of alginates, phlorotannins, fucoxanthin and fucoidans have the potential for utilisation as food supplements or in functional diets and have shown associated beneficial effects which support their use as a treatment of obesity (Wan-Loy and Siew-Moi, 2016). **Table 1.4** illustrates the mechanism of action of these bioactive potential ingredients from marine algae.

Algal Compounds	Mechanism of Action	Reference
Fucoxanthin *Alginates* *Phlorotannins*	Inhibition of pancreatic lipase	Matsumoto *et al.,* 2010; Wilcox *et al.*, 2014 Eom *et al.,* 2013
Fucoxanthin	• Enhanced ß-oxidation through increased expression of uncoupling protein 1 (UCP-1). • Suppression of inflammation in white adipose tissue (WAT). • Increased activities of key enzymes in lipid metabolism—AMP-activated protein kinase (AMPK) & acetyl CoA carboxylase.	Grasa-López *et al.,* 2016 Maeda *et al.*, 2009 Kang *et al.*, 2012
Fucoxanthin *Phlorotannins*	Suppression of adipocyte differentiation	Maeda *et al.*, 2006
Alginates	Delayed gastric clearance, stimulation of gastric stretch receptors and attenuated nutrient absorption	Pelkman *et al.,* 2007
Fucoidans	Downregulation of gene expression of key adipogenic markers and inflammatory-related genes in adipocytes	Wu *et al.*, 2016

Table 1.4 The mechanism of action of bioactive potential ingredients from marine algae adapted from Chu Wan-Loy and Phang Siew-Moi, 2016.

Recent data has demonstrated that sodium alginate has the potential to be an effective therapeutic agent for gastrointestinal mucositis caused by chemotherapy. Also, sodium alginate effectively prevents small intestinal bleeding, speeds mucosal healing, and it acts with a muco-protective application on the surface of the upper gastrointestinal tract. Alginate exerts a haemostatic effects on intestinal bleeding caused by methotrexate treatment. It carries out this action by precipitating fibrinogen increasing fibrin polymerisation and enhancing platelet aggregation (Yamamoto *et al.*, 2013). Additionally, dietary alginate has been associated with several biological, chemical, physical and sensory effects. One of the important physicochemical property is the ability to become viscous in the acidic stomach following ingestion which enhance its palatability (Brownlee *et al.*, 2005; Cassidy *et al.*, 2018). Besides that, this effect has the potential to delay gastric clearance rate and attenuate nutrient absorption, controlling postprandial glucose level and insulin values which shows a tendency to increase satiety (Kristensen and Jensen, 2011; Huang *et al.*, 2019). Furthermore, alginate acts as a bulking agent due to its water-holding capacity which lowers the time required to transfer the digested food to the colon. Therefore, this viscous polysaccharide could provide protection against colon cancer (MacArtain *et al.*, 2007).

Importantly, alginate has shown a beneficial effect in reducing cholesterol levels. In a study investigating the administration sodium alginate (7.5 g/d) in a low fibre diet for 6 ileostomy subjects, a relationship between alginate intake and attenuated dietary lipid absorption was observed. It was associated with lower bile acid release and increased fat extraction from the small intestine (Sandberg *et al.*, 1994). This result was supported by a recent study that evaluated the effect of alginate enriched bread on fat digestion which reported that alginate intake attenuated lipid absorption and reduced plasma triglyceride levels (Houghton *et al.*, 2019).

1.8.3.3 Inhibitory and modifying action on gastrointestinal enzymes activity

Dietary fibre has a considerable influence upon the action of digestive enzymes and as a result, has an impact on the absorption and digestion process in the alimentary canal (Ikeda and Kusano, 1983). Alginates and fucoidans are essential polysaccharides in brown marine seaweed which are mainly impervious to human and animal digestive enzymes, however, they are partly fermented by the action of colon bacteria (Seal and Mathers, 2001b; Younes *et al.*, 2017). The inhibition of absorption of some dietary elements such as dietary lipids, is one of the major

methods of combating obesity. For instance, the inhibitory action of seaweed bio-active derivatives on gastric and pancreatic lipase (Balasubramaniam *et al.*, 2013; Wan-Loy and Siew-Moi, 2016). Also, polyphenols derived from several brown seaweeds such as *Pelvetia canaliculata* and *Ascophyllum nodosum*, as well as many phenolic compounds and antioxidants derived from red algae such as *Kappaphycus alvarezii* and *Eucheuma denticulatum* are potent inhibitor of the activity of lipase, α-amylase, and trypsin (Chater, 2014a). The inhibitory ability of alginate is related to the structure and the molecular weight of alginate. For example, alginate extracted from the *Laminaria hyperborean* marine algae has a high content of guluronic acid (G) and has been shown to effectively inhibit pancreatic lipase by 75% compared to enriched-M alginates extracted from the *Lessonia nigrescens* seaweeds (Chater, 2014a; Wilcox *et al.*, 2014).

Additionally, it has been demonstrated by(Brownlee *et al.*, 2005; Houghton *et al.*, 2014) that alginate is a potent inhibitor of digestive enzyme activity, mainly in the upper gastrointestinal tract, and this reduction relies on alginate's structure. Therefore, several alginates can inhibit the activity of pancreatic lipase by around 75% and 85%, and *in vitro* alginates inhibit pepsin activity up to 80%. Alginates only have a small inhibitory effect on trypsin activity of around 10.4% and 7.5% inhibition(Chater *et al.*, 2015a; Houghton *et al.*, 2015).

1.9 Fat digestion in humans (the role of pancreatic lipase)

In this context, lipase is the main enzyme for lipid digestion that involves the digestion of triacylglycerol and phospholipids (Mukherjee, 2003). The hydrolysis of triacylglycerol (TAG) lipid results in free fatty acids as well as mono- and diacylglycerol and free glycerol; the hydrolysis of phospholipids leads to the production of free fatty acids and lysophospho-lipids **(Figure 1.4).** Efficient lipid hydrolysis involves colipase and bile salts as well as triglyceride lipases (Lowe, 2002). The human lipases which include lingual lipase in the saliva and gastric lipase secreted in the gastric juice, both have a lower pH stability and small molecular weights. There are also pancreatic, lipoprotein, endothelial and hepatic lipase. Pancreatic lipase is generated by the pancreatic acinar cells and is considered the main enzyme involved in triglyceride lipid digestion due to the fact that it is accountable for 50-70% of overall lipid digestion (Mukherjee, 2003; Birari and Bhutani, 2007; Zhang *et al.*, 2014). **Figure 1.5** shows the physiological function of dietary fat metabolism in the digestion and absorption of TAG. Therefore, ingestion of alginate as a dietary fibre in daily foods may be an effective adjunct to weight loss treatment.

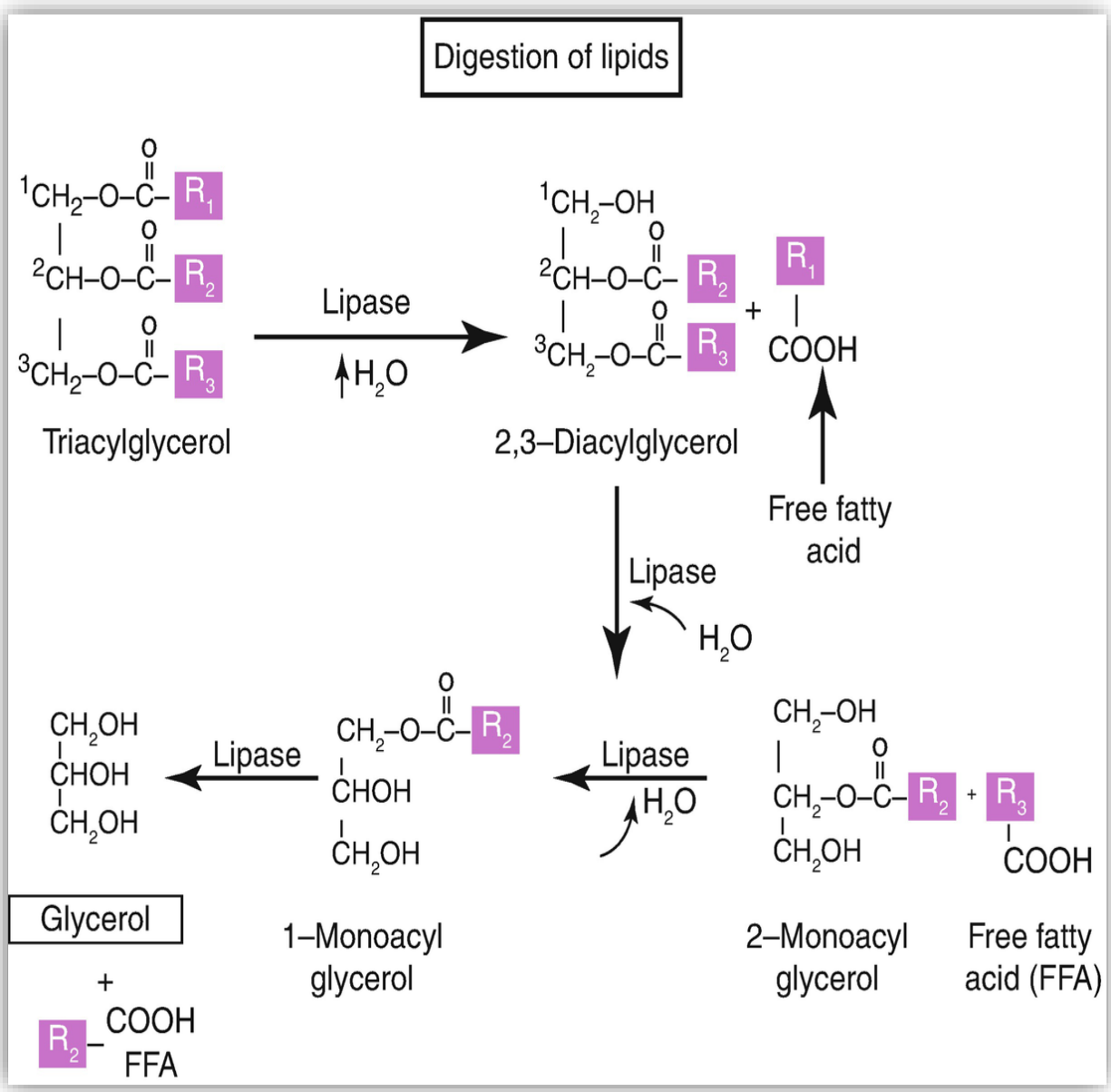


Figure 1.4 The hydrolysis process of triacylglycerol by lipase into diacylglycerols and fatty acid and then into monoacylglycerol and fatty acid, and then into monoacylglycerol and finally by lipase into glycerol and free fatty acid. This figure was obtained from (Gupta, 2019).

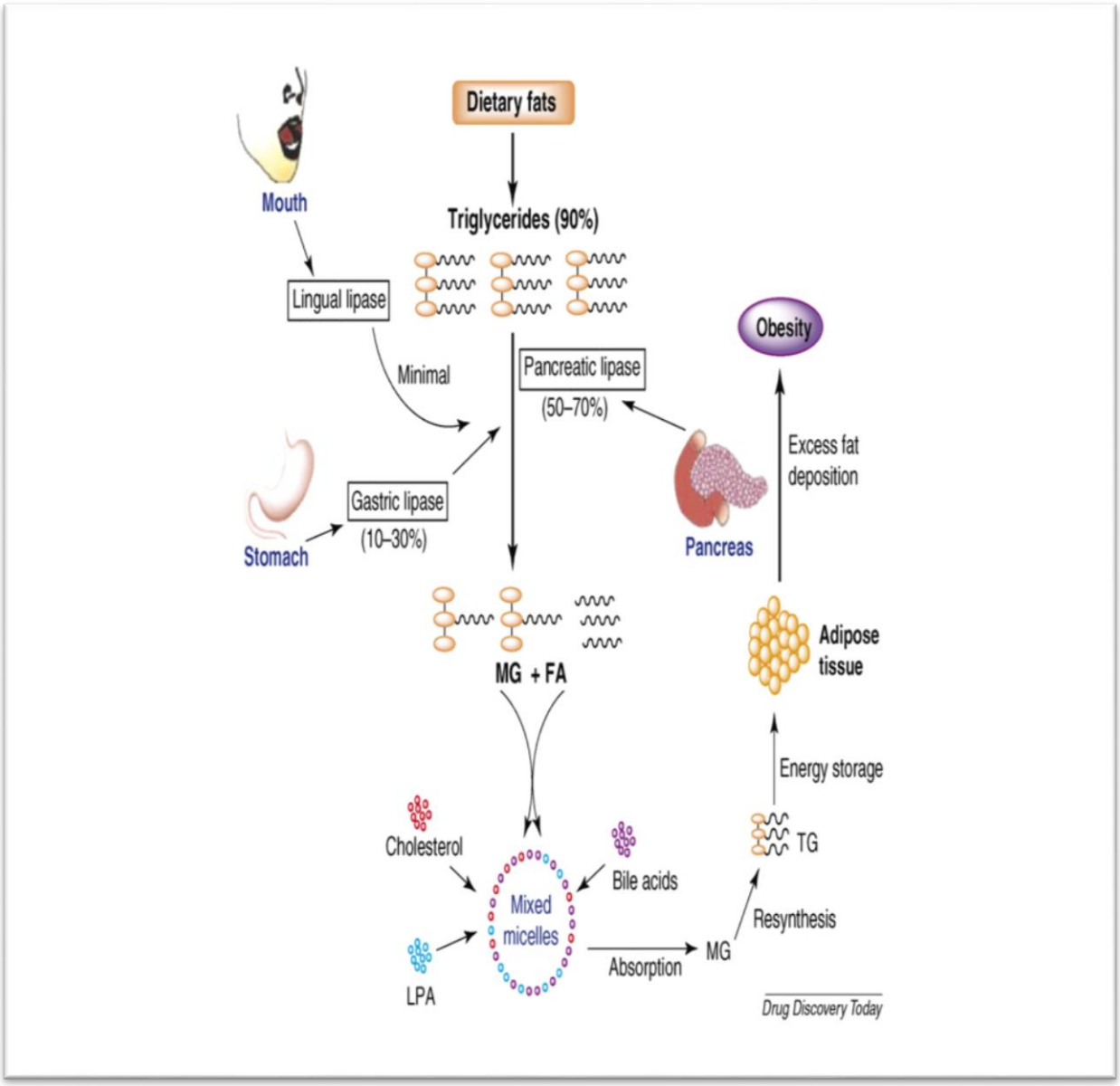


Figure1.5 A diagrammatic representation of the components involved in the digestion and absorption of TAG. This figure is adapted from(Birari and Bhutani, 2007).

1.10 The model gut & modifying digestion

Many studies have demonstrated that a gut model system is a useful approach for investigation of dietary intervention research and for developing new medications. Thereby, this research demonstrated the digestion of foods products that incorporated seaweed and seaweed bio-active components (alginate) using the model gut *in vitro* digestion system; this simulates digestion in the mouth, stomach and small intestines and has been developed at Newcastle University. It is believed to mimic *in vivo* human digestion in the alimentary tract (Hur *et al.*, 2011; Chater, 2014a; Bohn *et al.*, 2018). This artificial model is time, effort and cost effective compared to using *in vivo* models. Chapter 4 presents the usage of MG (model gut) in this research.

1.11 Project Hypotheses, Aims and Objectives of the study

There is a fundamental demand to use a sustainable, safe, and healthy alternative to anti-obesity medications that cause serious side effects in the obese population and are expensive. There is little research that specifically addresses the effect of incorporation of seaweed bioactive compounds in food vehicles. The incorporation of seaweed bioactive compounds into the diet are of importance to enhance general health, body weight loss and increase the daily fibre intake. Therefore, this principal has generated new targets for further investigation of using these bioactive compounds in the normal diet.

1.11.1 The purpose of this research was to test the hypotheses that:

1- The addition of 4% of brown seaweed and alginate into cheese and pork sausage are acceptable without association with wellbeing side effects in the healthy subject population.

2- *In vitro* digestion, seaweed and alginate incorporated foods have the ability to inhibit fat digestion and attenuate the release of glycerol.

3- Seaweed bioactives and alginate will be released during *in vitro* digestion.

4- Blood glucose and triglyceride levels will be modified in human volunteers at two hours and four hours after consumption of an alginate containing pork sausage meal compared to controls.

The seaweeds and alginates used in this thesis are shown in table 1.5.

1.11.2 Aims and Objectives

The primary aim of this thesis was to investigate the effects of adding seaweed and alginate foodstuffs on the lipid digestion in vitro, by comparing between the glycerol released from control, seaweed and alginate food samples taken from the model gut system. Moreover, the secondary aim was in conjunction with the in vitro digestion, I aimed to further investigate and quantify the release rate of alginate, seaweed and carbohydrate from the study's food vehicles throughout the samples taken during the in vitro digestion using the PAS assay.

Subsequently, the final aim was to highlight the potential for the prior and postprandial effect of alginate and control sausages as a meal on carbohydrate and fat digestion in healthy volunteers.

The overarching aim of this thesis and work in this area is to enhance and increase the daily consumption of functional seaweed bioactive ingredients such as fibre, in the Western societies. It is believed that the incorporation of seaweed bio-active components in some novel foods (cheeses and pork sausage) will continue to provide a fruitful approach in nutrition research and food industry setting. Additionally, this incorporation has the potential to attenuate the dietary lipid digestion. Thus, the research assessed the effect of consumption of brown seaweed and alginate in healthy human subjects and examine to what extent these foods are acceptable in terms of palatability, lack of effect on gut function and ease of incorporation into habitual diet.

The objectives were:

1- To test the acceptability of food incorporated with seaweed and alginate.
2- To collect food diary measurements for seven days for each week of intervention applied for the cheese and alginate acceptability study.
3- To use a daily and weekly well-being questionnaire to identify gastrointestinal and general health related behaviour.

The objectives were achieved by:

1- The total diet eaten was measured by 7 day food diaries at four weeks of intervention for each acceptability study.
2- A daily and weekly well-being questionnaire was used to identify gastrointestinal and general health related behaviour.

Food type	Seaweed/alginate incorporated	Ratio M / G	Source of material
Seaweed cheese	Ascophyllum nodosum	Not available	Seaweed and Co Northumbria cheese company
Alginate cheese	GHB	0.6	FMC
Alginate sausage	Unknown alginate	Unknown	Ruitenberg
Alginate bread	CC01(alginate)	0.8	Coca Cola

Table 1.5 The seaweeds and alginates foods used in this research. M (Manuronic acid) and G (Guluronic acid).

Chapter 2: Investigation to determine the acceptability of cheese and pork sausage containing seaweed extracts

2.1 Introduction

Obesity is a worldwide epidemic. As (Lapointe *et al.*, 2010) pointed out the cure of obesity and overweightness relies on reducing energy intake and dietary lipids to limit an excess accumulation of body fat, thereby, reducing body weight, decreasing the risk of type 2 diabetes mellitus and improving metabolism. Moreover, achieving a sustainable healthy food environment and supporting individual choice towards a healthy diet would greatly assist in obesity prevention (Swinburn *et al.*, 2015). There is a plethora of research demonstrating the potential benefits of dietary fibre in the improvement of metabolic health and in lowering the risk of developing obesity. An increased intake of dietary fibre (functional fibre as an indigestible carbohydrate with effective physiological consequences) is related to an increase in satiety sensations, and a decrease in the total macronutrients and energy uptake. Thus, resulting in weight loss (Slavin and Green, 2007; Wanders *et al.*, 2011; Reimer *et al.*, 2013; Solah *et al.*, 2016).

Broadly, there is increasing interest towards using naturally derived materials in foods, as well as in the pharmaceutical and cosmetic industries. Marine macro algae (seaweed) and marine-derived nutrients appear to be important candidates for bioactive materials and functional food ingredients due to their safety, antioxidant properties and their role as enzyme inhibitors. For example, alginate, a polysaccharide, is an effective inhibitor of pancreatic lipase; and it has been extensively used as a food additive and as an agent with reduced toxicity. Other examples include alginate-derived oligosaccharides, chito-oligosaccharides and phlorotannin-rich extracts which have been suggested to be important inhibitors of α-amylase and α-glucosidase (Tønnesen and Karlsen, 2002; Nick Pantidos and McDougall, 2014; Chater *et al.*, 2015; Houghton *et al.*, 2015; Jutur;Nesamma and Shaikh, 2016). Thus, adding seaweed and alginate bio-active compounds are likely to make an important contribution in the development of functional food products for the market. The consumption of edible seaweed has shown increased popularity in Western countries related to their health values and the increased consumption of Asian food. In addition, seaweed extracts especially viscous polysaccharides have been shown to be important agents to enhance the food products' acceptability and increase their shelf life (Brownlee *et al.*, 2012).

Alginate is a seaweed-derived polysaccharide indigestible in the upper human alimentary tract, therefore, it is classed as a dietary fibre. This marine-derived polysaccharide is mainly generated from brown algae species and makes up the extracellular matrix belonging to *Azotobacter* and *Pseudomonas* bacteria. In addition, alginate has an important manufactural application in the food and medical sectors as a viscous emulsifier with a gelling ability, as well as edible coatings and films improving the quality of food products (Evans and Linker, 1973; Brownlee *et al.*, 2005; Donati;Paoletti and Skjaak Braek, 2015; Skjåk-Bræk;Donati and Paoletti, 2015; Senturk Parreidt;Muller and Schmid, 2018).

2.2 Beneficial effects of seaweed and alginate enriched food and drink vehicles

In consideration of the potential use of alginate in food and drink vehicles, it has been shown to increase the physiological functionality in the alimentary tract such as an influence on colonic health and intestinal uptake rates of dietary nutrients. Moreover, alginate forms an acidic gel in the acid environment of the stomach which has the potential to alter feeding behaviour via slowed gastric emptying, improved gastric expansion receptor responses and impaired nutrient uptake (Dettmar;Strugala and Richardson, 2011). These unique nutritional and health properties of dietary seaweed and alginate are being investigated in several food and drink products as weight management tools. A recent pilot study in humans with an ileostomy investigated the effects of an alginate-enriched bread meal on lipid digestion; they demonstrated that there was a high lipid content in ileal effluent which is associated with a reduction in plasma triglyceride levels (Houghton *et al.*, 2019).

Moreover, in Japan, a recent randomized crossover pilot study investigated the influence of consuming 6g of roasted kombu seaweed (*Laminaria japonica*) which has a rich dietary fibre content including alginate, for each day during 4 weeks on 48 participants with lifestyle-related illnesses, has emphasised the beneficial effect of a decrease in plasma triglyceride levels and a lower incidence of constipation, diarrhoea, and hard stools (Nishiumi *et al.*, 2019). Additionally, the incorporation of seaweed and seaweed bio-active ingredients, more specifically alginate, as an algal fibre into daily food systems has been shown to have considerable health benefits that are widely demonstrated in the scientific literature (Fuller *et al.*, 2016). Also, seaweed food products are not a new idea with them being used in several Asian regions and some European countries. However, many seaweed and alginate-enriched food vehicles have palatability issues which may strongly influence their integration into an ordinary diet in several countries around the globe (Mahadevan, 2015).

As far as food acceptability studies are concerned, the sensory properties of food products, mainly taste and flavour, are essential factors that influence and modulate an individual's food choice (Clark, 1998), as well as food palatability (Yeomans *et al.*, 2008). Recent reports have concluded that, over the last decade, an intensive effort has been made to improve generally accepted food products, with an adequate shelf life and an enriched nutritional profile that incorporate particularly brown algae species and their marine natural compounds such as polysaccharides, iodine and fucoxanthin. However, there is not enough research investigating their potential bioactive benefits in *in vivo* studies. (Afonso *et al.*, 2019). Moreover, a further obstacle reported in several studies (Apling *et al.*, 1977; Ellis *et al.*, 1981; Georg Jensen *et al.*, 2012) is that diets containing large amounts of dietary fibre tend to result in highly viscous foodstuffs leading to lower palatability compared with a control food. They have also been shown to cause slight flatulence and abdominal inflation. A recent acceptability study investigating the effect on sensory quality including taste, colour and flavour of brown seaweed tea reported that the brown seaweed tea has a distinguishing smell of fish. This is considered a major obstacle in the consumption of these food products (Sinurat;Basmal and Suryaningrum, 2019). A reduced palatability score has limited the functional food products industry (Cassidy;McSorley and Allsopp, 2018).

In contrast, considerable earlier research has indicated that the enrichment of seaweed and seaweed hydrocolloids into foodstuffs is commonly acceptable and associated with improved nutritional profiles as well as an increase in the food product's shelf life and sensorial quality (Brownlee *et al.*, 2012). An acceptability study investigating a wholemeal bread containing seaweed has reported a greater acceptability of the 4% *Ascophyllum nodosum* seaweed wholemeal bread compared with a 1% *Ascophyllum nodosum* seaweed bread (Hall *et al.*, 2012). Moreover, Prabhasankar et al (Prabhasankar *et al.*, 2009) reported that 10% Wakame brown seaweed enriched pasta has acceptable sensory ratings with a slight seaweed flavour, and an enhanced nutritional value of fat, fibre and protein compared with a 20% Wakame enriched pasta. In addition, 3.6g of brown seaweed, *Ascophyllum nodosum* (Seagreens®), was added into whole-wheat flour (the dough) of Margherita pizza original recipe to improve the nutritional value of this pizza and to achieve the nutritional recommended level for public health. 49 adults and 63 children consumed a pizza portion that provided 1674 kJ (400 kcal) as a single meal and they reported higher sensory acceptance characteristics in terms of taste and appearance (Combet *et al.*, 2014). Furthermore, authors investigated the incorporation of 10% seaweed within wheat flour in noodles. This resulted in an increase in the nutritional content of protein, fat, and dietary fibre, as well as a more acceptable sensory perception by the consumers

regarding colour, taste, aroma, and texture when compared noodles made with wheat flour enriched with 30% seaweed (Kumoro;Johnny and Alfilovita, 2016). Moreover, it is found that several seaweed species obtained from the Caribbean and Pacific coasts of Costa Rica to be used as ingredient within their traditional food recipes and be consumed as fresh or a dry seaweed. It was reported that foods with more than 20% seaweed content in a food's dry weight, the food has been considered less acceptable by panellists when they tested the food. Also, the addition of high amounts of seaweed affects the traditional palatability of foods made from the Costa Rica recipes as well (Radulovich *et al.*, 2015).

According to recent literature, "no study on the impact of adding seaweeds to cheese acceptability has been reported" (Hell;Labrie and Beaulieu, 2018). It is important to note that recent attention has focused on the selection of popular food products that are widely consumed in the UK. Cheese is a highly favourable food product of the habitual diet with an increasing consumption level per capita, and this is associated with increased fat intake (Sharkasi and Kilara, 1994). For example, the majority of fat and saturated fat content are derived from the cheese topping of a pizza (Combet *et al.*, 2014). Cheese has acceptable nutritional properties that include a rich content of protein, several vitamins such as A, D and B12, calcium, lipid and riboflavin (Weinberg;Berner and Groves, 2004). There are a small number of cheeses containing seaweed; red seaweed *Palmaria palmata* and brown seaweed *Saccharina longicruris* known as *Kombu* are available to consumers such as 'Le Ti Pavez', and 'Tomme d'Iroise aux algues', both from Brittany, France (Hell;Labrie and Beaulieu, 2018). Therefore, there is a growing demand to provide healthy seaweed-based food products. The global seaweed market was $14.11 billion in 2020 and is projected to rise to $24.92 billion by 2028 (*Commercial Seaweed Market Size, Share & COVID-19 Impact Analysis, By Type (Red Seaweed, Brown Seaweed, and Green Seaweed), Form (Flakes, Powder, and Liquid), End-uses (Food & Beverages, Agricultural Fertilizers, Animal Feed Additives, Pharmaceuticals, and Cosmetics & Personal Care), and Regional Forecast, 2021-2028*, 2021).

Pork sausage is one of the popular traditional processed meat products in the UK with an elevated fat content that can reach up to 50% (Higgs, 2000). In general, dietary meat and meat products can have a reputation for being unhealthy in terms of high consumption and the link to health problems such as obesity, cancer and heart disease. Consequently, production and development of meat products including functional food ingredients will "improve the image of meat" and enhance its nutritional value (Jiménez-Colmenero, 2007). Further to this, the

addition of seaweed functional ingredients into popular meat products would be nutritionally effective as dietary meat has a low fibre content (Roohinejad *et al.*, 2017).

The unique aspect of this study was to provide food products which have a high nutritional value, are healthier and have favourable sensory attributes. According to the literature, no research has investigated the acceptability of 4% of brown seaweed and alginate incorporated within cheddar cheese and pork sausage in terms of palatability and lack of effect on gastrointestinal function on healthy participants. This could provide a new potential enriched functional food for the food market which is low cost and easily consumed as part of an everyday diet. This research includes a novel investigation of the acceptability of staple food products in the British diet – cheddar cheese and pork sausage – with the aim of developing healthy, palatable and cost-effective novel food products.

2.3 Hypotheses, Aims, and Objectives

The cheese and pork sausage acceptability studies were similar in terms of the study protocol and the measurement tool which included the same well-being questionnaire and the same online food diary. However, they had different participants and naturally different study foods. The primary aim of the present chapter is to evaluate the acceptability of a brown seaweed and an alginate cheese and pork sausage compared with their controls in a pilot study

The hypotheses were:

1- Incorporation of 4% of brown seaweed and 4% of alginate in cheese and pork sausages would be acceptable in terms of ease of incorporation into a habitual diet.
2- Incorporation of 4% of brown seaweed and 4% of alginate in cheese and pork sausages would be acceptable in terms of palatability and lack of effect on gastrointestinal function.

The aims were to test hypotheses by achieving the following objectives:

1- To address if is it possible for brown seaweed and alginate to be incorporated into a variety of food products such as cheddar cheese and pork sausage.
2- To test whether the addition of a higher amount of brown seaweed and alginate at 4 % in two different food vehicles (cheese and sausage) can be eaten within everyday breakfast, lunch, as a snack or dinner meal in the healthy subjects.
3- To address the acceptability of brown seaweed and alginate foods on 4 weeks gastrointestinal wellbeing in the healthy participant on a daily and weekly basis.

2.4 Material and Methods:

Cheese acceptability study

2.4.1- The Cheese making process

Cheese, alginate, and seaweed cheese were produced by the Northumberland Cheese Company as suitable for vegetarians and stored refrigerated, below 5˚C. **Figure 2.1** shows all study cheeses. Pasteurised cow's milk was used to produce the three different types of cheese. Two varieties of cheese, the alginate cheese and seaweed cheese, were made by adding the alginate (GHB) and brown seaweed *Ascophyllum nodsum* as dried whole seaweed during cheese making after 8 hours (added to the curd) respectively, and well mixed by hand. The alginate and seaweed cheese were put into a circular shape template, hard-pressed into cylindrical moulds and left for one day to dry. After that, the two study cheeses were left in brine for two days and then left to ripen for three months in a ripening room inside the cheese factory which is especially designed for drying the cheese and allowing maturation of the cheese.

Additionally, the control cheese 1 was cheddar cheese made from full fat cow's milk mixed with nettle, chive, parsley, onion, and garlic to mimic the appearance of the seaweed cheese. The control cheese 1 may contains some bioactive compounds. For example, onion, parsley have a polyphenol and antioxidants content(Pérez-Jiménez *et al.*, 2010). However these components would be present in much lower amounts than the alginate or seaweed. The control cheese 2 was a plain cheddar cheese made from full fat cow's milk. All the cheeses were sliced into a standard size of 30 g, packed into transparent plastic packaging and kept in the fridge at 4ºC. **Table 2.1** shows the nutritional information of 100g and for a 30g daily portion of cheese for the four study cheeses (alginate, seaweed, and control cheese 1&2).

Figure 2.1 The different cheeses used in the study.

Nutritional information	Seaweed cheese		Alginate cheese		Control cheese 1(Nettle)		Control cheese 2	
	Per 100g	Per serving 30 g	Per 100g	Per serving 30 g	Per 100g	Per serving 30 g	Per 100g	Per serving 30 g
Calories (kcal)	371.2	111.4	364.8	109.4	368	110.4	380	114
Fat (g)	29.8	8.9	29.8	8.9	31.1	9.3	31.0	9.0
Saturated fat (g)	20.6	6.2	20.6	6.2	21.7	6.5	21.5	6.4
Carbohydrate (g)	6.2	1.9	8	2.4	2.5	0.7	4.2	1.3
Fibre (g)	2	0.6	4	1.2	*	*	*	*
Sugars (g)	0.3	0.1	0.1	0	0.1	0	0.2	0
Protein (g)	20.5	6.1	20.2	6.0	19.4	5.8	21.1	6.3
Salt (g)	2.1	0.6	2.1	0.6	2.4	720	2.2	660
Sodium (mg)	980.8	294.2	844.8	253.4	960	288	880	264

Table 2.1 Nutritional profiles for seaweed, alginate, and control cheese and ∗ the fibre content was not reported for control cheese 1 and 2.

Nutritional Information	Brown Seaweed		Alginate	
	Per 100g	Per 4 g	Per 100g	Per 4 g
Calories (kcal)	161.0	6.4	0	0
Fat (g)	1.6	0.1	0	0
saturated fat (g)	0.4	0.0	0	0
Carbohydrate (g)	55.5	2.2	100	4
Fibre (g)	50.0	2.0	100	4
Sugars (g)	4.0	0.2	0	0
Protein (g)	6.0	0.2	0	0
Salt (g)	8.5	0.34	0.02	0
Sodium (mg)	3400.0	136.0	11	0.44

Table 2.2 Nutritional profile of brown seaweed and alginate incorporated into the cheese. The control cheese 1 may contains some small amounts of bioactive compounds such as polyphenols and antioxidants. These are unlikely to have any effects on the study.

2.4.2- Participants and sample size

The total numbers of participants included in the trial was thirty-seven individuals from the Newcastle region. They completed the cheese acceptability pilot study in a 4-week period. There were 16 males with a median age of 36.5 ± 9.6 years and 21 females with a median age of 38 ± 10.9 years, overall ranging between 21 and 64 years old. They were recruited through advertisements across the Medical School of Newcastle University via posters presented in different locations, through an email and on the Newcastle University staff website. In terms of the eligibility criteria, they had to be over 18 years old, a non-smoker, non-pregnant or breast feeding, healthy, not taking any medication and not allergic to the study foods, particularly free from lactose intolerance. Moreover, they were willing to try novel seaweed and alginate cheese. There was also no change in commonly consumed foods, physical activity or in body weight over the past 6 months and during the study. They were also required to actively use the internet to record their food diary on myfitnesspal.com. When the participants were considered eligible and met the inclusion criteria to participate in the cheese study after their reading for the study information sheet by email and were given an appointment to participate. They were invited to sign the study consent form and were given a study code, a paper copy of written subject information sheet about the study, as well as the opportunity to ask any question they may have and then a written consent form. The participants acknowledged that they have the right to withdraw from the study at any time.

2.4.3- Study design

Prior to each study week, each participant was provided with the choice as to when to attend to pick up the study cheese. In the first week (**week 1**), the participants were asked to consume their normal habitual diet, keep a food diary, and complete a Well-Being Gastrointestinal Health Questionnaire (WBGI-Q) at the end of each day. In the following week (**week 2**), participants were asked to include the first study cheese containing alginate into their normal diet. Again, the participants were required to keep food diaries and complete a well-being questionnaire at the end of each day. At the end of this week (**week 2**), volunteers were given a second study cheese (seaweed) to replace the first. This again was substituted into the volunteers' diet in place of the other cheese over the course of the week (**week 3**). The final cheese (control) was given in the last week (**week 4**) to replace the previous study cheese.

Again, the participants were required to keep food diaries and complete a well-being questionnaire at the end of each day. After the 4-week period, participants returned to receive their honorarium £10 per week and any travel expenses upon completion of the study. All questionnaire and diary data were fully anonymised at source, as each participant had a unique identifier number that was used to identify all study data. The procedure for this study is described in detail in **Figure 2.2.**

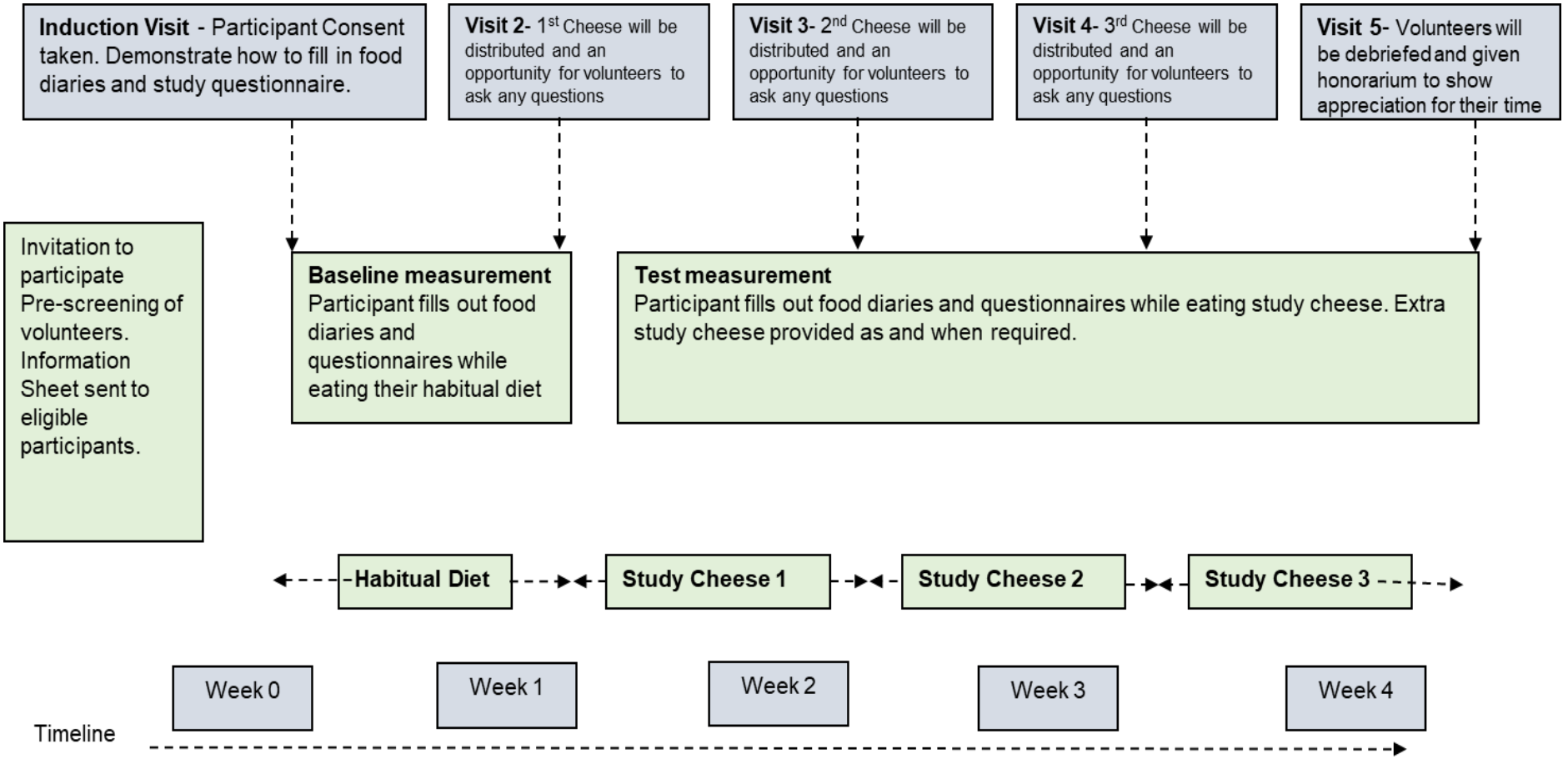


Figure 2.2 The cheese acceptability study protocol flowchart.

2.4.4- Food diary collection

The measurement of each participant's dietary intake per day was recorded on MyfitnessPal.com by using a 7-day diet record method. Participants were required to record all food details including the type of beverages and food, estimated portion size and cooking methods. The estimated amount of daily dietary intake including protein, carbohydrate, fat, cholesterol, fibre, sugar and sodium was converted into grams, and the food energy intake was measured in calories. Recording the food diaries took an average of 10 minutes for each participant to complete. The volunteers were advised not to consume their preferred cheese and to instead adhere to the study cheese provided. All food diaries were maintained and saved online. They received instruction on how to complete this food diary and how to insert the nutritional values of the cheese into their diary. MyfitnessPal.com contains almost all common food items such as vegetables, fruits, cereal and cereal products, milk and milk products, fats, oils, meat and meat products, fish and fish products, eggs, alcoholic and non-alcoholic beverages, seeds, nuts, soups, snacks, and sauces. The alginate, seaweed and control chesses were coded as 7531 Alginate Cheese, 30 g, 7531 Seaweed Cheese, 30 g and 7531 Control Cheese either 1 or 2, 30 g and added to the food list as a 30 g portion size that included all the nutritional values to this website. During each day of the cheese acceptability study, the food and drink consumed were reported at breakfast, lunch and dinner as well as any snacks taken during the day. Thirty grams of cheese per day was simply incorporated into participants' food intake with 7 slices of cheese per week during the three weeks of the study.

2.4.5- Well-being questionnaire

It is stated that regular clinical examination could effectively contribute to a gain in knowledge related to aetiology (Aitken;Zealley and Rosenthal, 1969). The Well-being Gastrointestinal Health Questionnaire (WBGI-Q) had been used in the form of a visual analogy scale (VAS) to record the expression of symptoms related to gastrointestinal health in both the cheese and pork sausage acceptability studies. This scale provided an accurate measurement tool to observe the participants' wellbeing. This questionnaire has been validated and shown to have a high degree of reliability. It has been previously used in several research studies (Aitken;Zealley and Rosenthal, 1969; Houghton *et al.*, 2019).

Each participant completed the daily questionnaire and the weekly questionnaire was completed on the last day of each intervention week. The SurveyMonkey.com website was used in this research and filling out the WBGI-Q took a maximum of 5 minutes to complete.

As shown in figures 2.3, 2.4 and 2.5, this survey combines three types of questions;

(i) ***How have you been feeling today?***

(ii) ***Have you been suffering from?***

(iii) ***Overall, how would you say the following have been?***

The first part of the WBGI-Q is made up of 7 questions with self-rated responses designed to measure the well-being quality of the participants per day during the four weeks of the study in the form of a VAS method. The VAS is 100 mm in length with a word anchored at each end, presenting the most favourable and the most unfavourable scoring as two opposing statements, measured from left to right. This was utilized to report on gastrointestinal satisfaction (**Figure 2.3**). The seven standardized questions concerning well-being were: ***How have you been feeling today?*** ("Alert" to "Sleepy"), ("Fine " to "Nauseous"); ("Full" to "Starving"); ("Not bloated" to "Bloated"); ("Not flatulent" to "Flatulent"); ("Calm" to "Irritable"); ("Relaxed" to "Anxious"). This VAS was filled in each day of the study by using SurveyMonkey.com. The second part of the WBGI-Q is 8 questions utilized to rate the respondent's general well-being in terms of maintaining adequate symptoms of general well-being that were scored as ("Not at all" to "Very"). The question was ***Have you been suffering from?*** and these items concerned dizziness, blurred vision, a difficulty to concentrate, a difficulty to think, excessive thirst, headaches/migraines, cravings for sweets and abdominal discomfort.

The third part of the WBGI-Q is 4 questions to assess the four factors that refer to general overall well-being as associated with the gastrointestinal symptoms (**Figure 2.5**). The four standardized questions were ***how would you say the following have been?*** including; bowel habit, urgency to pass stools, abdominal pain or discomfort, and amount of flatulence. The second and third parts formed the weekly WBGI-Q that was used at the end of each intervention week during the cheese study. Additionally, the participants were asked about any other unusual symptoms or discomfort, any additional comments or any other changes to their wellbeing they would like us to know about.

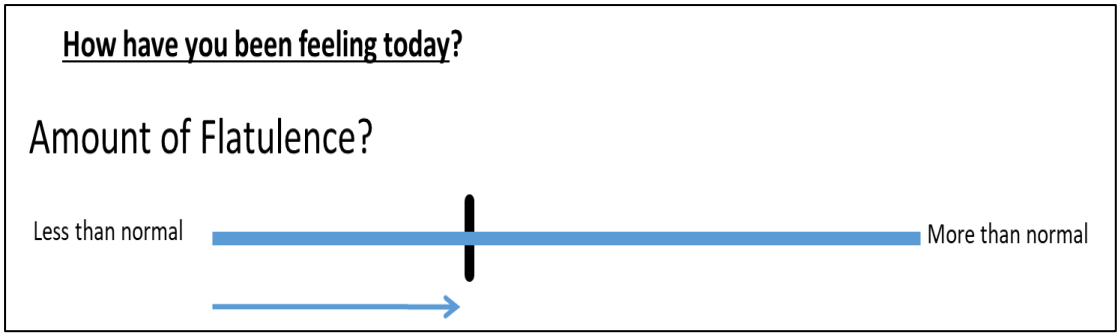


Figure 2.3 An example of a question from the wellbeing questionnaire. Where along the 100 mm line is crossed (on an analogue scale) refers to the response to this question.

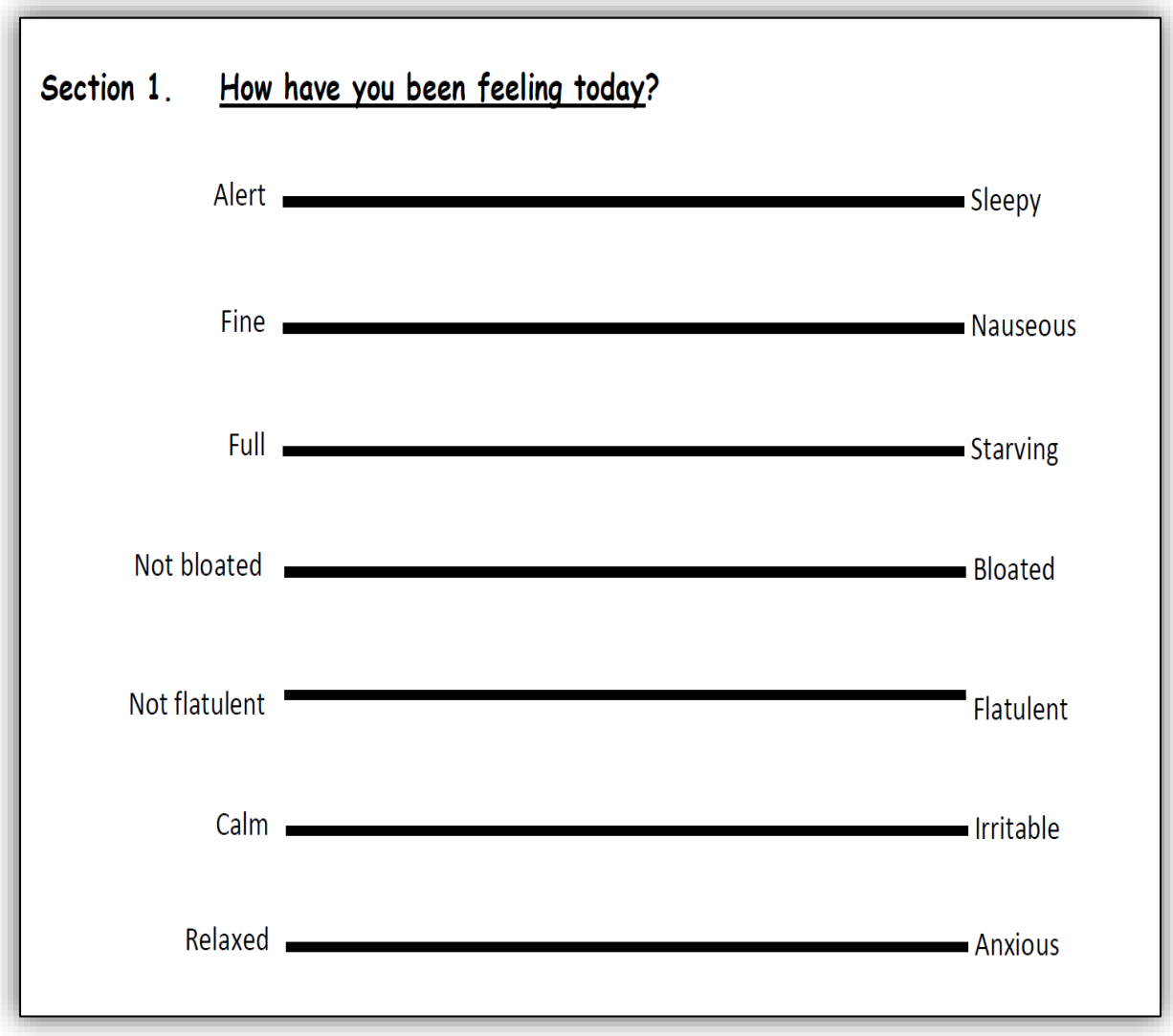


Figure 2.4 The wellbeing questionnaire with responses assessed on an analogue scale per day in both the cheese and sausage acceptability studies.

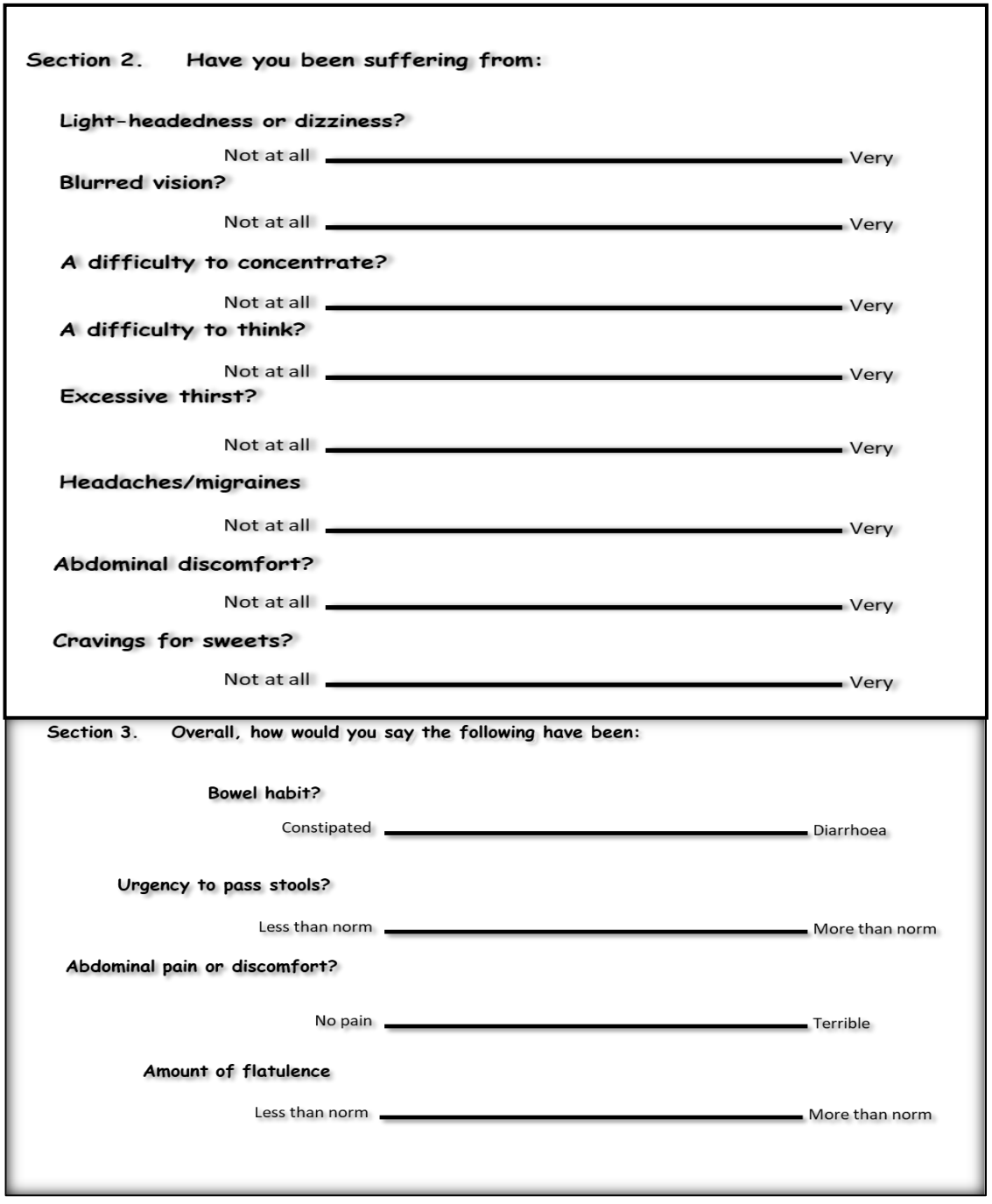


Figure 2.5 Sections 2 and 3 of the wellbeing questionnaires with responses assessed on an analogue scale at the end of each week of the study.

Pork sausage acceptability study

The aim of this pilot study was to test whether pork sausages containing alginate are acceptable in terms of palatability, lack of effect on gut function and ease of incorporation into habitual diet.

2.4.6- Pork sausage manufacture

The alginate and control sausages were produced by an expert in alginate technology at Ruitenberg Innovation Manufacturing, Ruitenberg Ingredients B.V., Griftstraat 8, Netherlands. The sausage consisted of pork, calcium acetate, water, sodium alginate (E401), salt, pepper, nutmeg, and potato starch. Table 3 presents the nutritional values of the alginate and control sausages per portion (one piece of sausage). The analysis of the chemical nutrient composition of the control pork sausage is presented in **Appendix 2.1**.

Nutritional Information	Control sausage per 1 sausage (46.4g)	Alginate sausage Per sausage (48.5g)	Control sausage per 100g	Alginate sausage Per 100g
Calories (kcal)	90.5	94.6	195	195
Carbohydrate (g)	0.4	0.5	1.0	1.0
Fat (g)	6.6	6.9	14.2	14.2
Protein (g)	7.5	7.8	16.1	16.1
Sugars (g)	0	0	0.01	0.01
Fibre (g)	0.7	2.6	1.5	5.3
Salt (g)	0.6	0.6	1.31	1.3
Sodium (mg)	0.2	0.4	0.5	0.9

Table 2.3 The nutritional profiles for alginate and control sausage.

2.4.7- Participants and sample size

A total of 35 participants 18 males and 17 females aged between 18 and 64 years, generally healthy, receiving no medical treatment were recruited. They were also non-smokers, not pregnant or breast-feeding women and had not been diagnosed with a known sensitivity to the ingredients of the pork sausage. They were actively using the internet and able to record their food diary on MyfitnessPal.com.

It was also possible for them to include a novel alginate sausage into their normal diet which had not changed in the 6 months prior to the study. Recruitment was achieved through emails as many of them participated in our previous research, an electronic advert on the Newcastle University staff website, an advert poster displayed in the Medical School, Newcastle University, as well as in a life event including direct contact with the medical students (Physiology Day) which is held in Newcastle University. All participants were invited to an induction meeting and were provided with a study code, informed consent form and a written subject information sheet. They were also asked to raise any concerns or questions about the research, and they were informed that they were free to withdraw from the study at any time.

2.4.8-Study design

In the first week (**week 1**), the participants were asked to consume their normal habitual diet, keep a food diary, and complete a well-being questionnaire at the end of each day. In the following week (**week 2**), participants were required to include the first study pork sausage into their normal diet. The volunteers were ask to consume three sausages per day. In the next week (**week 3**), the participants were asked to return to their habitual diet, keep a food diary and complete a well-being questionnaire at the end of each day. Again, in the following week (**week 4**), participants were asked to consume the second type of study pork sausage to replace the first (three per day) and they were required to keep their food diary and complete a well-being questionnaire every day. All food diaries were maintained on myfitnesspal.com and questionnaires had been designed on survey.answers.com. After the 4-week period, participants returned to receive their honorarium £10 per week and any travel expenses upon completion of the study. **Figure 2.6** shows the procedure for this study.

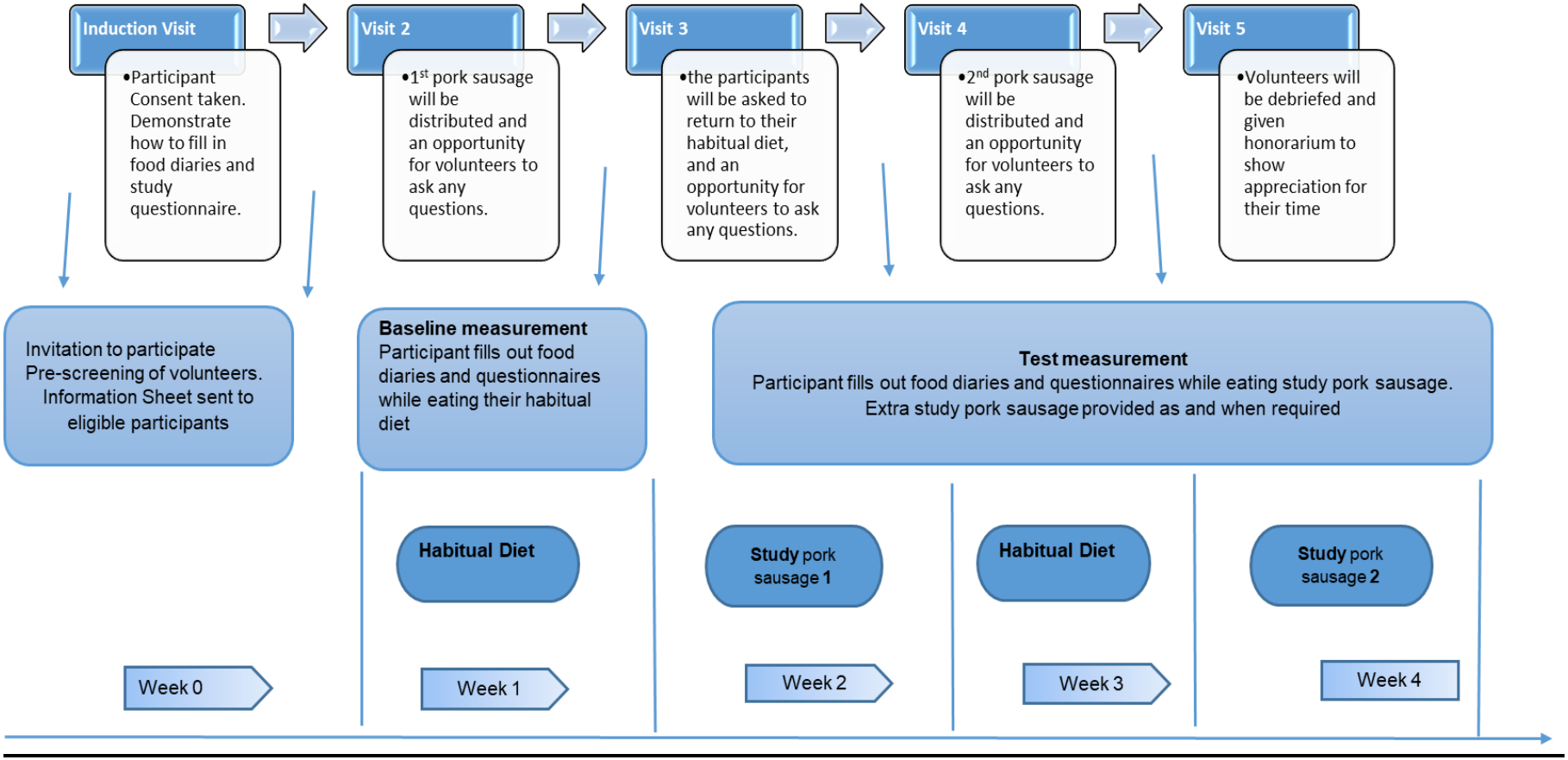


Figure 2.6 Flowchart of the study design for the acceptability study of alginate containing pork sausages.

2.4.9- Food diary collection

The participants followed the same procedure as described in section 2.4.4 (Food diary collection in the cheese study). The volunteers were advised not to consume their preferred sausage, but instead adhere to the study sausage provided prepared via any cooking method they preferred. The alginate and control pork sausages were added to the food diary list as alginate sausage (48.5 g) and control sausage (46.4 g). They were asked to consume 3 sausages per day.

2.4.10- Well-being questionnaire

The Well-being Gastrointestinal Health Questionnaire (WBGI-Q) had been used in this study as shown in **Figures 2.4** and **2.5**. The participants followed the same procedure as mentioned above in section 2.4.5.

2.4.11- Statistical analysis

An initial power calculation was carried out to determine the sample size needed to investigate any effects on gastrointestinal well-being. Thus, in both studies, 40 volunteers would be enough to determine this and allow a 10 % dropout or incomplete data collection from the pilot studies. All statistical analysis was carried out utilizing GraphPad Prism software (version 8.3.1). Every attempt was made to recruit 50 healthy males and females to the studies, as well as to reject the null hypothesis that there is no difference between the variables. An α level of 0.05 was considered statistically significant. The trend of association was assessed according to Pearson's correlation to determine the association between the dietary variables and the well-being scores within each intervention week of the two studies. All data was expressed as mean ± standard deviation. Data analysis was performed using ordinary one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons.

2.4.12- Ethical Considerations

The Faculty of Medical Sciences Ethical Review Committee of the Newcastle University reviewed and approved all study materials of the acceptability study for cheese containing seaweed & alginate (01088/2016), as well as the acceptability of alginate containing pork sausages (1268/13317/2017). This included the study protocol, information sheet for volunteers, informed consent form, poster adverts and the participant's debrief sheet. This study has been approved and was fully funded by the Biotechnology, Biosciences Research Council, U.K. as well as the Saudi Arabian Government. **Appendix 2.2** shows the study protocol, information sheet, consent form, poster adverts and the debrief sheet for cheese acceptability study, and, **Appendix 2.3,** shows the information of pork sausage acceptability study.

2.4.13- Reward for participation

A voucher of the value of £40 was given on the final visit to all subjects who completed all parts of the cheese and sausage acceptability study on the condition they had completed 7 days food diaries each week and completed the daily and weekly well-being questionnaire.

2.5 Results:

Cheese acceptability study

2.5.1- Subject characteristics

65 participants expressed interest in participating in this study. Twenty eight of these were excluded as they withdrew before starting the study (n = 9), withdrew during the study without providing a reason (n = 2), competing time commitments (n = 5), were taking medication (n = 5), were lactose intolerant (n = 2), not willing to fill in the food diary every day (n = 2), inability to comply with the food diary (n = 3). Thus, 37 males and females completed the study and were included in the final analysis.

2.5.2- Energy and macronutrient intake

The difference in the average daily energy and the macronutrients consumed in the baseline week and when the 37 participants consumed all the control, seaweed and alginate cheeses is presented in **Figure 2.7**. Nutrient diversity was scored by summing the total daily amount of specific food nutrients at breakfast, lunch, dinner, and snacks including staple servings of the study cheese per day, divided by the number of the specific days including their eating of cheese. The analysed macronutrients data indicated that no significant differences ($P > 0.05$) was observed in nutrients consumed by participants including calories, carbohydrate, protein, fat, cholesterol, fibre, sugar, and sodium for each meal during breakfast, lunch, dinner and snacks in the baseline, alginate, seaweed and control cheese week. **Figure 2.7** shows the effect of consuming the alginate, seaweed and control cheese on overall food consumption; no significant differences were observed between the three cheeses for any of the nutrient outcomes measured, except the increased intake of Na. Tukey`s multiple comparisons test showed there was a significant difference between average sodium intake in baseline week vs seaweed cheese week ($P < 0.05$)and baseline week vs control cheese week ($P < 0.05$) but not between the baseline and the alginate cheese week.

In addition, as is shown in **Figure 2.7**, the highest fibre intake was obtained when the participants consumed alginate cheese, however, it did not reach a significant level ($P > 0.05$). There were two types of control cheese, the first being cheddar cheese with nettle to mimic the appearance of the seaweed cheese and the second one was a plain cheddar cheese. There was no significant difference in the nutritional profile between the two cheeses ($P > 0.05$). **Table 2.4** represents the average calories and nutrients consumed with baseline, alginate, seaweed and the control cheese. This refers to, the average of control cheese 1 and 2 together. They are the same cheddar cheese with different flavour. There was no difference in terms of subject acceptability/symptoms/nutritional content during the consumption of control cheese 1 and 2.

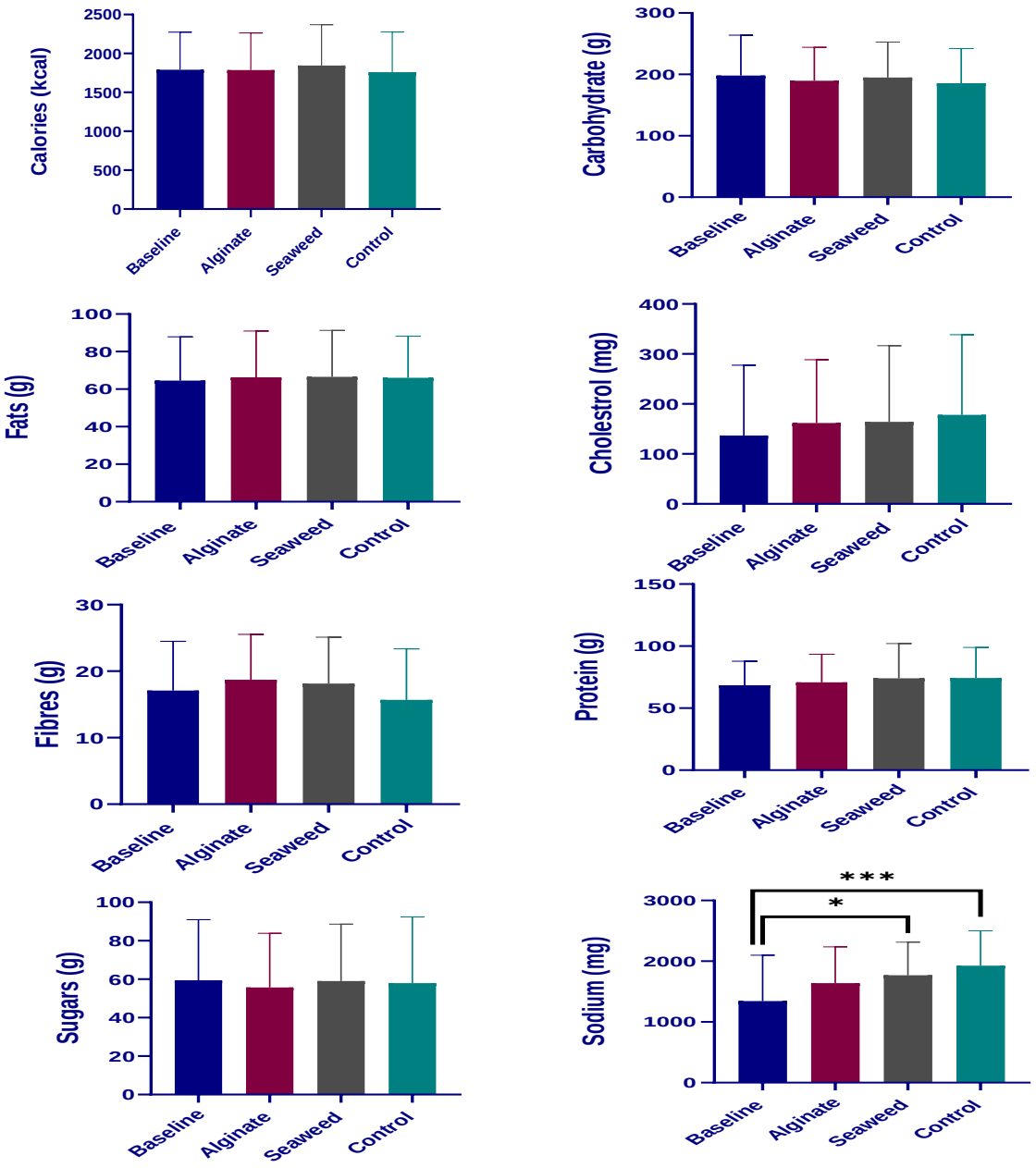


Figure 2.7 The variation between the average amounts of the food nutrients consumed from the first week of study, baseline week, until the last week, control cheese week. This includes calories intake (kcal), protein, carbohydrate, fat, fibre and sugar (g), cholesterol and sodium (mg). The data shows the mean value for 37 participants for 4 weeks of food diary data with standard deviation. No significant difference between the nutrients was observed ($P > 0.05$), except between baseline vs control cheese and seaweed cheese for sodium consumed as mentioned below in Table 2.4. *P* values <0.05 are represented by * and <0.0005 are represented by ***. The term (control cheese) refers to the average of control cheese 1 and 2.

Nutrient intake	Baseline week (n= 37)	Alginate cheese (n= 37)	Seaweed cheese (n= 34)	Control cheese (n= 37)	*P* value
Calories (kcal)	1789 ± 545	1785 ± 46 4	1783 ± 608	1756 ± 515	0.9
Carbohydrate (g)	197 ± 74	191 ± 54	190 ± 66	187 ± 62	0.8
Fat (g)	65 ± 25	66 ± 25	64 ± 28	69 ± 3	0.9
Cholesterol (mg)	134 ± 1	122 ± 131	118 ± 140	152 ± 165	0.6
Fibre (g)	18 ± 8	17 ± 7	16 ± 78	19 ± 21	0.3
Sugars (g)	59± 32	56 ± 29	66 ± 31	58 ± 35	0.9
Protein(g)	70 ± 21	71 ± 23	73 ± 30	77 ± 34	0.6
Sodium (mg)	1325 ± 698	1572 ± 610	1673 ± 612	1874 ± 525	*0.001

Table 2.4 A statistical summary of the difference of mean ± SD for the average daily intake of nutrients in the baseline, alginate, seaweed, and the control cheese week. *P* value was derived from Ordinary one-way ANOVA with multiple comparisons and shows no significant difference ($P > 0.05$) between the mean calories and all the nutrients consumed during the study except the intake of Na, as * represents the significant value obtained from Ordinary one-way ANOVA of treatment between the 4 columns (all study weeks) ($P < 0.05$).

2.5.3- Daily wellbeing questionnaire

The participants' responses to the daily wellbeing questions (**Figure 2.4**) in terms of being alert or sleepy, fine or nauseous, and all the other wellbeing factors are shown in **Figure 2.8**. There was no significant difference in the participants' feeling of being alert, nausea, bloated, irritable and anxious all $P > 0.05$. However, the wellbeing scores of flatulence increased when they consumed alginate cheese compared to baseline week ($P< 0.05$), seaweed cheese and baseline ($P < 0.05$) and between control cheese and baseline week ($P < 0.05$). In addition, they felt much fuller when they consumed alginate cheese compared to the baseline week ($P< 0.05$); this was also observed when the seaweed cheese was consumed compared to the baseline week ($P< 0.05$) and when they had control cheese compared to the baseline week ($P < 0.05$).

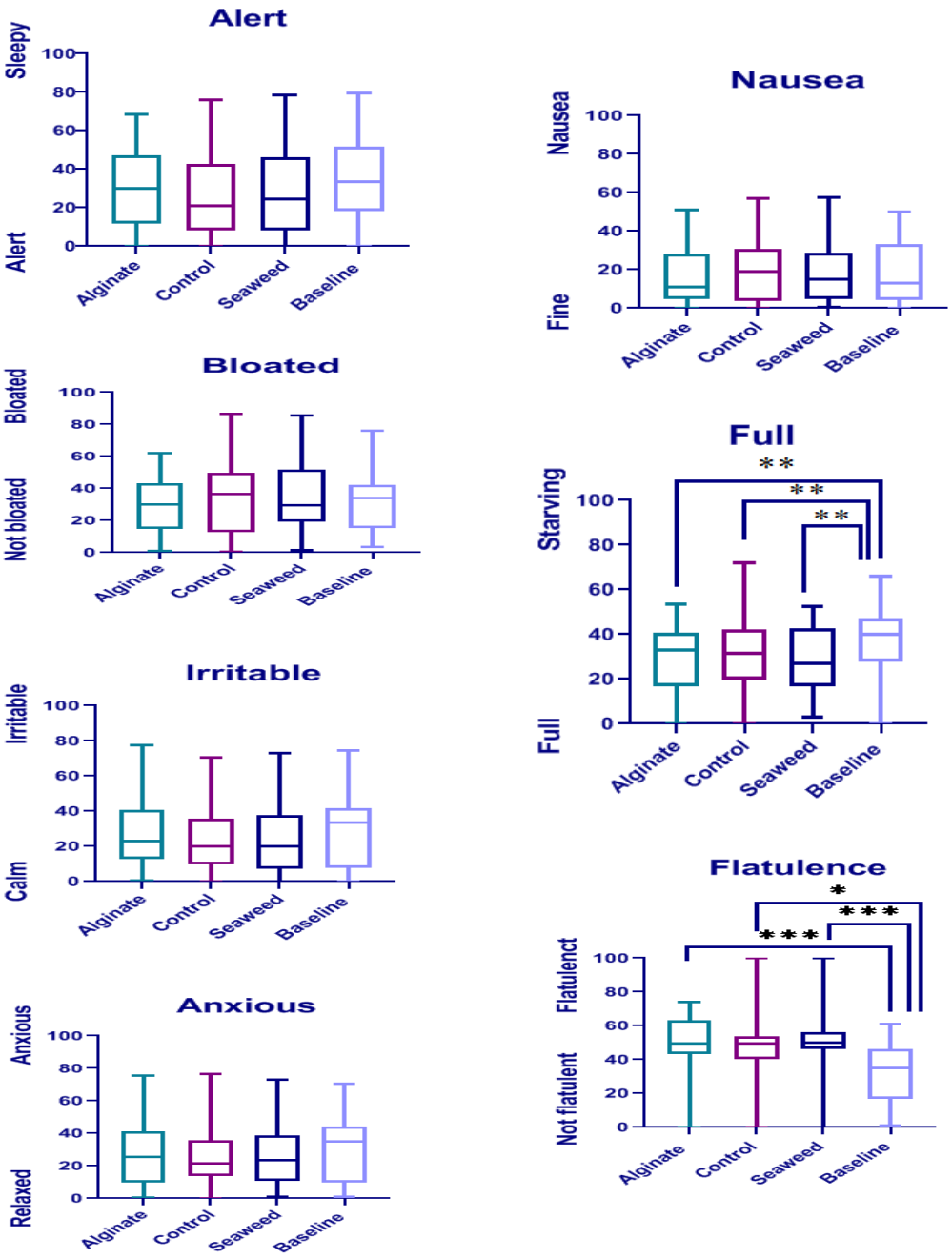
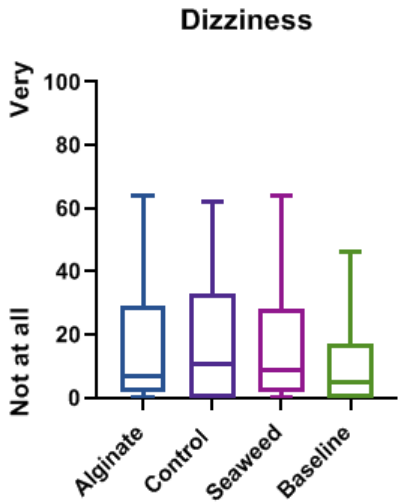


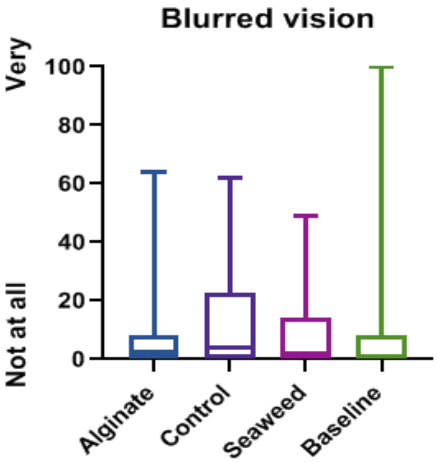
Figure 2.8 The variation between the average daily wellbeing scores including baseline, alginate, seaweed, and control cheese week. *P* values <0.05 are represented by *, *P* values <0.005 are represented by ** and <0.0005 are represented by ***.

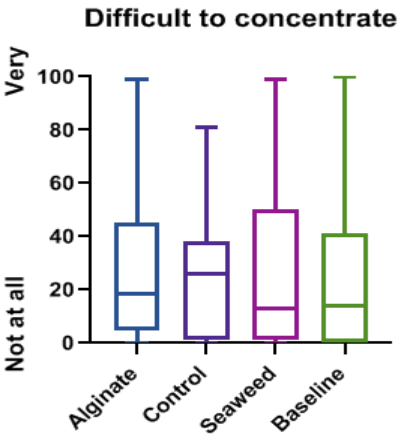
2.5.4- Weekly wellbeing questionnaire

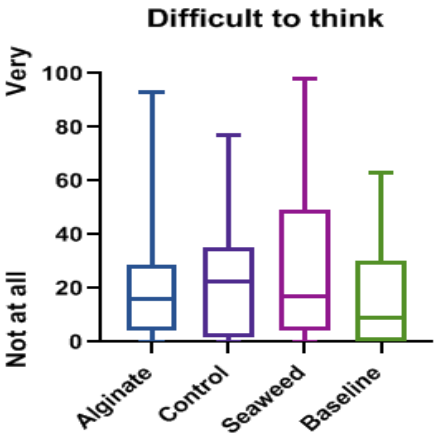
Tukey's multiple comparison test was used to compare the average weekly wellbeing responses to the 12 questions used in **Figure 2.5**, during the baseline, alginate, seaweed, and control cheese week are presented in **Figure 2.9**. There was no significant difference ($P>0.05$) in terms of dizziness, thirst, headaches, cravings for sweets, abdominal discomfort, blurred vision, abdominal pain, difficult to concentrate, difficult to think and bowel habit.

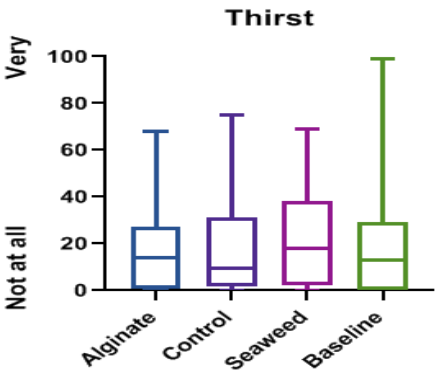
However, with regards to the urgency to pass stool, there was a significant difference when the participants consumed alginate versus control cheese ($P< 0.05$) as they had less flatulence with control cheese intake compared with alginate cheese week, seaweed versus alginate cheese ($P< 0.05$) they were less urgent to pass stool with seaweed cheese consumption compared to alginate cheese intake, seaweed versus baseline week ($P< 0.05$) they were significantly less urgent to pass stool with seaweed intake compared with baseline intake and control versus baseline week($P< 0.05$) they felt less urgent to pass stool with control cheese intake compare to baseline week. Additionally, there was a significant difference for being flatulent during the alginate week compare to baseline ($P< 0.05$)as they were slightly more flatulent with alginate cheese intake compare with baseline week and between seaweed and baseline($P< 0.05$) they had more flatulence with seaweed cheese intake compared to baseline week.

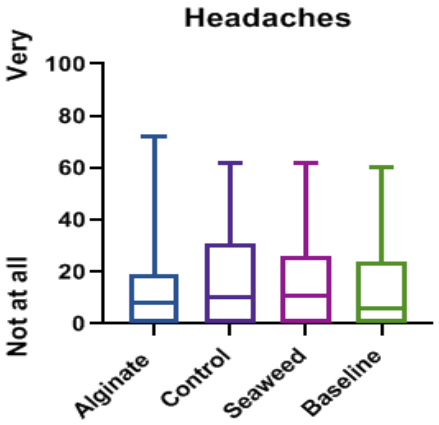

Dizziness
Very
Not at all
100
80
60
40
20
0
Alginate
Control
Seaweed
Baseline


Blurred vision
Very
Not at all
100
80
60
40
20
0
Alginate
Control
Seaweed
Baseline


Difficult to concentrate
Very
Not at all
100
80
60
40
20
0
Alginate
Control
Seaweed
Baseline


Difficult to think
Very
Not at all
100
80
60
40
20
0
Alginate
Control
Seaweed
Baseline


Thirst
Very
Not at all
100
80
60
40
20
0
Alginate
Control
Seaweed
Baseline


Headaches
Very
Not at all
100
80
60
40
20
0
Alginate
Control
Seaweed
Baseline

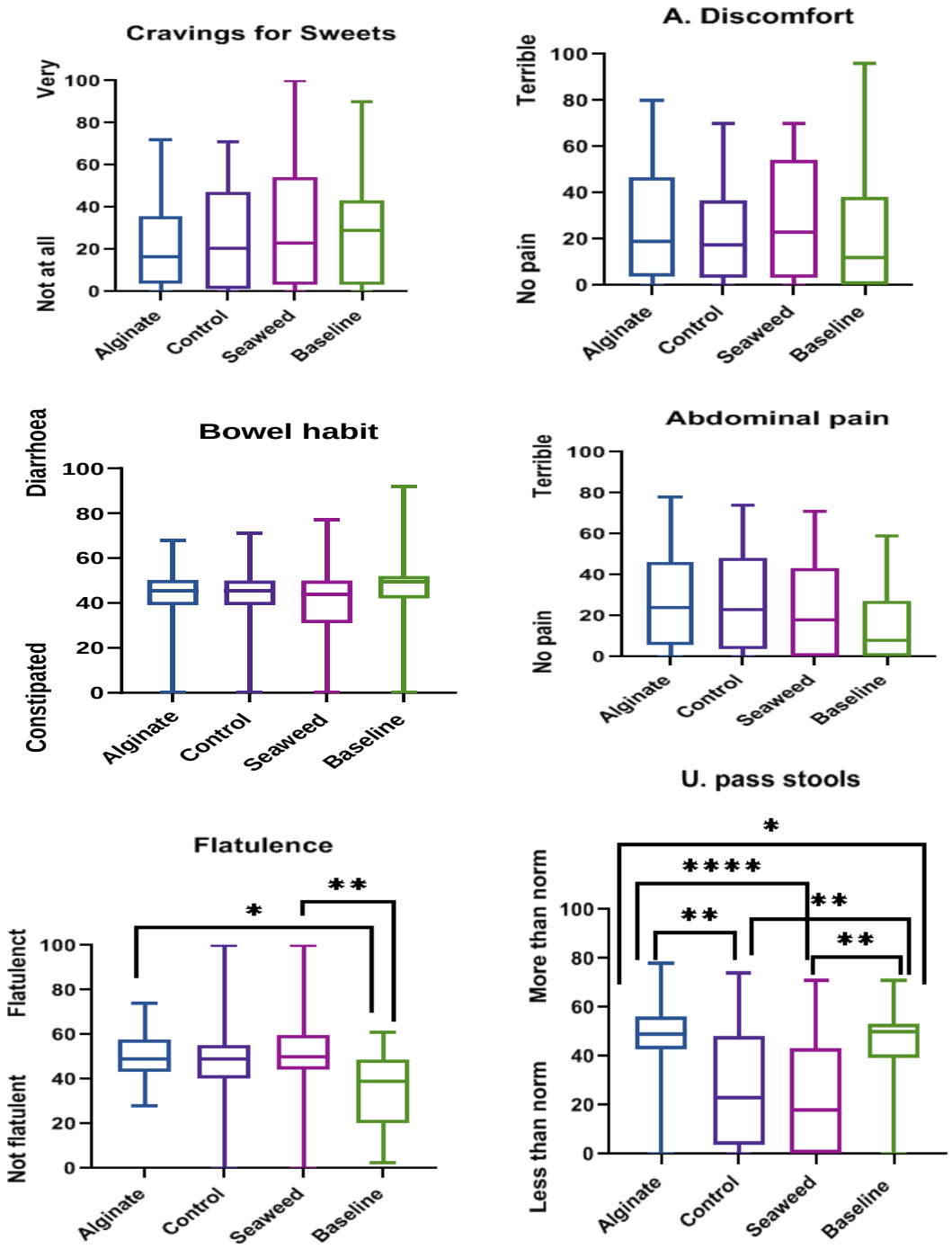


Figure 2.9 The variation between the average weekly wellbeing scores including baseline, alginate, seaweed, and control cheese week. U. indicates urgency to pass stools and A. indicates abdominal discomfort. *P* values <0.05 are represented by *, *P* values <0.005 are represented by ** and <0.00005 are represented by ****. In terms of the normal responses only bowel habit, flatulence and urgency to pass stools would be expected to be around 50 on the y axis. All the other measures would be expected to be close to zero.

2.5.5- Correlation between wellbeing and nutrients consumed daily and weekly

The correlation between the average daily nutrient consumption and the average wellbeing scores obtained when the participants consumed their ordinary diet at the baseline, alginate, seaweed and control cheese is presented in **Table 2.5**. The correlation between the average of nutrients consumed and the responses to the extra questions asked at the end of each week of the cheese consumption is shown in **Table 2.6**. The relationship between the average food consumed including the study foods per day and the participants wellbeing responses were determine by calculating the coefficient correlation (r). Overall, there were no significant correlation between the intake of calories, carbohydrate, fat, cholesterol, protein, sodium, sugars and fibre when the participants ate both the alginate and seaweed cheeses, and their feeling of being alert or sleepy, nauseous or fine, full or starving, bloated or not, flatulent or not, irritable or calm and anxious or relaxed every day. However, when they had their habitual diet without the study cheese, their feeling of being flatulence in association with dietary Na intake was significantly increased ($P< 0.05$) and r = 0.34 which indicates a positive correlation between these two variables. Moreover, compared to control cheese intake, there was a positive correlation between the fibre intake and an increased feeling of nausea significantly as r = 0.33 ($P< 0.05$). In other words, whenever their Na and fibre intake increased, their feelings of flatulence and nausea increased respectively. The effect of simultaneous increases in sodium and fibre intake was not tested on feelings of flatulence and nausea.

With regards to the questionnaires completed at the end of each week (**Figure 2.5**). During the week of seaweed cheese intake, a significant negative correlation (r = - 0.41) has been observed between the average participant`s intake of sugar and being flatulent ($P < 0.05$), which revealed that, whenever sugar intake increased, this was associated with feeling less flatulent than normal. This a potential indication of no adverse effects of seaweed cheese intake on being flatulent.

Moreover, during alginate cheese consumption, there was a strong positive correlation r = 0.63 between sugar intake and the feeling of dizziness ($P < 0.05$), having blurred vision ($P <0.05$), and having headaches ($P< 0.05$); this was based on the VAS response of the participants with answers located between 0 to 10 – 0 representing not at all and 100mm representing severe.

The feeling with difficulty concentrating ($P = 0.05$) and having abdominal discomfort ($P< 0.05$) had VAS located between 0 to 20mm. In addition, the dietary protein intake was significantly positively correlated with the increased urgency to pass stools ($P = 0.05$). Despite this correlation with the VAS responses being located between 40 and 60 mm on the scale which is right in the middle and as 0 indicates less than norm and 100 shows more than norm, this demonstrates that the alginate cheese has not created a problem in urgency to pass stools. Importantly, the dietary fibre intake significantly correlated with bowel habits ($P< 0.05$) as the VAS was located between 40 and 60 mm on the scale and showed no change in the bowel habits feeling, as 0 represented constipated and 100 indicated diarrhoea. Generally, the consumption of alginate cheese did not change the participants' wellbeing.

Regarding to the control cheese intake, there was a positive correlation between the urgency to pass stools and sugar intake ($P< 0.05$), the VAS presented between 40 and 60 mm, indicating normal feelings. However, there was a negative correlation between sugar intake and bowel habit ($P< 0.05$) as the VAS range in between 40 and 60 mm on the scale with a slight trend toward having constipation. This result could be related to the fact that the participants had the lowest fibre intake (**Figure 2.7**) in this week compared with the other weeks.

Finally, in the baseline week, there was a positive correlation toward protein intake and abdominal discomfort ($P< 0.05$); the VAS was located between 0 and 20 mm on the scale with 0 representing Not at all and 100 showing Very. Also, the same nutrient significantly correlated with the feeling of abdominal pain ($P <0.05$); the VAS range in between 0 to 30 with 0 representing no pain and 100 described as Terrible. The same result of feeling of abdominal pain and the VAS was also seen with cholesterol intake ($P = 0.05$). There was a negative correlation between blurred vision and the intake of fat ($P< 0.05$); with the VAS range in between 0 to 15 mm on the scale. Therefore, increased fat intake correlated with reduced blurred vision. Also, they felt less abdominal discomfort and less pain with protein and cholesterol intake during their habitual diet.

All significant correlation between the daily wellbeing scores **Figure 2.10A**, and weekly wellbeing scores and the nutrients consumed in baseline, alginate, seaweed, and the control cheese weeks are presented in **Figure 2.10 B**.

This data is the correlation between the average diet intakes for example, the average protein intake during the baseline week and what level of abdominal discomfort they reported. The

figure shows the majority of participants had low levels of abdominal discomfort. Regarding to protein intake, most of the values were around 60-70g. This fits with, the current average protein consumed per day of 76g/day for adults between 19-64 years old in the UK However several subjects had levels of 90g and above exceeding the average intake. Most of the values in this study exceeded the daily required levels which are ~45g/day for women and ~56g/day for men (https://www.nutrition.org.uk/healthy-sustainable-diets/protein/?level=Health%20professional).

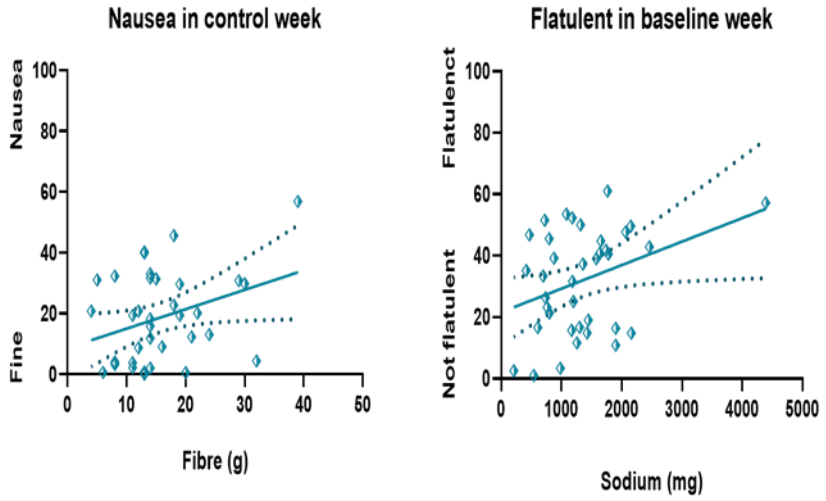
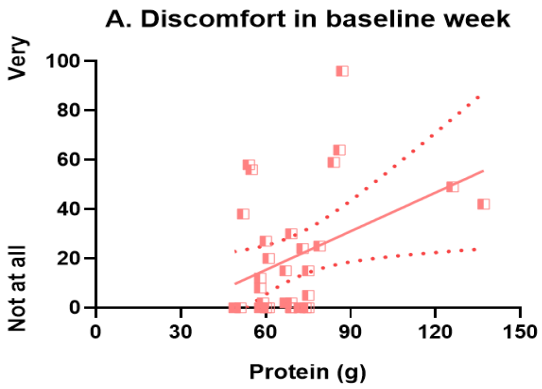
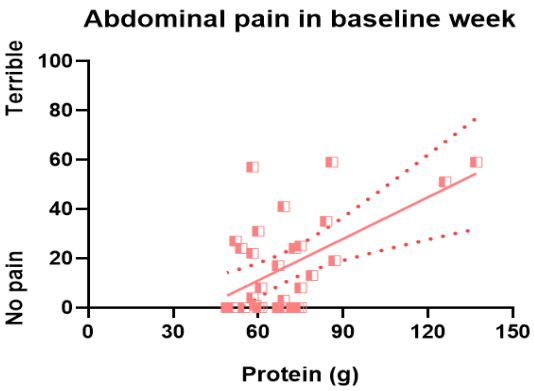
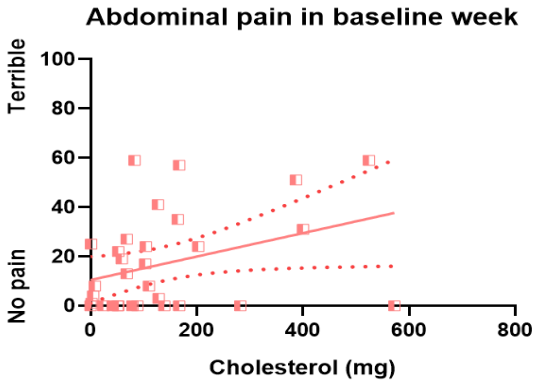
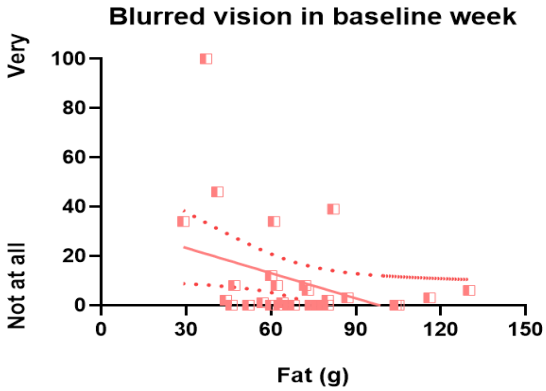


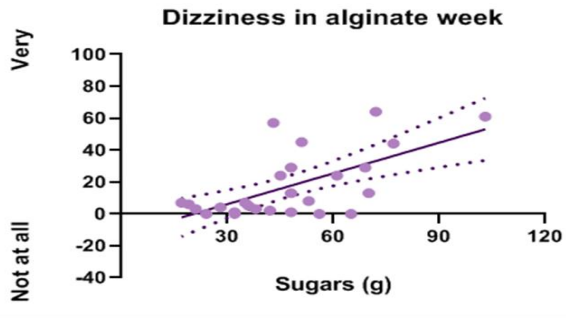
Figure 2.10 A Two significant correlation between the specific nutrients correlated with specific symptoms of daily wellbeing. It shows a significant correlation between the average daily fibre intake and the feeling of nausea during the control cheese intake as well as it presents the significant correlation between the feelings of being flatulent in association with dietary Na intake during the habitual diet intake. Each point represents a single participant.

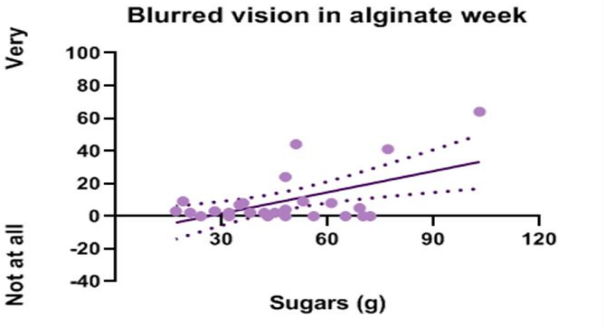

A. Discomfort in baseline week
Very
Not at all
100
80
60
40
20
0
0
30
60
90
120
150
Protein (g)

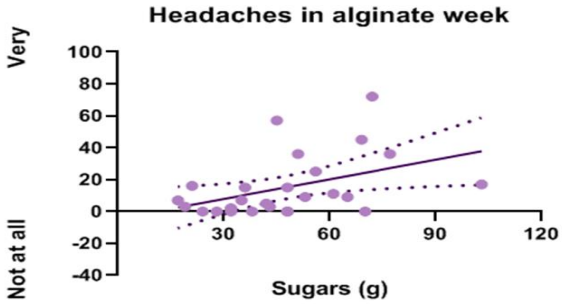

Abdominal pain in baseline week
Terrible
No pain
100
80
60
40
20
0
0
30
60
90
120
150
Protein (g)

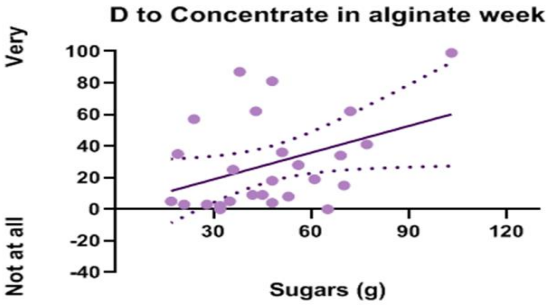

Abdominal pain in baseline week
Terrible
No pain
100
80
60
40
20
0
0
200
400
600
800
Cholesterol (mg)

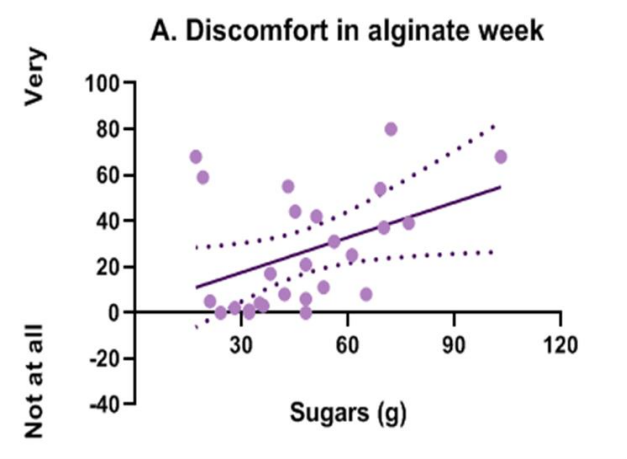

Blurred vision in baseline week
Very
Not at all
100
80
60
40
20
0
0
30
60
90
120
150
Fat (g)

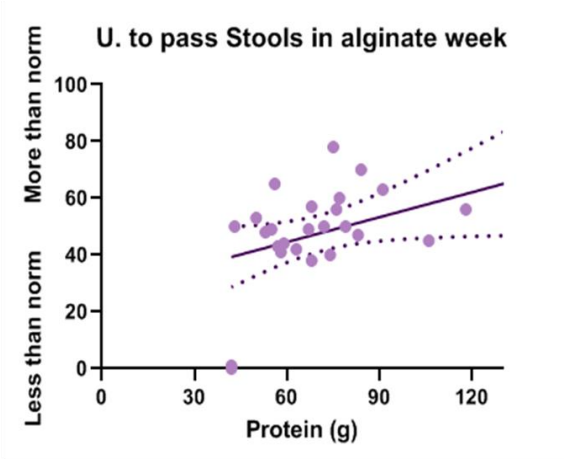

Dizziness in alginate week
Very
Not at all
100
80
60
40
20
0
-20
-40
30
60
90
120
Sugars (g)

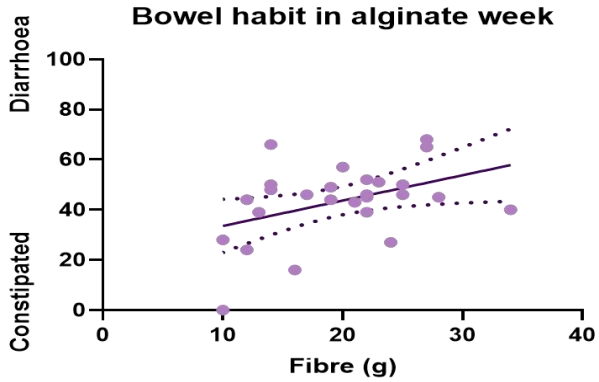

Blurred vision in alginate week
Very
Not at all
100
80
60
40
20
0
-20
-40
30
60
90
120
Sugars (g)


Headaches in alginate week
Very
Not at all
100
80
60
40
20
0
-20
-40
30
60
90
120
Sugars (g)


D to Concentrate in alginate week
Very
Not at all
100
80
60
40
20
0
-20
-40
30
60
90
120
Sugars (g)


A. Discomfort in alginate week
Very
Not at all
100
80
60
40
20
0
-20
-40
30
60
90
120
Sugars (g)


U. to pass Stools in alginate week
More than norm
Less than norm
100
80
60
40
20
0
0
30
60
90
120
Protein (g)


Bowel habit in alginate week
Diarrhoea
Constipated
100
80
60
40
20
0
0
10
20
30
40
Fibre (g)

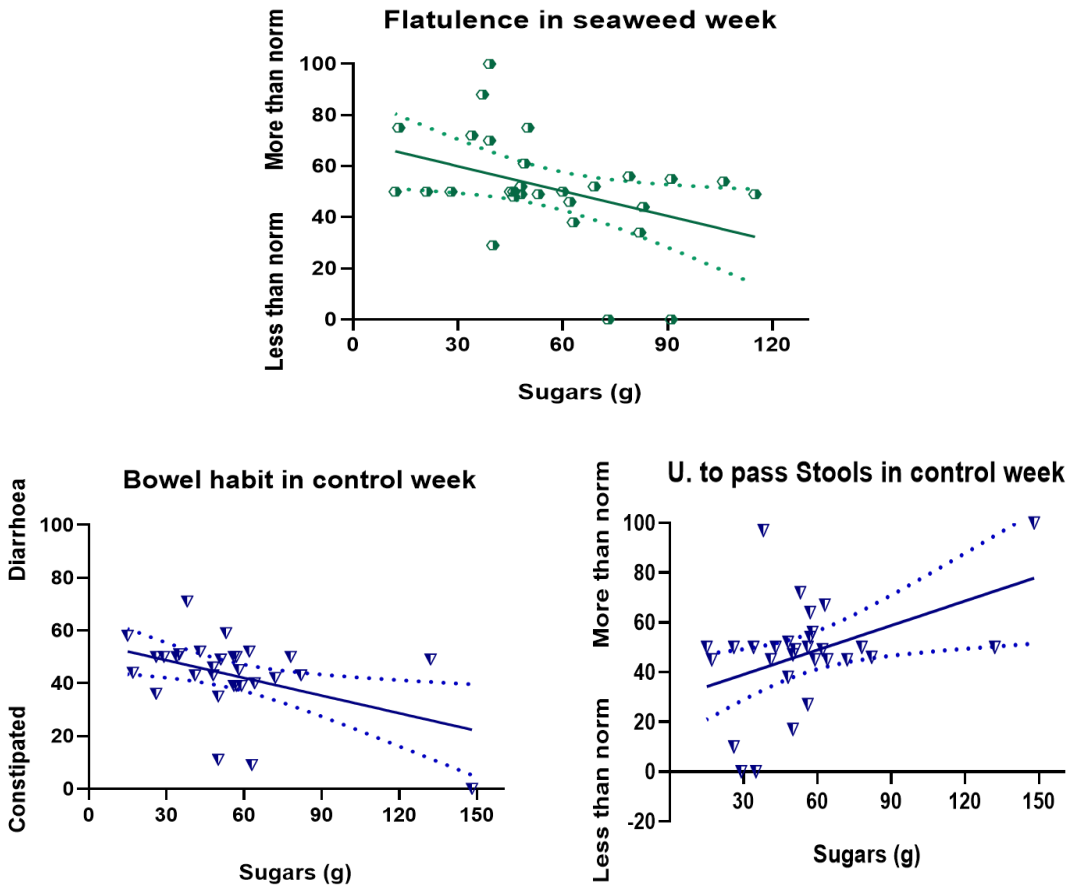


Figure 2.10 B Significant correlation between the specific nutrients correlated with specific symptoms of weekly wellbeing during the consumption of habitual diet in baseline, alginate, seaweed, and the control cheese weeks respectively. This figure is separated to four different week and each week has a different colour. The first is the baseline week 1 which has a pink colour; the second is alginate week with a purple colour; the third week is seaweed week with green colour and the last week is control week with dark blue colour. The solid line a linear regression between the dietary variables and the well-being scores. The dotted lines represent the 95% confidence bands of the linear regression best fit.

	Alert	Nausea	Full	Bloated	Flatulent	Irritable	Anxious
Bassline week							
Calories (kcal)	ns	ns	ns	ns	ns	ns	ns
Carbohydrate (g)	ns	ns	ns	ns	ns	ns	ns
Fat (g)	ns	ns	ns	ns	ns	ns	ns
Cholesterol (mg)	ns	ns	ns	ns	ns	ns	ns
Protein (g)	ns	ns	ns	ns	ns	ns	ns
Sodium (mg)	ns	ns	ns	ns	*0.343	ns	ns
Sugars (g)	ns	ns	ns	ns	ns	ns	ns
Fibre (g)	ns	ns	ns	ns	ns	ns	ns
Alginate cheese week							
Calories (kcal)	ns	ns	ns	ns	ns	ns	ns
Carbohydrate (g)	ns	ns	ns	ns	ns	ns	ns
Fat (g)	ns	ns	ns	ns	ns	ns	ns
Cholesterol (mg)	ns	ns	ns	ns	ns	ns	ns
Protein (g)	ns	ns	ns	ns	ns	ns	ns
Sodium (mg)	ns	ns	ns	ns	ns	ns	ns
Sugars (g)	ns	ns	ns	ns	ns	ns	ns
Fibre (g)	ns	ns	ns	ns	ns	ns	ns
Seaweed cheese week							
Calories (kcal)	ns	ns	ns	ns	ns	ns	ns
Carbohydrate (g)	ns	ns	ns	ns	ns	ns	ns
Fat (g)	ns	ns	ns	ns	ns	ns	ns
Cholesterol (mg)	ns	ns	ns	ns	ns	ns	ns
Protein (g)	ns	ns	ns	ns	ns	ns	ns
Sodium (mg)	ns	ns	ns	ns	ns	ns	ns
Sugars (g)	ns	ns	ns	ns	ns	ns	ns
Fibre (g)	ns	ns	ns	ns	ns	ns	ns
Control cheese week							
Calories (kcal)	ns	ns	ns	ns	ns	ns	ns
Carbohydrate (g)	ns	ns	ns	ns	ns	ns	ns
Fat (g)	ns	ns	ns	ns	ns	ns	ns
Cholesterol (mg)	ns	ns	ns	ns	ns	ns	ns
Protein (g)	ns	ns	ns	ns	ns	ns	ns
Sodium (mg)	ns	ns	ns	ns	ns	ns	ns
Sugars (g)	ns	ns	ns	ns	ns	ns	ns
Fibre (g)	ns	*0.333	ns	ns	ns	ns	ns

Table 2.5 shows the correlation between the daily average nutrients consumed in the baseline, alginate, seaweed and control cheese weeks, and the average change in daily wellbeing scores from the daily questionnaire. * states the significant correlation with $P < 0.05$ between the specific wellbeing and the specific dietary nutrient. All the numbers represent the r values of Pearson coefficient and ns refers to no significant correlation.

	Dizziness	Blurred vision	Difficulty to concentrate	Difficulty to think	Excessive thirst	Headaches migraines	Cravings for sweets	Abdominal discomfort	Bowel habit	Urgency to pass stools	Abdominal pain	Amount of Flatulence
Baseline week												
Calories (kcal)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Carbohydrate (g)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Fat (g)	ns	*- 0.319	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cholesterol (mg)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*0.357	ns
Protein (g)	ns	ns	ns	ns	ns	ns	ns	*0.396	ns	ns	*0.549	ns
Sodium (mg)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Sugars (g)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Fibre (g)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Alginate cheese week												
Calories (kcal)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Carbohydrate (g)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Fat (g)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cholesterol (mg)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Protein (g)	ns	ns	ns	ns	ns	ns	ns	ns	ns	*0.390	ns	ns
Sodium (mg)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Sugars (g)	*0.634	*0.547	*0.393	ns	ns	*0.435	ns	*0.409	ns	ns	ns	ns
Fibre (g)	ns	ns	ns	ns	ns	ns	ns	ns	*0.417	ns	ns	ns
Seaweed cheese week												
Calories (kcal)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Carbohydrate (g)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Fat (g)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cholesterol (mg)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Protein (g)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Sodium (mg)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Sugars (g)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*- 0.406
Fibre (g)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Control cheese week												
Calories (kcal)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Carbohydrate (g)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Fat (g)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cholesterol (mg)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Protein (g)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Sodium (mg)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Sugars (g)	ns	ns	ns	ns	ns	ns	ns	ns	*0.43	*0.423	ns	ns
Fibre (g)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 2.6 The correlation between the average nutrients consumed per day and the weekly wellbeing score during the consumption of alginate, seaweed and the control cheese. * states the significant correlation with $P < 0.05$ between the variables and ns refers to no significant correlation.

2.5.6- Palatability and acceptance of seaweed and alginate cheese

Thirty-seven participants consumed the three cheeses in this study, except for the seaweed cheese. Two female participants; one had wellbeing issues following seaweed cheese intake (diarrhoea and light headedness approximately 1.5 hours after eating the cheese). Thus, she was immediately asked to stop and the second one did not take seaweed cheese because the supply had run out. Also, many participants observed the seaweed cheese became mouldy after a few days of consumption and as such, were given a new seaweed cheese to consume instead. Just twenty-two participants out of 37 provided comments during the study about their opinions of the cheese products. Three participants reported that they were overall satisfied with the alginate, seaweed and the control cheese provided in this study and the other 19 participants provided their opinion between like and dislike as presented in the next section.

As far as GI wellbeing is concerned, with alginate cheese intake, one subject reported “I had a rather loose stool”. Another subject described an increase in flatulence associated with increased use of the toilet. On the other hand, a participant reported feeling less hungry after alginate cheese intake. Additionally, two subjects specifically reported feelings of minor nausea and not much energy during the day, as well as flatulence following seaweed cheese intake; they also reported slight nausea on consumption of the control cheese. One volunteer reported “The urgency to pass stools has increased more than normal” associated with seaweed cheese consumption. In addition, “one of the main challenges I have faced during the study was how quickly the seaweed cheese went mouldy”. One participant observed that “the seaweed cheese portion had white patches over it, so I didn't eat it!”. However, regarding the feeling of wellness, one participant said that “At first, I ate the cheese for my lunch, but I changed it and make it as my snacks with tea. My body feels more healthy and even though I have full schedules, I usually get to sleep easier than before” was reported during consumption of the seaweed cheese.

Regarding palatability, two participants really enjoyed the seaweed cheese because they were familiar with seaweed, but they disliked the alginate cheese; they describe it as “the worst cheese they ever had”, and one of them said the “flavour of cheese was acrid”. In addition, a further two subjects preferred the seaweed cheese and one said, “I’d definitely buy that if I saw it”. However, five participants described the seaweed cheese respectively: “Cheese number 2 was really disgusting!”, “Not my favourite cheese”, “Seaweed cheese is quite unpleasant, it is not nice”, “Seaweed cheese was not very palatable”, and also the fifth one said: “Not my favourite cheese” as well. Moreover, two participants mentioned that alginate cheese was fine, and one of them observed an increased appetite with alginate cheese. Additionally, two subjects preferred just the control cheese, one of them said “a third cheese with green bits which was also palatable” and that they did not like alginate or seaweed cheese. Because the aroma from the seaweed cheese it was difficult to blind the subjects to the cheese type Therefore in this study, all participants had knowledge which type of cheese that would be consumed each week.

Pork sausage acceptability study

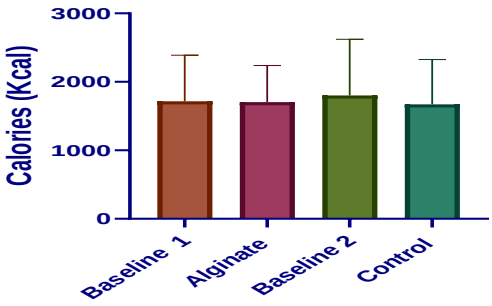
2.5.7- Subject characteristics

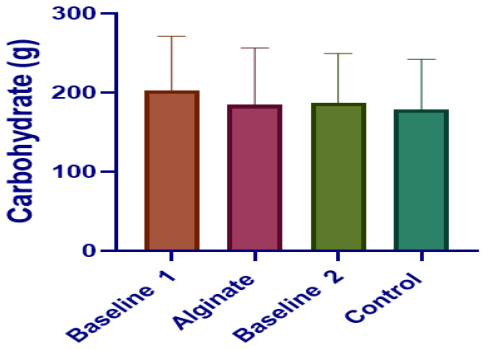
Thirty-eight volunteers were eligible to participate in the pork sausage acceptability study, however, some participants were lost as they withdrew before starting the study (n = 1), withdrew during the study due to an inability to comply with the food diary (n = 1), and feeling sick, dizzy and with very bloated stomach (n = 1). Therefore, 35 participants completed the study: 18 male age (35.5 ± 11.2) and 17 females age (24 ± 13.1).

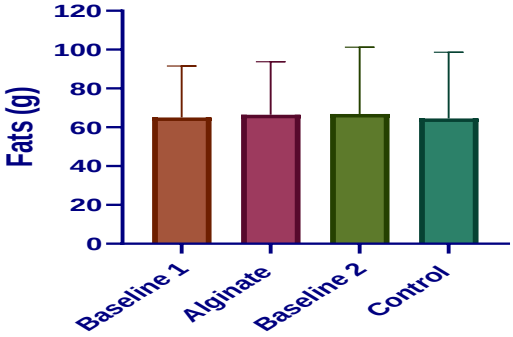
2.5.8- Energy and macronutrient intake

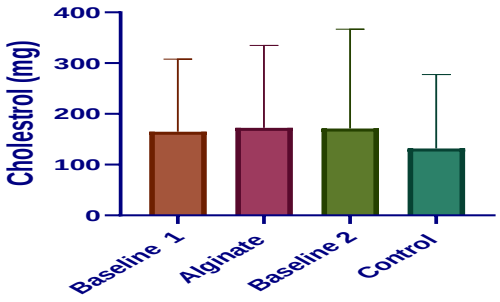
The average daily intake of calories, protein, fat, cholesterol, carbohydrate, sugar, sodium, and dietary fibre by the 35 eligible subjects are shown in **Figure 2.11** Repeated measures one-way ANOVA indicated that the participants' intake of food in their habitual diet, alginate sausage, control sausage and in the baseline 2 (washout week) on the intervention were not significantly different in terms of the consumption of energy, carbohydrate, protein, fat, cholesterol and sodium all P values > 0.05.

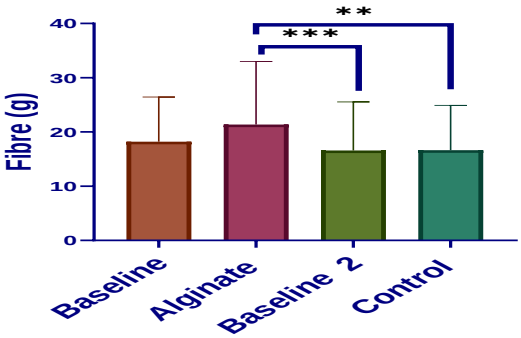
The intake of sodium from the diet including the alginate sausage appeared to be lower comparing to the sodium intake from the control sausage week, and the first and second baseline weeks, but these differences were not statistically significant (P>0.05). The intake of fibre during the alginate sausage week was statistically higher than in the baseline 2 week (P< 0.05) and in the control sausage week (P< 0.05), but not when compared with the fibre intake from the habitual diet (P > 0.05). Almost all the fibre intake during this week came from the consumption of alginate, thus alginate was the main contributor to the increase in their dietary fibre intake. The participants in their habitual diet consumed higher sugar levels compared with what they did during the control sausage intake (P< 0.05). **Table 2.7** represents the average calories and nutrients consumed with baseline week 1 and 2, alginate sausage, and the control sausage.

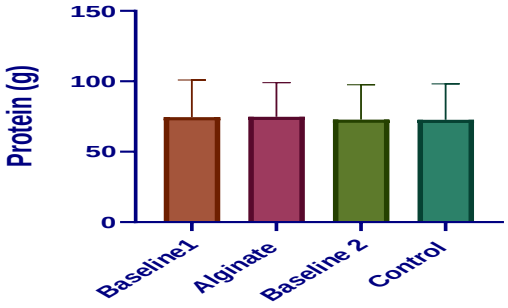

Calories (Kcal)
3000
2000
1000
0
Baseline 1
Alginate
Baseline 2
Control

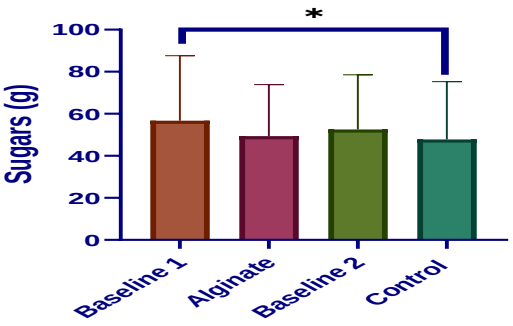

Carbohydrate (g)
300
200
100
0
Baseline 1
Alginate
Baseline 2
Control


Fats (g)
120
100
80
60
40
20
0
Baseline 1
Alginate
Baseline 2
Control


Cholestrol (mg)
400
300
200
100
0
Baseline 1
Alginate
Baseline 2
Control


Fibre (g)
**
***
40
30
20
10
0
Baseline
Alginate
Baseline 2
Control


Protein (g)
150
100
50
0
Baseline1
Alginate
Baseline 2
Control


Sugars (g)
*
100
80
60
40
20
0
Baseline 1
Alginate
Baseline 2
Control

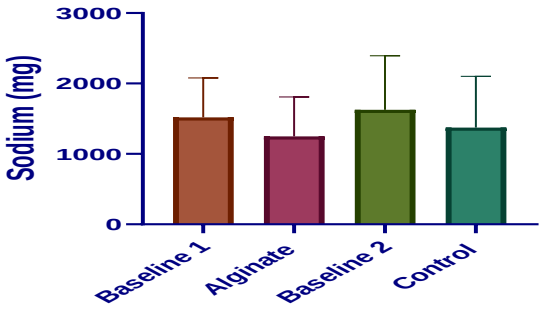

Sodium (mg)
3000
2000
1000
0
Baseline 1
Alginate
Baseline 2
Control

Figure 2.11 The variation between the average amounts of the food nutrients consumed from baseline week 1, alginate sausage week, baseline week 2 and control sausage week including calories intake (kcal), protein, carbohydrate, fat, fibre, sugar (g), cholesterol and sodium (mg).

The data shows the mean with standard deviation (±SD) of the 35 participants during the 4 weeks of the study. *P* values <0.05 are represented by * *P* values <0.005 are represented by ** and <0.0005 are represented by ***. For fibre, the method used was a repeat measures One-Way ANOVA with Tukey's multiple comparison test. This compared the mean of each column with the mean of every other column.

There were 35 volunteers, each with seven data points per sample, therefore up to 245 data points for each comparator, allowing the analysis to show statistical differences, even with overlapping standard deviations. The data could be shown as standard error of the mean, giving much lower error bars but as all other figures are presented as standard deviation, this might lead to confusion for the reader. For sugar, the method used was a repeat measures One-Way ANOVA with Tukey's multiple comparison test. This compared the mean of each column with the mean of every other column.

Nutrient intake	Baseline week 1 (n= 35)	Alginate sausage (n= 35)	Baseline week 2 (n= 35)	Control sausage (n= 35)	*P* value
Calories (kcal)	1771± 591	1702 ± 491	1751 ± 538	1665 ± 534	**0.58**
Carbohydrate (g)	203 ± 69	185 ± 72	187 ± 62	179 ± 63	**0.10**
Fat (g)	65 ± 27	66 ± 27	67 ± 34	64 ± 34	**0.87**
Cholesterol (mg)	165 ± 143	173 ± 162	171 ± 196	132 ± 146	**0.19**
Fibre (g)	18 ± 8	21 ± 12	17± 9	17 ± 8	***0.00**
Sugars (g)	57 ± 31	49 ± 25	53 ± 26	48 ± 28	***0.04**
Protein(g)	75 ± 26	75 ± 24	73 ± 25	73 ± 25	**0.28**
Sodium (mg)	1518 ± 559	1248 ± 561	1627 ± 766	1374 ± 727	**0.09**

Table 2.7 Shows a statistical summary of the difference of mean ± SD for the average daily intake of nutrients in the baseline week 1, alginate sausage week, baseline week 2, and the control sausage week. P value was derived from Ordinary one-way ANOVA with multiple comparisons and shows no significant difference (P >0.05) between the mean calories and all the nutrients consumed during the study except the intake of fibre and sugar, as * represents the significant value obtained from Ordinary one-way ANOVA of treatment between the 4 columns (all study weeks) (P < 0.05).

2.5.9- Daily wellbeing questionnaire

Overall, answers to the wellbeing questions used from **Figure 2.4** which were answered every day were not significantly different between the baseline 1, baseline 2, alginate and control sausage weeks. The correlations are presented in **Figure 2.12**. Tukey's multiple comparisons test showed no significant differences were observed between the wellbeing measurements for each week: alertness ($P>0.05$), nausea ($P>0.05$), fullness ($P>0.05$), bloating ($P>0.05$), flatulence ($P>0.05$), irritability ($P>0.05$) and anxiousness ($P>0.05$).

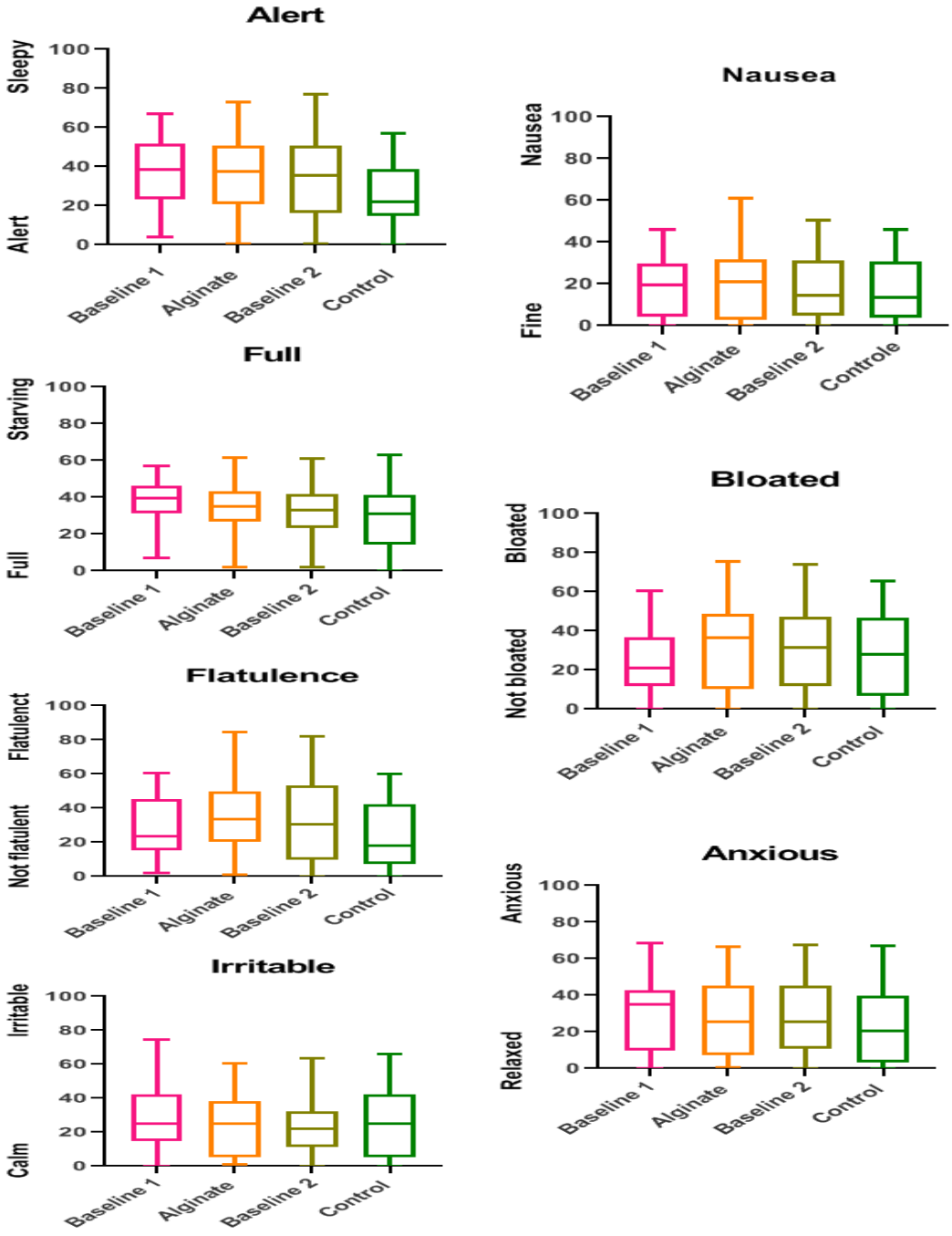
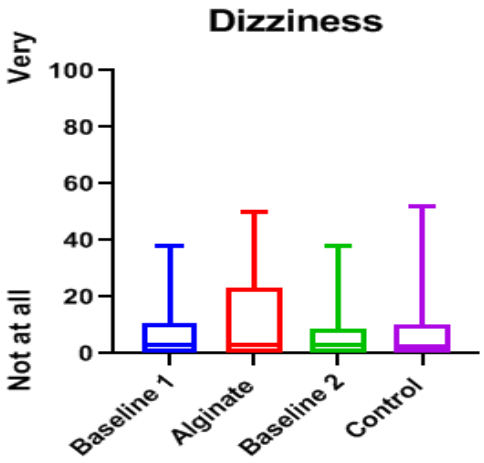
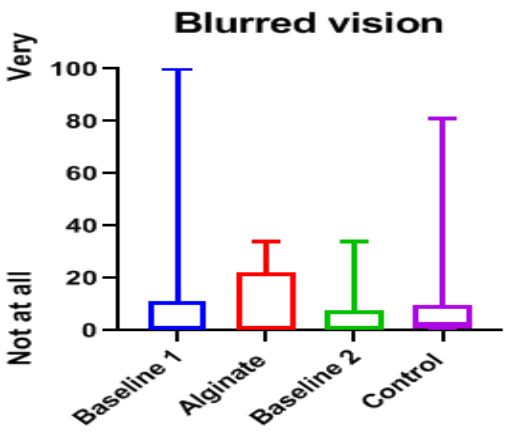


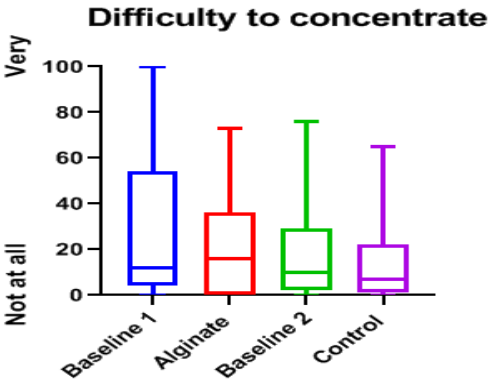
Figure 2.12 The variation between the average daily wellbeing scores including baseline 1, baseline 2, alginate, and control sausage weeks.

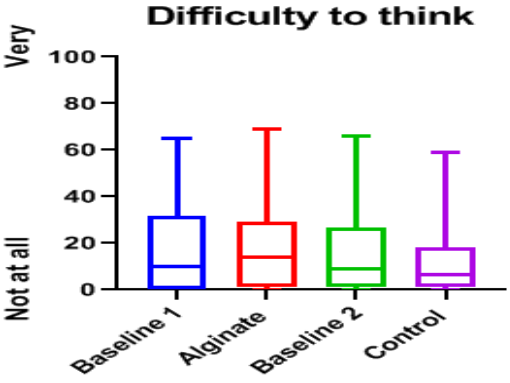
2.5.10- Weekly wellbeing questionnaire

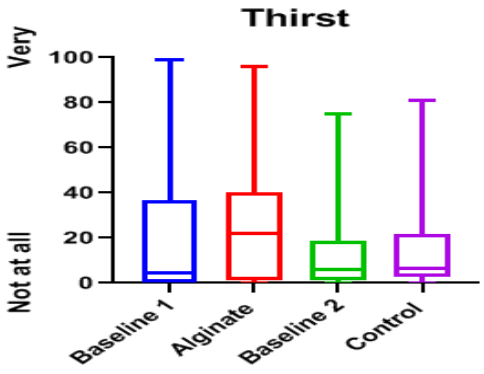
The scores for the weekly wellbeing questions were averaged between the baseline 1, baseline 2, alginate and control sausage week; all these responses to questions are presented in **Figure 2.13**. No significant difference was observed between the average scores for all questions ($P>0.05$) including dizziness, blurred vision, difficulty to concentrate, thirst, headaches, craving for sweet, abdominal discomfort, bowel habit, urgency to pass stools, abdominal pain and the amount of flatulence. Regarding stool softness, a very similar pattern was found between the alginate, control sausage and both baseline weeks among the participants. The feelings of thirst, abdominal discomfort and flatulence were the highest with alginate sausage intake compared to the other weeks, however they did not reach a level of significance.

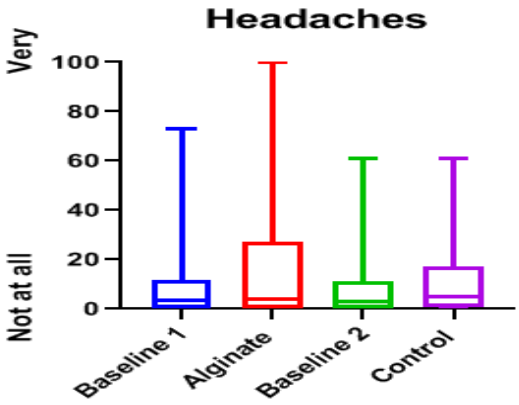

Dizziness
Very
Not at all
100
80
60
40
20
0
Baseline 1
Alginate
Baseline 2
Control


Blurred vision
Very
Not at all
100
80
60
40
20
0
Baseline 1
Alginate
Baseline 2
Control


Difficulty to concentrate
Very
Not at all
100
80
60
40
20
0
Baseline 1
Alginate
Baseline 2
Control


Difficulty to think
Very
Not at all
100
80
60
40
20
0
Baseline 1
Alginate
Baseline 2
Control


Thirst
Very
Not at all
100
80
60
40
20
0
Baseline 1
Alginate
Baseline 2
Control


Headaches
Very
Not at all
100
80
60
40
20
0
Baseline 1
Alginate
Baseline 2
Control

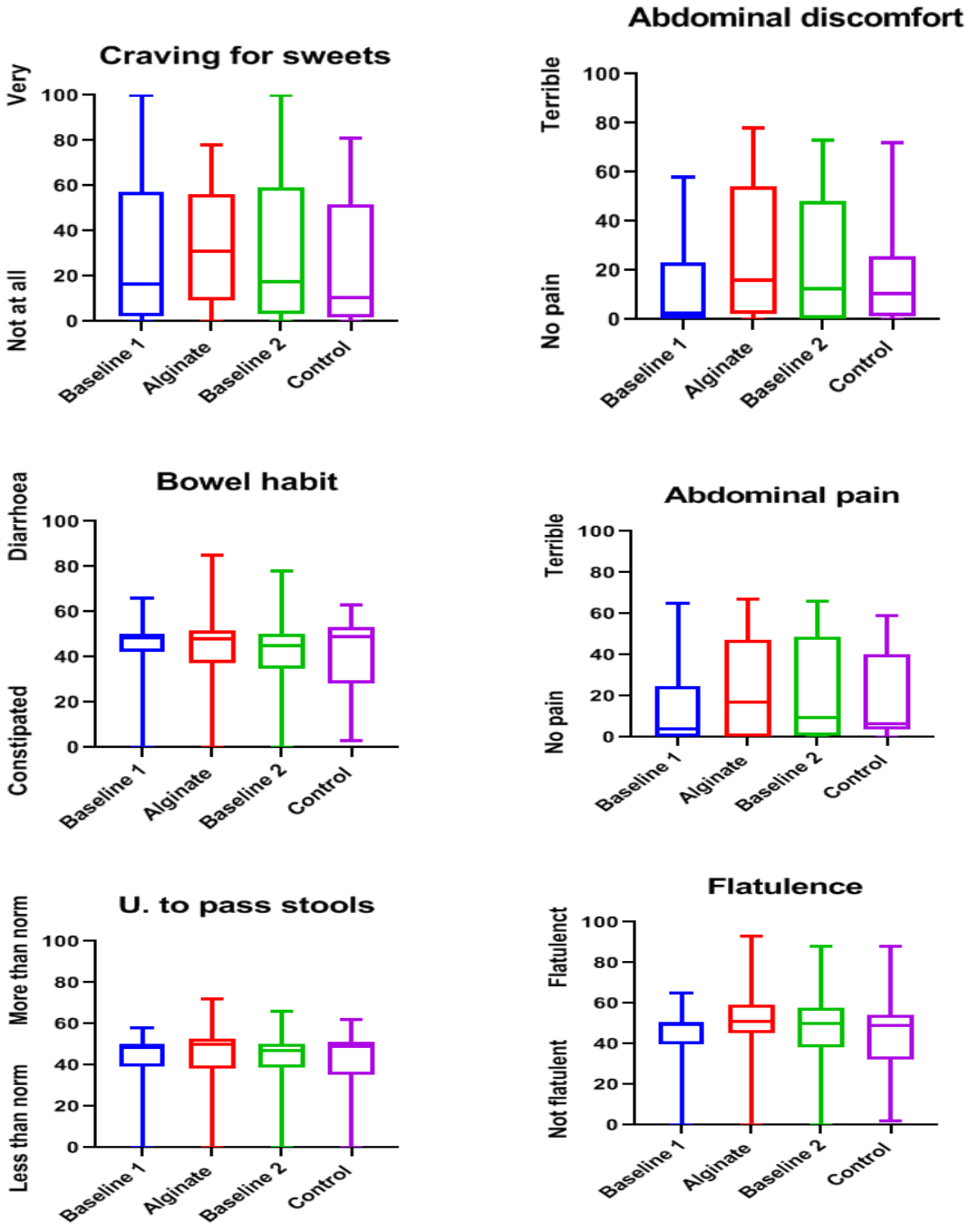


Figure 2.13 The average weekly wellbeing scores including baseline week 1, alginate sausage week, baseline week 2 and the control sausage week.

2.5.11- Correlation between wellbeing and nutrients consumed

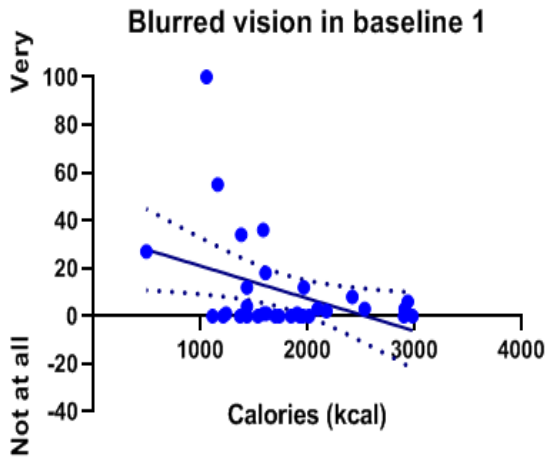
The correlation between the average daily consumption of nutrients including dietary energy, carbohydrate, protein, fat, cholesterol, sugar, fibre and sodium, and the daily wellbeing responses concerning bloating, irritability, and other wellbeing characteristics (**Figure 2.4**) are presented in **Table 2.8**. Pearson correlation coefficients showed no significant association between the average nutrients consumed including eating alginate and control pork sausages and any change in the average wellbeing scores throughout the whole study at each day ($P>0.05$).

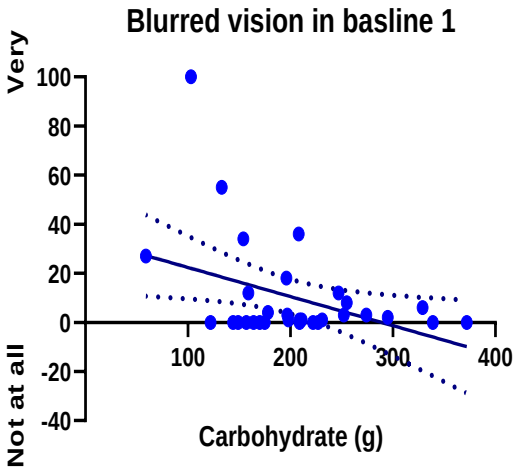
At the end of each week, some of the nutrients had a significant impact on several characteristics of wellbeing, **Table 2.9** represents the r value of correlation and* shows the statistical significance between the variables. During the alginate sausage week, the participants reported blurred vision, and this was negatively associated with calories, carbohydrate, protein, sugar and fibre. In addition, difficulty to think was negatively correlated with the intake of calories ($P<0.05$), protein ($P<0.05$) and sugar ($P<0.05$). A significant negative correlation was calculated between sugar consumed and headaches ($P<0.05$) and abdominal discomfort ($P<0.05$). An excessive thirst was negatively correlated with the amount of carbohydrate consumed ($P<0.05$) and a negative correlation was observed between protein intake and the feeling of dizziness ($P<0.05$) and difficult to concentrate was negatively correlated with calories consumed ($P<0.05$).

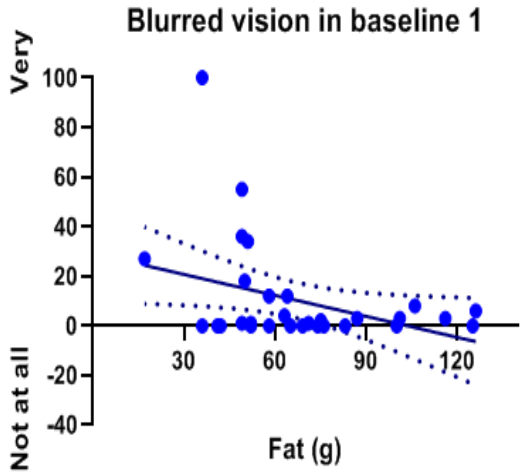
In comparison with the correlation between the normal food consumed at the baseline week 1 and wellbeing of that week, the average daily amounts of calories ($P<0.05$), carbohydrate ($P<0.05$), fat ($P<0.05$) and fibre consumed ($P<0.05$) were shown to negatively correlated with blurred vision. Moreover, there was a negative correlation described between the average daily calories ($P<0.05$), carbohydrate ($P<0.05$), fat ($P<0.05$) and sodium consumed ($P<0.05$) and the amount the subjects suffered from headaches or migraines. In addition, the average intake of sodium was negatively correlated with feeling of dizziness ($P<0.05$) and difficulty to think ($P<0.05$) and the carbohydrate intake was negatively correlated with being difficult to concentrate ($P<0.05$) as presented in **Table 2.9**.

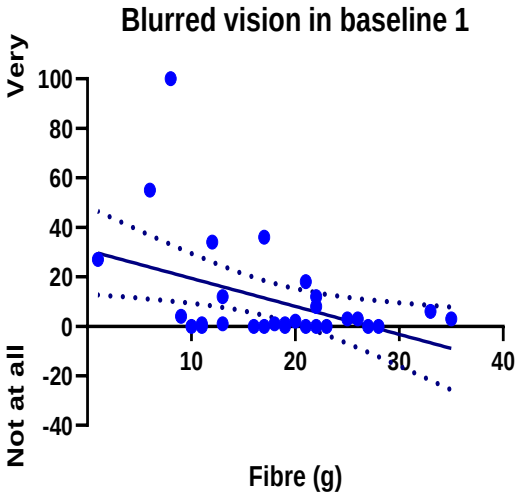
Additionally, during the control sausage week, the daily average amount of calories ($P<0.05$), carbohydrate ($P<0.05$), protein ($P<0.05$) and sugar ($P<0.05$) were negatively associated with excessive thirst. A significant negative correlation was calculated between dizziness ($P<0.05$), abdominal discomfort ($P<0.05$) and the amount of flatulence ($P<0.05$), and the average amount of protein consumed daily. Moreover, $P<0.05$ demonstrated a negative correlation between energy consumption and dizziness, and blurred vision respectively. There was also a negative correlation between the intake of sodium and changes in bowel habits ($P<0.05$).

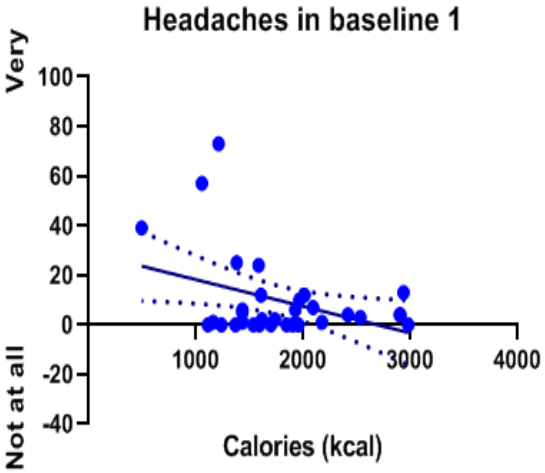
However, during the baseline week 2, a significant correlation was observed between the amounts of flatulence and the average intake of calories ($P<0.05$), carbohydrate ($P<0.05$), fat ($P<0.05$), protein ($P<0.05$) and sugar ($P<0.05$). The experience of flatulence in this week was related to the increased consumption of some foods such as broccoli, red kidney beans, cabbage, lentils and beans more than the intake of the same food items in both alginate and control sausage week. In addition, the intake of calories ($P<0.05$), fat ($P<0.05$) and cholesterol ($P<0.05$) were correlated with headaches. Cholesterol was also positively correlated with blurred vision ($P<0.05$).

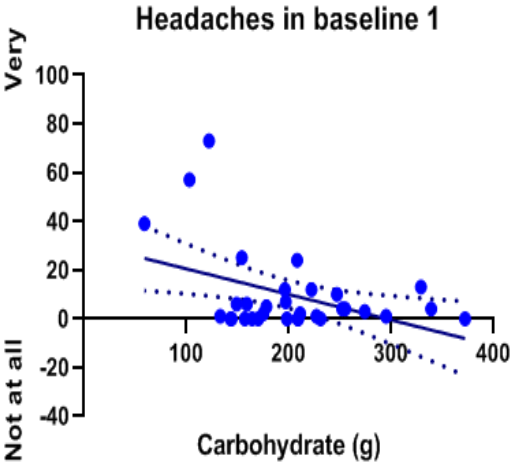

Blurred vision in baseline 1
Very
Not at all
100
80
60
40
20
0
-20
-40
1000
2000
3000
4000
Calories (kcal)


Blurred vision in basline 1
Very
Not at all
100
80
60
40
20
0
-20
-40
100
200
300
400
Carbohydrate (g)


Blurred vision in baseline 1
Very
Not at all
100
80
60
40
20
0
-20
-40
30
60
90
120
Fat (g)


Blurred vision in baseline 1
Very
Not at all
100
80
60
40
20
0
-20
-40
10
20
30
40
Fibre (g)


Headaches in baseline 1
Very
Not at all
100
80
60
40
20
0
-20
-40
1000
2000
3000
4000
Calories (kcal)


Headaches in baseline 1
Very
Not at all
100
80
60
40
20
0
-20
-40
100
200
300
400
Carbohydrate (g)

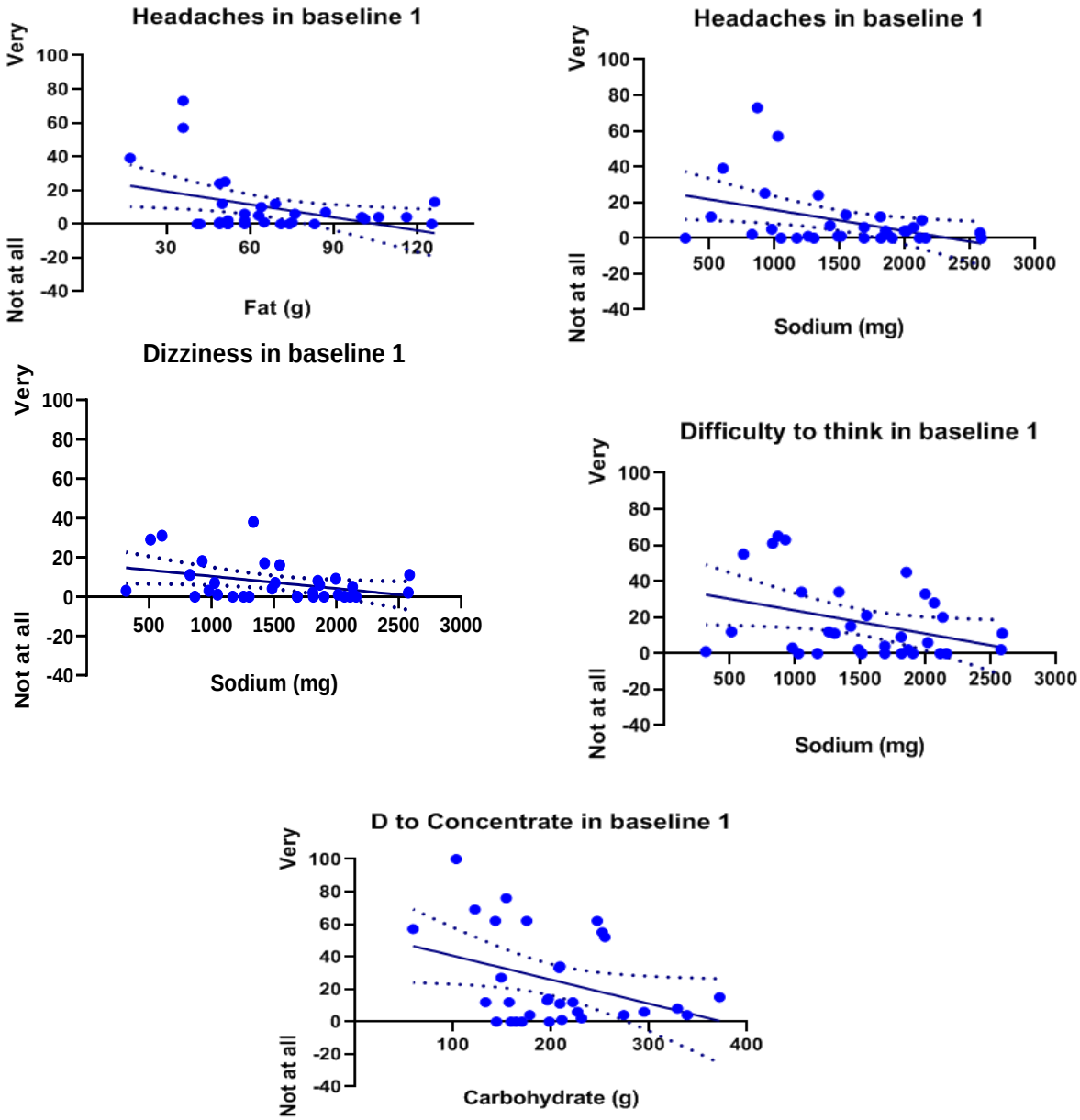
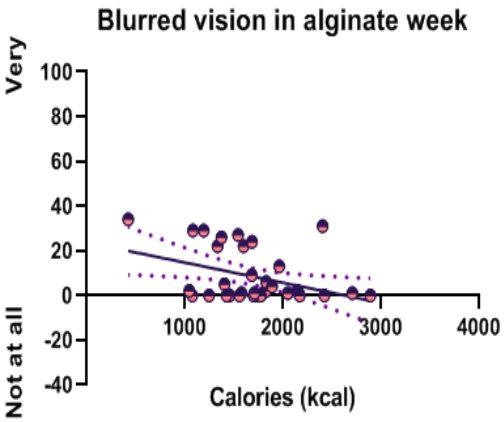
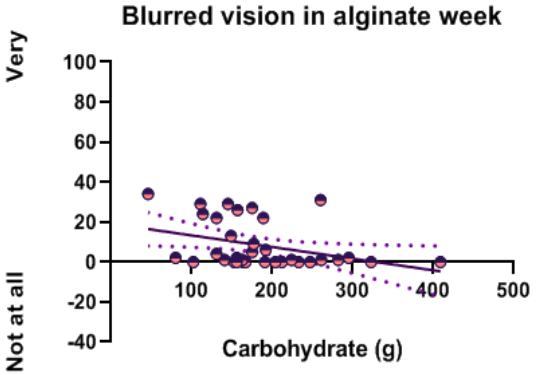
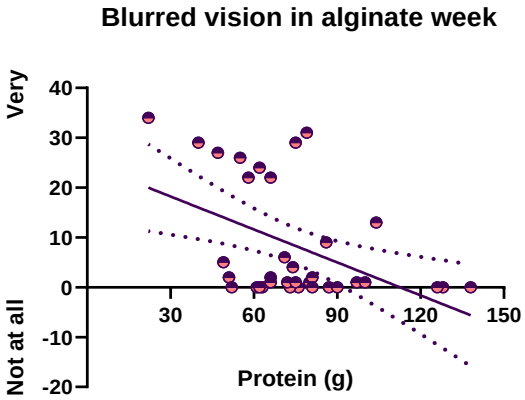
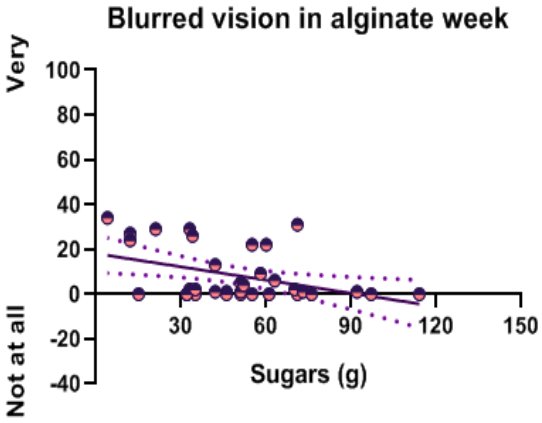


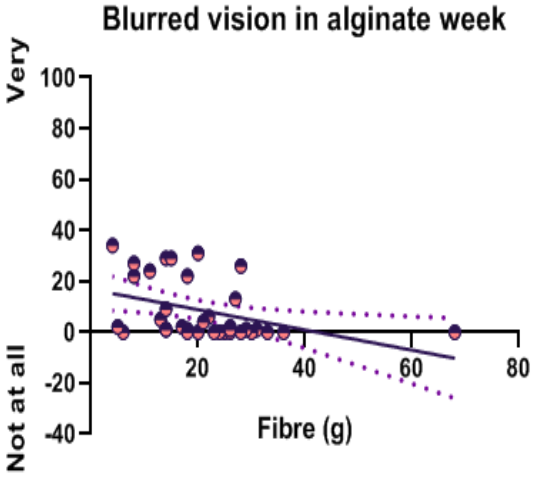
Figure 2.14 Correlations between the specific nutrients and specific symptoms of weekly wellbeing scores during the consumption of habitual diet in the first baseline week. For example, Correlation between some nutrients consumed in baseline week 1 and many symptoms of wellbeing that affected the participants on the last day of this week. There was no specific reason between a nutrients consumed such as carbohydrate and the feeling of headache in the baseline 1 week. The majority of participants did not report a headache and just two participants had a headache. The solid line a linear regression between the dietary variables and the well-being scores. The dotted lines represent the 95% confidence bands of the linear regression best fit.

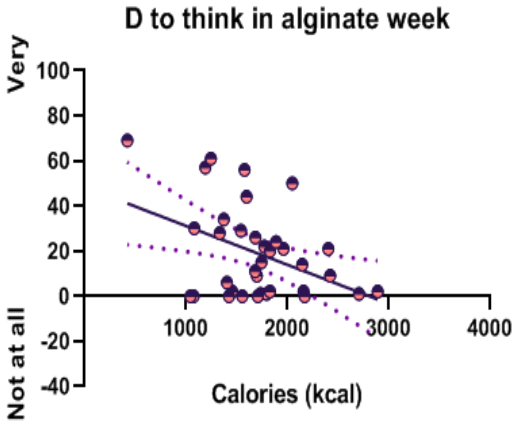

Blurred vision in alginate week
Very
Not at all
100
80
60
40
20
0
-20
-40
1000
2000
3000
4000
Calories (kcal)

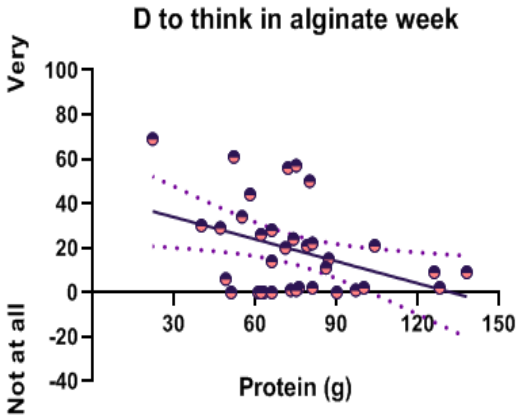

Blurred vision in alginate week
Very
Not at all
100
80
60
40
20
0
-20
-40
100
200
300
400
500
Carbohydrate (g)

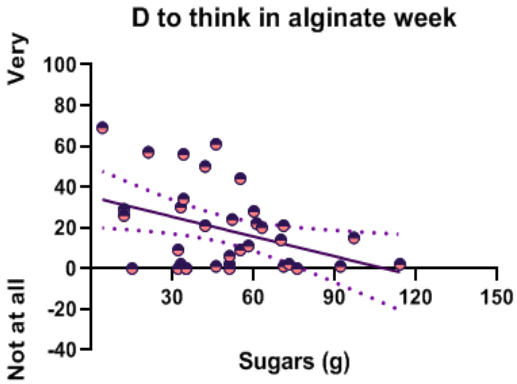

Blurred vision in alginate week
Very
Not at all
40
30
20
10
0
-10
-20
30
60
90
120
150
Protein (g)

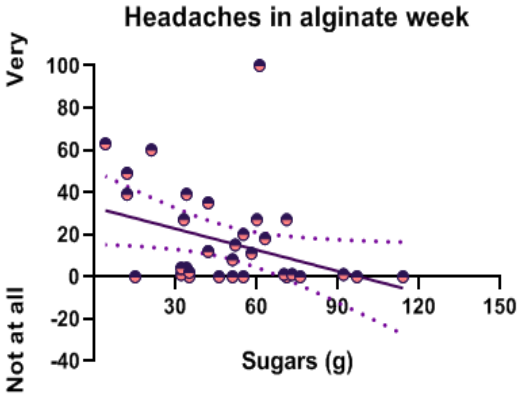

Blurred vision in alginate week
Very
Not at all
100
80
60
40
20
0
-20
-40
30
60
90
120
150
Sugars (g)

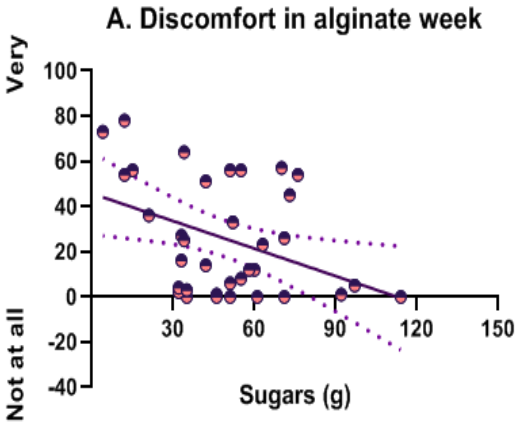

Blurred vision in alginate week
Very
Not at all
100
80
60
40
20
0
-20
-40
20
40
60
80
Fibre (g)


D to think in alginate week
Very
Not at all
100
80
60
40
20
0
-20
-40
1000
2000
3000
4000
Calories (kcal)


D to think in alginate week
Very
Not at all
100
80
60
40
20
0
-20
-40
30
60
90
120
150
Protein (g)


D to think in alginate week
Very
Not at all
100
80
60
40
20
0
-20
-40
30
60
90
120
150
Sugars (g)


Headaches in alginate week
Very
Not at all
100
80
60
40
20
0
-20
-40
30
60
90
120
150
Sugars (g)


A. Discomfort in alginate week
Very
Not at all
100
80
60
40
20
0
-20
-40
30
60
90
120
150
Sugars (g)

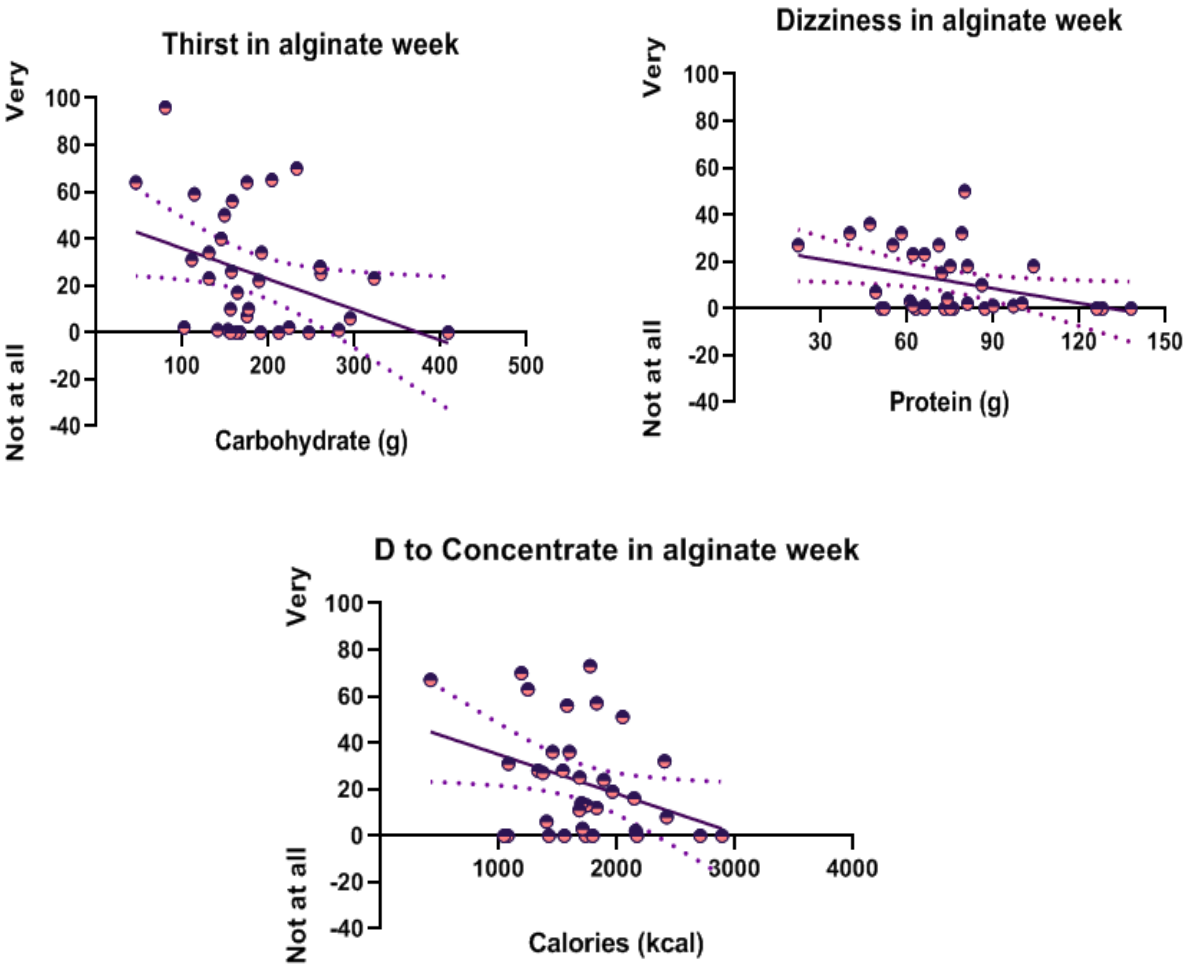
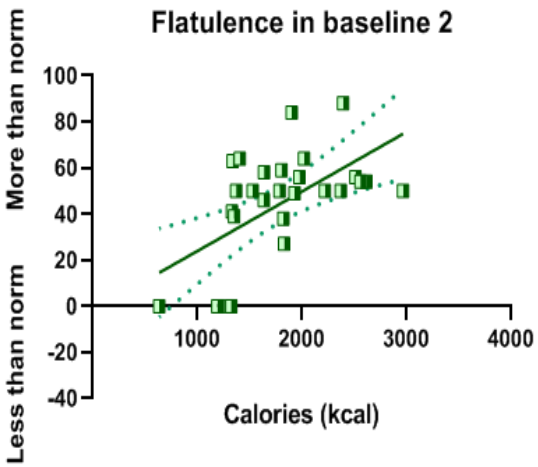
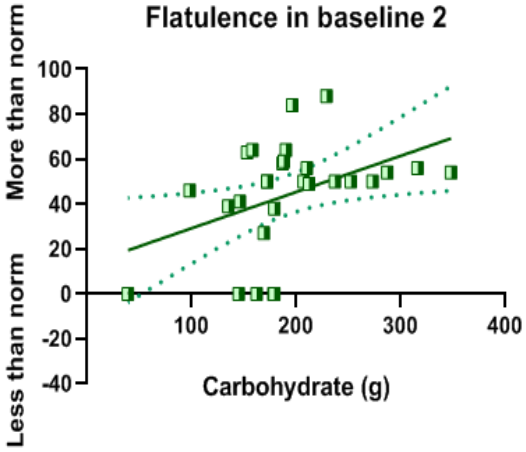
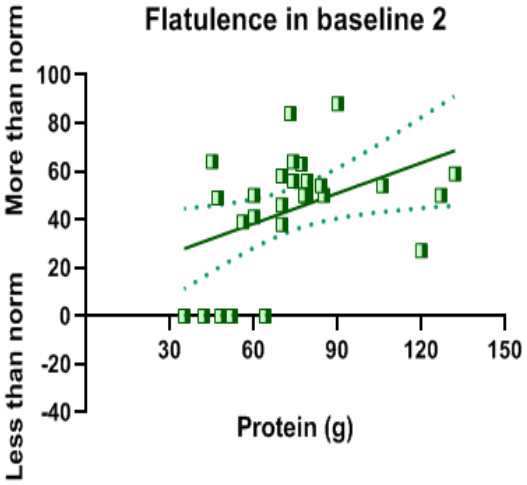
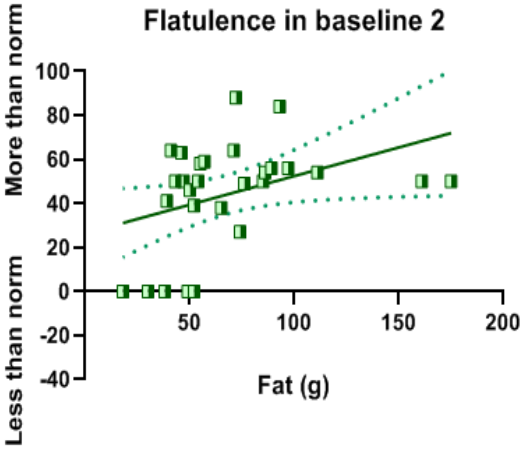


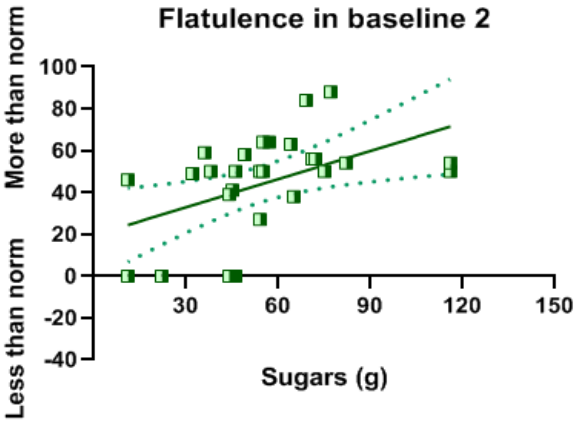
Figure 2.15 Correlations between nutrients and weekly wellbeing scores during the consumption of alginate pork sausage.


Flatulence in baseline 2
More than norm
Less than norm
100
80
60
40
20
0
-20
-40
1000
2000
3000
4000
Calories (kcal)


Flatulence in baseline 2
More than norm
Less than norm
100
80
60
40
20
0
-20
-40
100
200
300
400
Carbohydrate (g)


Flatulence in baseline 2
More than norm
Less than norm
100
80
60
40
20
0
-20
-40
30
60
90
120
150
Protein (g)


Flatulence in baseline 2
More than norm
Less than norm
100
80
60
40
20
0
-20
-40
50
100
150
200
Fat (g)


Flatulence in baseline 2
More than norm
Less than norm
100
80
60
40
20
0
-20
-40
30
60
90
120
150
Sugars (g)

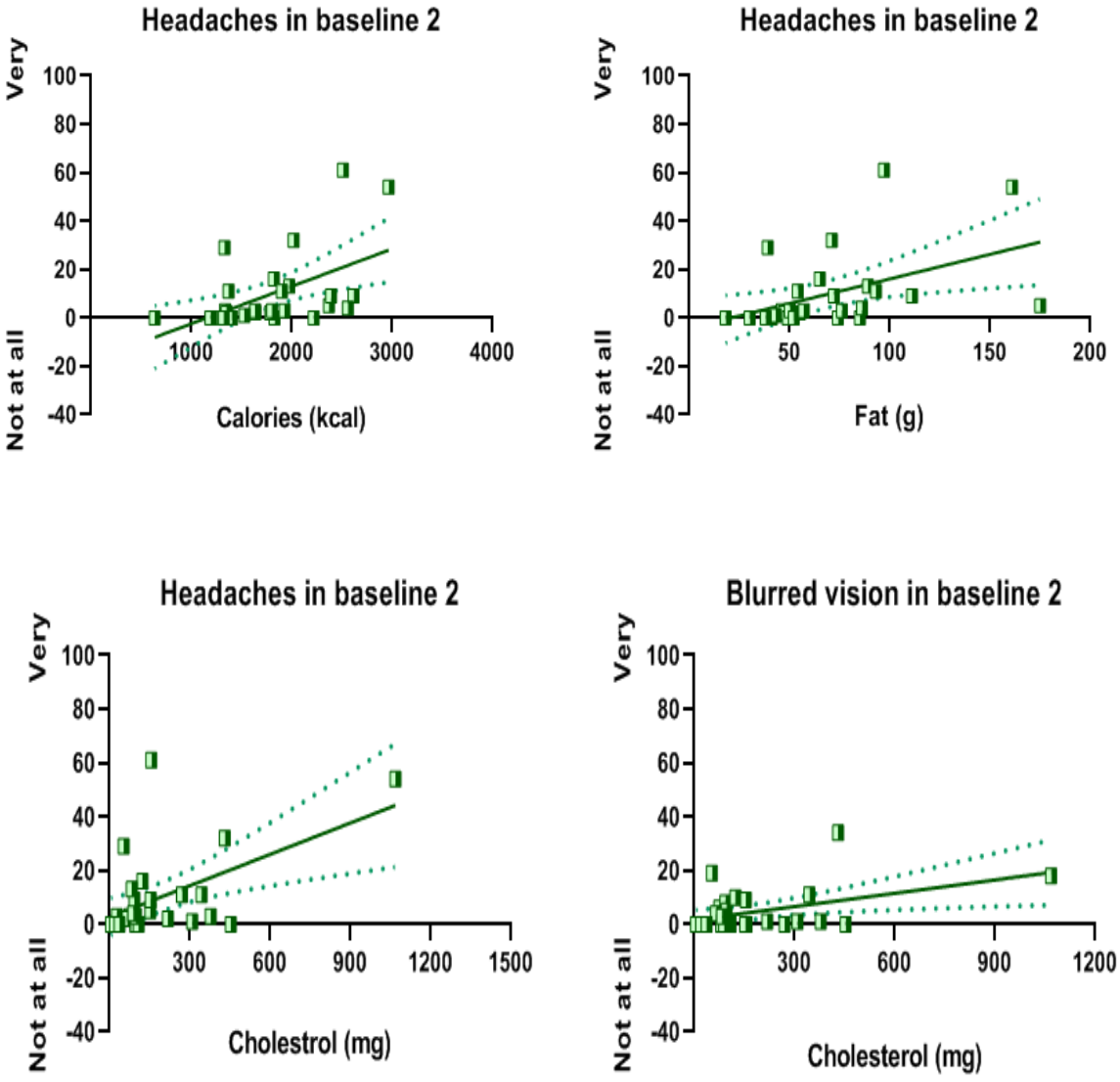
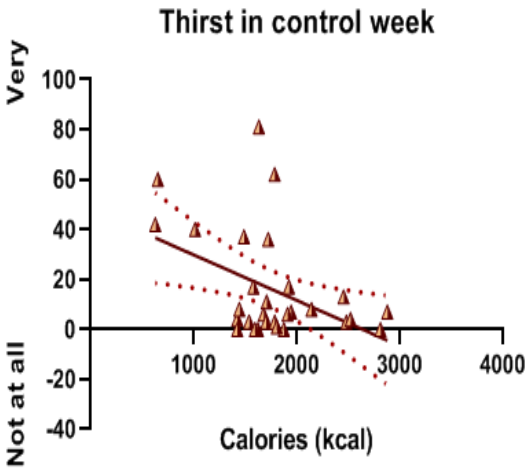
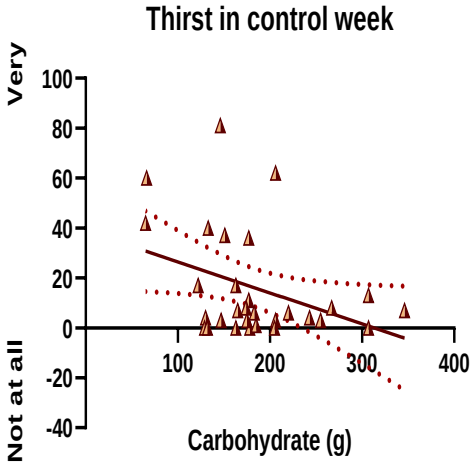
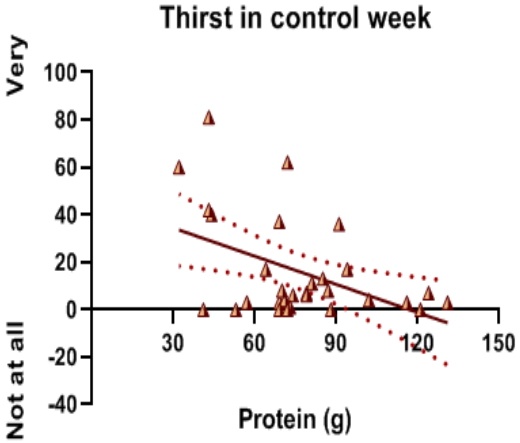
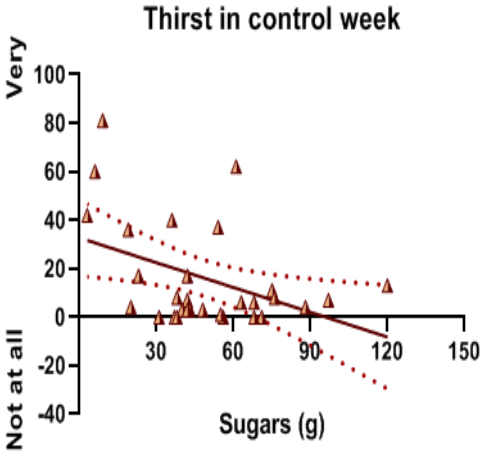


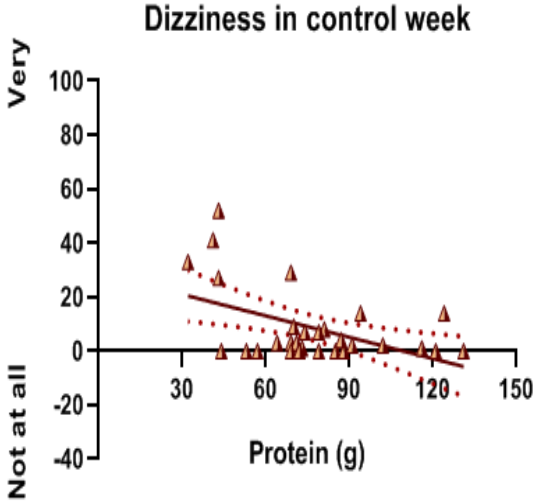
Figure 2.16 Correlations between nutrients and weekly wellbeing scores during the consumption of habitual diet in the second baseline week. In this week, the participants had experience of flatulence and this was probably due to the increased intake of food items such as steamed broccoli, red kidney beans, Chinese cabbage, lentils and beans compared with their intake for the same food items in both the alginate and control sausage weeks.


Thirst in control week
Very
Not at all
100
80
60
40
20
0
-20
-40
1000
2000
3000
4000
Calories (kcal)


Thirst in control week
Very
Not at all
100
80
60
40
20
0
-20
-40
100
200
300
400
Carbohydrate (g)


Thirst in control week
Very
Not at all
100
80
60
40
20
0
-20
-40
30
60
90
120
150
Protein (g)


Thirst in control week
Very
Not at all
100
80
60
40
20
0
-20
-40
30
60
90
120
150
Sugars (g)


Dizziness in control week
Very
Not at all
100
80
60
40
20
0
-20
-40
30
60
90
120
150
Protein (g)

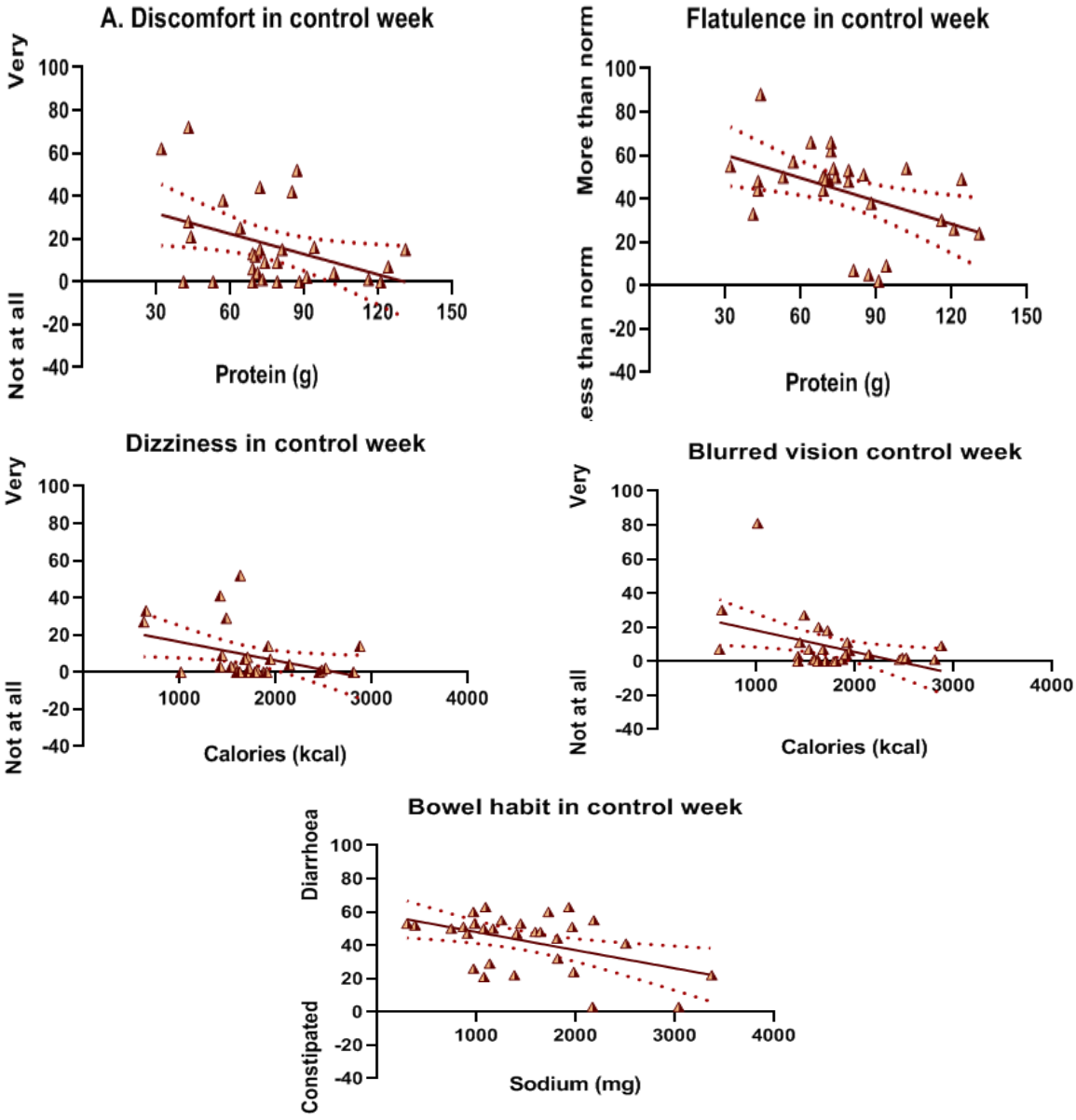


Figure 2.17 Correlations between nutrients and weekly wellbeing scores during the consumption of control pork sausage.

	Dizziness	Blurred vision	difficulty to concentrate	difficulty to think	Excessive thirst	Headaches/ migraines	Cravings for sweets	Abdominal discomfort	Bowel habit	Urgency to pass stools	Abdominal pain	Amount of Flatulence
Baseline 1 week												
Calories (kcal)	ns	*- 0.3903	ns	ns	ns	*- 0.378	ns	ns	ns	ns	ns	ns
Carbohydrate (g)	ns	*- 0.3909	*- 0.360	ns	ns	*- 0.424	ns	ns	ns	ns	ns	ns
Fat (g)	ns	*- 0.358	ns	ns	ns	*- 0.401	ns	ns	ns	ns	ns	ns
Cholesterol (mg)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Protein (g)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Sodium (mg)	*- 0.363	ns	ns	*0.356	ns	*- 0.399	ns	ns	ns	ns	ns	ns
Sugars (g)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Fibre (g)	ns	*- 0.424	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Alginate week												
Calories (kcal)	ns	*- 0.378	*- 0.355	*- 0.416	ns	ns	ns	ns	ns	ns	ns	ns
Carbohydrate (g)	ns	*- 0.357	ns	ns	*- 0.358	ns	ns	ns	ns	ns	ns	ns
Fat (g)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cholesterol (mg)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Protein (g)	*- 0.361	*- 0.459	ns	*- 0.396	ns	ns	ns	ns	ns	ns	ns	ns
Sodium (mg)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Sugars (g)	ns	*- 0.412	ns	*- 0.390	ns	*- 0.351	ns	*- 0.396	ns	ns	ns	ns
Fibre (g)	ns	*- 0.396	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Baseline 2 week												
Calories (kcal)	ns	ns	ns	ns	ns	*0.518	ns	ns	ns	ns	ns	*0.565
Carbohydrate (g)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*0.420
Fat (g)	ns	ns	ns	ns	ns	*0.457	ns	ns	ns	ns	ns	*0.381
Cholesterol (mg)	ns	*0.448	ns	ns	ns	*0.529	ns	ns	ns	ns	ns	ns
Protein (g)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*0.421
Sodium (mg)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Sugars (g)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*0.460
Fibre (g)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Control week												
Calories (kcal)	*- 0.379	*- 0.414	ns	ns	*- 0.436	ns	ns	ns	ns	ns	ns	ns
Carbohydrate (g)	ns	ns	ns	ns	*- 0.373	ns	ns	ns	ns	ns	ns	ns
Fat (g)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cholesterol (mg)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Protein (g)	*- 0.481	ns	ns	ns	*- 0.457	ns	ns	*- 0.398	ns	ns	ns	*- 0.453
Sodium (mg)	ns	ns	ns	ns	ns	ns	ns	ns	*- 0.463	ns	ns	ns
Sugars (g)	ns	ns	ns	ns	*- 0.423	ns	ns	ns	ns	ns	ns	ns
Fibre (g)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 2.9 The correlation between the average nutrients consumed per day and the weekly wellbeing score during the consumption of habitual diet, alginate sausage and the control sausage. *states the significant correlation with $P < 0.05$ between the variables and ns refers to no significant correlation.

2.5.12- Palatability and acceptance of the sausages

Only twenty out of thirty-five participants reported on their overall impressions of the alginate and control sausages. Regarding overall acceptance, three subjects stated that both sausages were acceptable and were unable to identify a difference between them. One participant was totally happy to include seaweed because it is healthy and rich in iodine. Another female participant considerably preferred alginate sausage because it was salty, and she welcomes alginate food products to consume in the future. Furthermore, another two argued that both sausages were fine and one of them said "the sausage overall was pleasant to eat", however, the control sausage was better than the alginate sausage, because the latter was saltier and drier, as well as having a more plastic consistency which may not be acceptable to the UK audience. One said, "I wouldn't go out of my way to search for it".

As far as general and GI wellbeing is concerned, one participant specifically mentioned that during her alginate sausage intake, she felt considerably fuller for longer following ingestion of the alginate sausage, with no cravings for sweets during the day; even after a long day at work she was not hungry, she was more energetic than usual and noticed an improvement in bowel movement. However, she experienced flatulence, several occurrences of reflux and more frequent burping than experienced on a regular basis. With the control sausage, the same participant reported that she was well with no gas reflux or constant flatulence but reported feeling less full compared with alginate sausage.

Similarly, four participants mentioned that they felt well in general, but were thirstier on consumption of the alginate sausage. One participant observed a bowel habit change including smelly and soft faeces with consumption of the alginate sausage. In addition, a further 4 participants reported slight increased flatulence, and one lady from them reported in the first baseline week "Stomach feels weird but not that obvious" and also reported with control sausage that "Flatulence is the main issue in my digestion system". In addition, the second one of them reported bloating, however, this could be due to a larger amount of food consumed compared to usual. One participant with alginate sausage intake, complained of a bad stomach and he said "Feeling that some of the food isn't passing down properly. Unusual sensation as if food is still sitting in my chest. Temporary feeling and burping more than normal" and the fourth one had a

few GI issues associated with the alginate sausage and he said, this may be “since I do not consume a lot of fibre”.

According to palatability, three participants stated that these sausages do not taste like normal sausage. Generally, the sausage was pleasant to eat, and although the alginate sausage was a little dry in texture, there was generally no difference in taste when compared to the control sausage as one participant reported that “the alginate sausage appeared a little drier and chewier compared to the control sausage”. However, another two participants extremely disliked the alginate sausage and said that, “The sausages didn't taste very nice and the smell when cooking them was awful!” and the other said “The sausages were disgusting! I didn't like the smell, texture or taste!” with no comment on the control sausage from these two participants.

2.6 Discussion

This research began with an open-ended question designed to explore if the addition of 4 % seaweed or seaweed extract (alginate) in cheeses and 4% alginate in pork sausage could affect the gastrointestinal well-being among apparently healthy volunteers. Also, to answer the question: is this cheese and sausage acceptable? In addition, an important note is that cheese and pork sausage are a main source of important nutrients and minerals and are frequently consumed in the UK. These food items are not high in dietary fibre; thus, their frequency of consumption would make them potential candidates for incorporation of seaweed and their extract alginate which would have the potential to increase total dietary fibre intake, as well as reduce fat digestion. Additionally, seaweed extracts, especially viscous hydrocolloids, play an increasingly important role in the food industry. It is therefore important to ensure food product acceptability and an enhanced shelf-life of these products. In particular, alginate compared to the other marine polysaccharides could be used in increased amounts in foods and beverages (Brownlee *et al.*, 2012).

Cheese acceptability study

The present study examined the effect of this cheese and alginate incorporated cheese on the average nutrients consumed and several characteristics of general wellbeing including gastrointestinal adverse events, in individuals who are apparently healthy. Additionally, it allows

the examination of the effect of adding seaweed and seaweed bio-actives into food products. Previous research reported that the addition of seaweed to a dietary product results in the dietary enrichment of important nutrients such as proteins, fibres and lipids (Mohamed;Hashim and Rahman, 2012). The administration of dietary alginate limits the uptake and hydrolysis of macronutrients in the diet (Brownlee *et al.*, 2009).

The results of the study suggest alginate cheese is acceptable as there was no significant difference observed between the daily VAS of general wellbeing that included some gastrointestinal side effects, except minor symptoms such as feeling with urgency to pass stool and amount of flatulence associated with seaweed intake. The average nutrient intake during the alginate, seaweed and control cheese consumption presented in **Table 6**. The 4% alginate and 4% seaweed used in this study are similar to the alginate percentage used in the alginate bread acceptability study that showed positive outcomes in terms of limiting the absorption of dietary lipids and increasing the feeling of fullness after a single meal intake (Houghton *et al.*, 2019).

A recent study investigated the effect of the incorporation of several concentrations (2-10%) of seaweed composite flour into a muffin cake product. The aim was to assess the muffin's organoleptic acceptability by using a 7-point Likert scale. It stated that seaweed powder could be used up to 6% to increase the total fibre intake with no unpleasant effect on the muffin's colour, flavour, or aroma properties. However, the control muffin was the most favourable among the candidates for aroma and taste. The participants reported that the seaweed muffin smelt fishy (Mamat *et al.*, 2018). This percentage can be considered as a high amount to be added into foodstuffs and lower percentages could improve the participants' acceptance.

Interestingly, the average daily intake of sugar (56.4g ± 28) showed positive association with the general weekly well-being of no dizziness, blurred vision, difficulty to concentrate, headaches, abdominal discomfort, and with normal bowel habit such as not being constipated or having diarrhoea and with normal urgency to pass stools when the participants consumed alginate cheese. A finding in this acceptability study is that the participants reported the lowest dietary intake of sugar during the alginate cheese week compared to the other weeks, although it was not significant. Overall, the ingestion of alginate did potentially lead to a minor change in bowel habit and a slightly increased faecal urgency for the participants.

In addition, the participants did not report any weekly abdominal pain or abdominal discomfort during the intake of alginate, seaweed, and control cheese. However, this finding was contradictory with the alginate and control bread outcomes that reported an increase in abdominal pain with alginate and control bread intake compared to the baseline (Houghton *et al.*, 2019). This could be due to the fact that the cheese study involved healthy participants whereas the bread study involved ileostomy volunteers. Generally, alginate as a viscous fibre has a lower fermentation ability compared to other viscous fibre (Wolf *et al.*, 2002; Brownlee *et al.*, 2005). This means that fibres are unable to be digested by the human digestive enzymes and are, therefore, not absorbed but there are slightly fermentable carbohydrates that pass through the GI tract with lower change and are eliminated in the faeces (Grabitske and Slavin, 2009). Regarding alginate, the lower digestible carbohydrate has a minor fermentation ability, this is important to reduce the carbohydrate digestion by the colonic bacteria and then reduce the production of gases such as carbon dioxide, and methane and short chain fatty acids (Cummings;Macfarlane and Englyst, 2001). In other word, this type of fibre could facilitate the reduction of flatulence and undesirable gastrointestinal effects and a moderate alginate intake has the potential to reduce the severity of the GI symptoms.

Furthermore, nondigestible fibres have been reported to be efficacious in the improvement of gastrointestinal health (Murphy *et al.*, 2012). Alginate is a dietary fibre (Brownlee *et al.*, 2005), and has been convincingly demonstrated to be nutritionally beneficial and have potential implications on improving GI health (Horniblow *et al.*, 2016). Importantly, a previous dietary treatment intervention provided two strawberry crisp bars per serving supplemented with soluble viscous fibre of 1.6 g of alginate and 5.5 g of guar gum compared to the control crispy bars. These bars were given to 48 healthy individuals and aimed to investigate the effects of this functional fibre on blood glucose concentrations and GI symptoms after a meal. The results showed a lower postprandial glycaemic response over 180 min of intervention in comparison with the control bars as well as showing similar increased GI symptoms of being nauseous, abdominal cramping, and flatulence compared to the control bars intake (Williams *et al.*, 2004). Further to this, the finding of minor gastrointestinal intolerance of the cheese study are consistent with the crisp bars containing alginate and guar gum fibres. Eating alginate food could cause minor change for GI symptoms that may makes the GI symptoms worse than normal. The finding from this research agrees with this literature

However, previous research had shown that the frequent use of food and drinks supplemented with alginate as a viscous fibre can result in several GI tolerance symptoms such as flatulence, burping, stomach ache, nausea (Torsdottir *et al.*, 1991; Sandberg *et al.*, 1994; Georg Jensen *et al.*, 2012) and a decrease in palatability as well (Vuksan *et al.*, 2009). Most notably, there was a lack of association between overall average nutrients consumed and the weekly wellbeing responses including gastrointestinal symptoms across the whole cohort. This is considered a robust indication for the use of seaweed in food products. Regarding to the consumption of seaweed cheese, the rating of flatulence was significantly increased with average sugar intake, a symptom which was mentioned by some of the participants. It could be that the presence of flatulence with seaweed cheese. A potential explanation for the presence of this symptom could be the lower amount of dietary fibre ingested during that week compared to the other weeks, also the average fibre intake was the lowest compared to other weeks. A potential explanation for the presence of this symptom could be the lower amount of dietary fibre ingested during that week compared to the other weeks, although the average fibre intake was the lowest. Another reason could be that, some participants during the seaweed cheese week increased their intake of legumes such as hommus, wholenut peanut butter, and cooked lentils were the highest compared with their intake during the other weeks.

Table 2.4 outlines the average amount of fibre consumed in the seaweed cheese week (16.3g ± 7.5) compared with the other cheeses. Moreover, another possible reason related to this symptom could be the intake of other seaweed active substances responsible for this intestinal flatulence. Regarding flatulence, algae consumption has been linked with an increase in faecal mass in rats (Gudiel-Urbano and Goñi, 2002). This may explain the trend seen during the present study. Moreover, it is also of interest to note that in healthy subjects, a slight increase in the postprandial blood glucose concentration affects gastrointestinal motility and sensory function. There is, accordingly, an increased simulation of duodenal waves that account for duodenal gut inflation (Lingenfelser *et al.*, 1999; Rayner *et al.*, 2001). This may account for the occurrence of this symptom among a minor number of participants. The positive correlation between the intake of sugar and being flatulent as seen in **Figure 2.10 B,** indicates that increased sugar intake is associated with feelings of flatulence. Additionally, it can be inferred that some oligosaccharides escape digestion in humans owing to the lack of α-galactosidase activity in human mucus and as a

result they are digested by the colonic bacteria, producing hydrogen, carbon dioxide and methane which mainly contribute to the formation of flatulence (Naczk;Amarowicz and Shahidi, 1997).

Moreover, unlike other well-being factors, a significant negative correlation was observed between the average sugar consumption and bowel habits. Participants complained of diarrhoea with an increased urgency to pass stools during the control cheese week, even though the highest dietary fibre intake was observed during this week. This symptom is not related to the intake of control cheese itself because this cheese is free from the functional seaweed and alginate, it could be because of eating more food than usual or changes in lifestyle for example intense physical activity has been associated with a number of adverse GI symptoms including: heartburn, nausea, vomiting, abdominal pain, and diarrhoea (de Oliveira and Burini, 2009). This thesis did not investigate any life style changes during the period of the study. It has been clearly established that consuming an appropriate diet improves health and GI functionality resulting in good health (Cencic and Chingwaru, 2010).

It was surprising that the higher fibre intake was not observed during the alginate cheese week, but it was related to the ingestion of the control cheese. The increase in dietary fibre intake was often related to the intake of fibre rich foods during that week. For example, one lady participant frequently consumed 100g of granola and 30g of Quaker oats in her breakfast meal in alginate week with the average intake of fibre (4.8 ± 3.3), however, she had a frequent intake of 60g of wholegrain bran flakes, 100g of wholemeal Fusilli pasta, 100g of fruits and nuts Muesli in the control cheese week with average intake of fibre (6.5 ± 1.6) with 1.8 g variation of fibre between the two weeks from the breakfast meals. Therefore, it is possible that any other minimal symptoms reported could be related to other dietary components or any life style changes rather than the study food.

The highest intake of fat, cholesterol, protein, and sodium among the participants was observed during the control cheese week when compared to the other weeks. The increased intake of dietary Na resulted in an increased average daily intake of Na from the participants diet during this week 1874 ± 525 mg, and 1673 ± 612 mg in seaweed cheese week compared with 1572 ± 610 mg in alginate cheese week and the lowest intake in the baseline week 1325 ± 698 mg of Na per day, as presented in **Table 2.4**. For example, a male participant consumed an average of 7 days of Na intake in baseline week 2066 mg per day, alginate 2417 mg, seaweed 2173 mg and in control

cheese 2707 mg per day. He consumed food with a higher Na content compared with the other weeks. However, a female had an average Na intake of 1635 mg per day during the baseline week, 1918 mg during the seaweed week, 1530 mg in the control week, and the highest Na intake during the alginate cheese week 2828 mg. The various food intake for the participants during the study contributed greatly to the apparent differences in the average Na consumed. As the sodium intake is lowest in the baseline week and increases through the seaweed and alginate weeks could it be that the salty taste of these modified cheeses increased the participants craving for salt?

The participants were not asked if they craved salty food and when they were given the cheese, and were asked to replace their normal cheese with the study cheese and they were free to eat the cheese alone or with anything like chutney or olives. For example, one participant said he used the cheese to make a pasta sauce. So, he added salt and some herbs with the cheese. Indeed, their food intake during each week was various

Regarding the seaweed cheese consumption, there was a noticeable challenge posed with seaweed cheese in terms of shelf-life and palatability. The addition of the brown seaweed did not negatively affect the flavour and texture of this cheese, however, a few participants complained of a bitter flavour, strong aroma, and mould growth at approximately 3 days post-collection of this cheese only as seen in **Figure 2.18.**

This, in turn, increased the undesirability of the cheese with relatively low levels of consumption in comparison to the alginate and control cheeses. This finding is in line with a previous study by Sheffield Hallam University. They used wholemeal bread enriched with 5% of *Ascophyllum nodosum* as a salt replacement. It was noted in this study that mould growth happened after 3 to 4 days (Brownlee *et al.*, 2012). As mould was not observed on the seaweed cheese stored at the University in a cold room at 4 ºC, this mould could be the result of the higher temperature used during the storage of this cheese by the participants, leading to an increased spoilage rate with this change taking place approximately 3 days after beginning the seaweed cheese week.

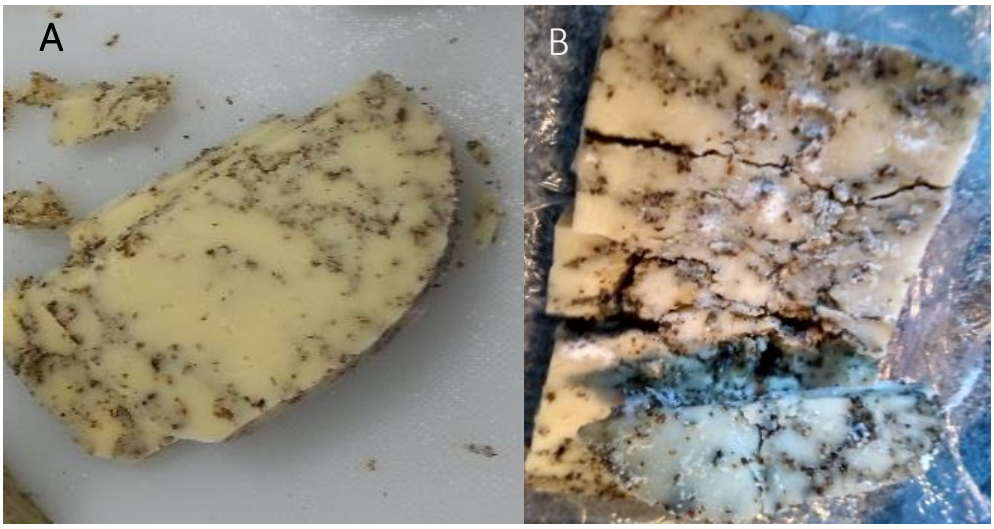


Figure 2.18A fresh seaweed cheese and 2.18B the mould growth in a 30 g of seaweed cheese containing 4% of *Ascophyllum nodosum*, 3-4 days after start of seaweed cheese week.

Pork sausage acceptability study

The alginate sausage was acceptable with just minor GI side effects and importantly with no withdrawal throughout the study. Generally, the addition of dietary fibre to meat products has been found to be associated with a greater fibre intake and is capable of inducing changes in the negative image of meat to be seen as a more nutritious food (Yadav *et al.*, 2016). Bearing this in mind, the current study revealed that pork sausage containing alginate was acceptable, demonstrating an association between the daily alginate pork sausage consumption and general well-being including gastrointestinal symptoms in healthy subjects. The positive results of this study suggest sodium alginate can be included in a food product and improve the feeling of wellbeing with just mild gastrointestinal symptoms compared with the results of the control sausage week and the baseline week 2.

Interestingly, the participants had significantly higher dietary fibre content during the alginate sausage week when compared to the control sausage week and habitual diet intake ($P<0.05$). Therefore, this finding demonstrates an acute increase in dietary fibre ingestion in the study population as a result of alginate ingestion during this week. The alginate is the most likely source of the fibre increase, however other potential changes in the diet that could have changed the fibre levels were not fully investigated. Regarding the acceptability finding, there was no significant correlation between the average food intake including calories, carbohydrate, fat, cholesterol, protein, fibre, sugar and sodium, and the general wellbeing of the participant's gastrointestinal issues, in the whole cohort. There was no statistical difference between all the nutrients consumed and the feeling of wellbeing at each day of the alginate and control sausage week. However, at the end of each week of this study, the participants observed several negative links between some nutrients consumed and their feeling of wellbeing with some side effects noted.

With alginate sausage consumption, there was a noticeable significant negative association of sugar on the feelings of blurred vision, difficulty to concentrate, headaches and abdominal discomfort, this can be seen from **Figure 2.15**. This result was not observed in the week before and after alginate sausage intake, including when the control sausage was consumed. During the alginate sausage intake, all these symptoms were negatively associated with a VAS scale range

from 0 that indicates Not at all to approximately 30mm of being concerned for the four previous wellbeing symptoms. Although a significant association a change between 0-30 on a 0-100 scale is probably not important.

Importantly, the participants did not report any functional gastrointestinal disorders during the alginate sausage consumption week which could be because of the potential benefits of alginate as a dietary fibre. Alginate is a safe dietary fibre and sodium alginate can be consumed in large quantities for a relatively long time without adverse effects in human or rodents (El Khoury;Goff and Anderson, 2015). Accordingly, this finding is in line with other studies which reported a similarity of gastrointestinal side effects between alginate foods and the control food products (Wolf *et al.*, 2002; Williams *et al.*, 2004; Pelkman *et al.*, 2007).

It is important to note that there was an intestinal gas complaint with alginate sausage intake, although there was no significant correlation between the diet in alginate sausage intake and being flatulent; however, the participants stated when they were when asked to provide feedback. Five participants complained ofrom being flatulence with alginate sausage intake and just one lady reported an improved wellbeing. They stated that "Possible slight increase in flatulence otherwise everything normal"; "Temporary feeling and burping more than normal"; "Flatulence is the main issue in my digestion system"; "I had a few GI issues with the sausages containing alginate, possibly since I do not consume a lot of fibre" and the last one said, "had an Indian buffet dinner; ate leftovers from the Chrismats meal; had a large Chrismas dinner so that probably caused the bloating and flatulencets". This example showsees how the other factors could contribute to some GI issues such as flatulence. However, one lady preferred alginate sausage and she said "I quite liked the sausage with the alginate as it kept me fuller for longer after consuming plus it really helped curb my sweet tooth cravings. This contributing to a much healthier me.

Feelings of flatulence were also observed during the second baseline week of the study. In addition, the participants experienced better wellbeing in terms of reduced suffering from headaches.

It is evident that diet can have an impact on some GI symptoms such as bloating, abdominal discomfort and flatulence (Fardy and Sullivan, 1988). In regards to increased flatulence, several researchers demonstrated that in a normal diet a volume of unabsorbed carbohydrate that arrives at the colon and acted on by the intestinal flora could cause an increase in gases released and other

GI side effect such as diarrhoea, bloating, and abdominal cramps (Davies, 1971; Anderson;Levine and Levitt, 1981). Moreover, the participants' feeling of being excessively thirsty during the control sausage week was reduced to "not at all" when they consumed more dietary carbohydrate in this week. In this week, increased carbohydrate intake was associated with being less thirsty. No clear reason was observed for this. Eight some participants reported being thirsty with alginate sausage and this may be because alginate sausage was saltier than the control sausage.

When considering the participants' sodium intake, they had the lowest daily average amount of sodium (1248 mg) during the alginate sausage week when compared with the control sausage week (1374 mg) and the baseline week 2 (1627 mg). Despite having the highest amount of salt ingested in baseline week 2, they did not complain of any excessive thirst at all. This could be related to a change into their salt intake after alginate sausage intake, some participants stated that alginate sausage was salty. Thus, when no longer consuming the alginate sausage, they reverted back to an increased salt intake.

Furthermore, it has been observed that in the alginate sausage week, increased sugar intake was negatively correlated with the ratings for abdominal discomfort. Thus, there was an increasing trend among the participants toward feelings of lower abdominal discomfort as the average score on the VAS showed Not at all. The same negative correlation was observed between the increased sugar consumed and having fewer less headaches, as well as finding it less difficult to think.

Also, the increased protein intake was correlated with less dizziness, less blurred vision and less difficulty to think. This result is not consistent with the previous reports with both alginate and control bread consumption (Houghton *et al.*, 2019). In addition, the result also show that ingestion of alginate sausage had no effect on the participants' wellbeing.

This finding of reduced abdominal discomfort with the alginate sausages of small chain fatty acids (SCFA) (Anderson *et al.*, 1991). Additionally, algal polysaccharides are less likely to be digested by the colonic microflora (Assoumani, 1976) and they are different to other polysaccharides (Michel and Macfarlane, 1996). Algal polysaccharides vary according to the content of sulphate, the glycosidic linkages, monosaccharide structure and molecular weight as a result of various

harvesting time and these differences could affect digestibility (Gotteland *et al.*, 2020). Moreover, a fermentability study of alginate conducted with human colonic bacteria reported a reduction in CO_2 and CH_4 gas production (Gibson;Macfarlane and Cummings, 1990).

A negative linkage indicated that there was a negative association between a specific nutrient's intake and the feeling of any wellbeing symptoms. In this study, a negative correlation was observed between several symptoms including reduced dizziness, blurred vision, and difficulty to think, and the increased consumption of protein during the alginate sausage week. Similarly, a negative correlation was observed between protein intake and a reduction in ratings of dizziness, abdominal discomfort, flatulence, and thirst during the control sausage week, **Figure 2.17**. The control sausage intake changed the participants' wellbeing in this study. In addition, there was no correlation between the intake of protein and any adverse symptoms of wellbeing including symptoms related to GI in the baseline week 1 and 2. Protein and amino acids should have been digested and absorbed before reaching the colon and should therefore have no direct effect on colonic health. If they did reach the colon and were degraded by colonic bacteria (Moore and Holdeman, 1974) this could have increased the feeling of flatulence and reduced wellbeing.

An important observation is that in the second baseline week, there was an increased frequency in the intake of some dietary items such as steamed broccoli, red kidney beans, Chinese cabbage (bok-choy), fried chicken, lentils, beans, vegetable stir fry and Thai stir-fried noodles. These items were consumed by the same participants in increased frequencies compared with their intake for the same food items in both alginate and control sausage week. These changes in food consumption could have had an influence on the data.

In other words, they consumed these specific food items in baseline week 2 more than their consumption for the exact food items in both alginate and control sausage week. It is clear that, the intake of foods with an increased content of nonabsorbable oligosaccharides such as legumes, peas, beans, and food items with high sulphate content for example sulphur-rich vegetables such as cruciferous vegetables that include: broccoli, cabbage, and cauliflower as well as onions, nuts, and spices are common leading causes for excessive intestinal gas production (Roudebush, 2001).

Therefore, from a nutritional point of view, this dietary evidence can explain the experience of flatulence during this week of intervention and this could be a result of the increased intake of these food items during this week and not related to the intake of alginate or control sausage at all. The participants' experience of a normal average of flatulence with a trend toward an increase in this symptom alongside an increase in the intake of calories, fat, protein, carbohydrate, and sugar in the baseline week 2 is presented in **Figure 2.16.**

Furthermore, it has been postulated that alterations in wellbeing symptoms could be related to other factors rather than the food intake itself, such as being under stress. As many of the participants were students during their exams, this might have influenced their food choices. This factor is important, but it cannot be seen or controlled by the researcher. "It might have been possible to change the questionnaire to ask about stress but this is a validated questionnaire and adding new questions would have required new validation studies."

A previous study has demonstrated how the feeling of stress is able to affect an individual's dietary intake (Serlachius;Hamer and Wardle, 2007). In the acceptability of alginate containing sausage, there was a mild GI symptom related to alginate sausage intake and a slightly higher VAS for some GI side effects in the second baseline week documented by some participants. Therefore, this food product was overall acceptable.

2.6.1 Strengths and limitations of the cheese and pork sausage acceptability studies

A strength of this dietary intervention is that the participants' intake for all nutrients allowed observation of their dietary patterns over the one-month period of the intervention either in the cheese or sausage study. Overall, the finding from the sausage study agree with the previous results of the cheese acceptability study. The seaweed and alginate cheese, and alginate pork sausage have provided evidence that it is possible to incorporate seaweed and seaweed extracts such as alginate with a higher percentage of 4%, into foods products which can be consumed. Both were acceptable, safe and well tolerated among the participants.

A further important point is that both the cheese and sausage acceptability studies showed a high compliance with the study procedure. Compliance is an important factor to determine the successfulness of health intervention research especially with foods containing an effective component (Williams *et al.*, 2004). The administration of an alginate dose into a diet often leads to some GI effects such as flatulence, stomach-ache, increased passing of stools, and flatulence (Pelkman *et al.*, 2007) and this may lead to an increased drop-out rate and lower compliance. However this was not the case in this study. Moreover, the validated 100 mm anchored visual analogue scale wellbeing questionnaire was used in association with an assessment of 7-day dietary intake records to investigate the association between all the participants' food intake and their feeling of wellbeing. This tool is easy to be used.

However, in this type of research involving human participants there will always be barriers and limitations. Importantly, obtaining ethical approval for the cheese acceptability study required a considerable amount of time and effort. This was also the case for the sausage and acute feeding studies.

Another limitation was that, regarding the two acceptability studies, the participants were varied with respect to adherence to the study procedure. Notably, between the participants in the both studies there was a different response in terms of the quantities of cheese or sausage required to be eaten each day of a study. Most of them consumed the required amounts per day, however, some of them consumed more or did not manage to consume enough. In addition, for participants to be included in the statistical analysis, they were required to complete the study; as some did not complete the study, they were excluded from the statistical analysis. This exclusion could have affected the findings. For example, the participants who did not completed the VAS daily and the food diary were excluded.

Generally, the obvious diversity of habitual dietary nutrients intake between the participants in these two acceptability studies may assist in explaining the presence of some mild gastrointestinal symptoms and other well-being side effects. In relation to this, it is difficult to determine if these symptoms are really related to the study's food, cheese or sausages, or as a result of any lifestyle changes or work stress which are essential factors that influence wellbeing and are out of the scope of this research. It is very important to mention again that 14 out of 35 participants in the alginate sausage acceptability study were Newcastle University students who started their exams during

the study. Busy students in their examination period may have provided a less accurate estimation of their daily food intake; furthermore, their habitual food intake may be different between the normal and exam days. Additionally, seaweed cheese has a lower acceptability score when compared to the other cheeses. This could be due to a fishy flavour present in seaweed and in conjunction with the growth of mould, this affected how much was consumed.

MyfitnessPal.com had been used to record the nutrient intake during both acceptability studies by the participants. It is considered a rich source of nutrient data, containing information on variety of plant and animal-based diets.

It is an easy website to download and subsequently use, and it is effective in saving and managing time and data. It also maintains the privacy and reduces the effort for the researcher in terms of transferring the data compared with a traditional manual tool. Despite all this, the dietary data used were provided by the volunteers themselves. The accuracy of this data obtained is dependent on the participants themselves in term of food measuring, weighing the food consumed, selecting the right food categories and the ability to remember what and when they ate the food. The accuracy of these measurements cannot be guaranteed, they depends on whatever the participants provided. It could be that a limitation on an individual scale may have arisen due to insufficient time to fill in the food diary or the reliance on their memory, especially for recording the food diary.

2.7 Conclusion:

The incorporation of 4% brown seaweed and its extracted bio active compounds 4% alginate, as functional ingredients in cheddar cheese and pork sausage was acceptable and have shown only a minor effect on general wellbeing and GI side effects; though these appeared to have no compliance issues during both the cheese and the pork sausage acceptability studies. The ingestion of alginate as a functional hydrocolloid has demonstrated an increased dietary fibre intake compared to the control food and baseline weeks in the acceptability study of alginate sausage. The highest intake of fibre was related to the alginate sausage week. From the dietary data, the average ingestion of fibre including alginate during the alginate sausage week was 21 g, and it was 17 g, 18 g and 17 g of fibre in control sausage, baseline weeks 1 and 2 respectively.

This would be an effective dietary approach to enhance and encourage fibre consumption. This has a great potential in improving health and enhancing general wellbeing especially GI wellbeing.

In this research, the intake of seaweed and alginate incorporated cheese significantly increased the feeling of fullness and reduced the feeling of starvation compared to the baseline week. In addition, this result was also demonstrated in alginate sausage compared to the baseline week. The reduction of hunger would have the potential to reduce the energy and nutrient digestion and absorption. In other words, this might help to reduce the amount of food consumed which considerably assists in controlling body weight and combating obesity as well as enhancing the interest in providing therapeutic nutritional foodstuffs and dietary treatment intervention for healthy food product markets.

In relation to this, an interesting point is that marine macroalga species in the form of edible seaweed provide important nutritional values of several essential nutrients such as protein, fats, carbohydrates, minerals and vitamins that all come from algal food sources which can be consumed by vegetarians and vegans. In other word, the consumption of this bioactive components can be used by a wide population.

Finally, the lack of association between the average daily diet consumed and the general and GI health symptoms either in seaweed and alginate cheese or in alginate pork sausage not only resulted in an improvement in their acceptability among the participants but also significantly supports the promotion of the production of functional foodstuffs for public consumption.

Further work is needed to investigate whether an increased volume of seaweed and alginate into cheese and pork sausage would be accepted or if the increased intake of more than three sausages per day or more than one 30g piece cheese a day would sufficiently increase fullness and still have little effect on GI function as well as being appropriate for incorporation into a normal diet. Moreover, several participants welcomed the idea of incorporation of seaweed and their extract such as alginate into their ordinary diet. Producing more popular food products which are easy to be consumed will encourage the health and wellbeing food market to produce healthier choices that improve the nutritional profiles such as increase the intake of dietary fibre and the other

functional components such as polyunsaturated fatty acids, proteins, minerals and vitamins will assist in improving peoples' dietary habits and enhance their wellbeing.

Chapter 3: The effect of alginate on carbohydrate and fat digestion in a mixed meal- A Pilot Trial

3.1 Obesity and the intervention approaches

Obesity is recognized as a metabolic abnormality of dietary lipids and is categorised by the development of excess adiposity and fat distribution. This is associated with a number of related health complications (Birari and Bhutani, 2007; Nakazono *et al.*, 2016). Obesity and overweightness are considered a major nutritional disorder in the United States (Yanovski *et al.*, 2002). The World Health Organisation reported in 2016 that, about 1.9 billion individuals over the age of 18 years old were overweight and more than 650 million were living with obesity around the world (WHO, 2018).

Several factors increase the prevalence of obesity in the world. For instance, one of the common factors in the Eastern Mediterranean Region, mostly the Arabian Countries is that, changing of dietary habits from traditional foods rich in whole grain cereals and fruits and vegetables into a Westernized diet such as consuming a diet rich in saturated fat, cholesterol, and refined carbohydrates with a low intake of dietary fibre and polyunsaturated fatty acids; this is frequently combined with a low level of physical activity (Galal, 2003; Ng *et al.*, 2011). In a review of thirteen epidemiological studies which investigated the connection between the prevalence of obesity and dietary fat consumption, eleven of the studies showed a statistically significant relationship between obesity and the energy density from fatty foods intake (Lissner and Heitmann, 1995). Moreover, later reviews have described how lipid-rich diets lead to increased fat accumulation in adipose tissue without fat oxidation (Schrauwen and Westerterp, 2000). Additionally, another example of what is meant by a negative change in dietary habit is included in a review by Malik and Hu (2015). It was discussed how an elevated consumption of sugar and sugar-sweetened beverages correlated with an increased risk of obesity, type 2 diabetes and cardiovascular disease in various populations (Malik and Hu, 2015). Consistent with these findings, data from three different cohort research studies have demonstrated that there is an association between intake of highly sugar-sweetened

beverages and an increased genetic predisposition to a high BMI and body weight in human adults (Qi *et al.*, 2012).

A similar finding was reported in epidemiological research; sugar-sweetened beverages contributed to an increased risk of developing obesity, type 2 diabetes and metabolic syndrome (Bray, 2009; Hu, 2009). Furthermore, research defines the diet of Western societies over the past four decades, as the "Western Diet'', this includes an increased intake of dietary lipid, protein, sugar and salt in combination with the frequent intake of processed and 'fast foods' and reduced intake of dietary fibre, vegetables and fruits (Thorogood *et al.*, 1994). This diet has a major influence on the development of obesity, metabolic syndrome, cardiovascular disease and many autoimmune diseases (Manzel *et al.*, 2013). Interestingly, therefore, to reduce the development of obesity, an effective approach targeting the interaction between individual interventions and the improvement of social and environmental factors is required (Blüher, 2019).

Accumulated evidence has clearly shown that adhering to a healthy dietary pattern, in association with an adequate level of physical activity, is an effective approach to weight loss in an adult obese population. However, this does not appear to be effective long term as many individuals regain weight. Thus, long term effective follow-up programs are required (Wadden, 1993; Curioni and Lourenço, 2005). For example, although weight loss is possible following a carbohydrate restricted diet (the ketogenic diet), evidence reported that people who are following this diet may be limiting their intake of several important foods such as legumes, whole grains, and fruits (Joshi *et al.*, 2019). Importantly, it is well established that dietary lipids provide extra energy of around 9 kcal per g compared to 4 kcal per g for both proteins and carbohydrates (Donato and Hegsted, 1985). Therefore, controlling lipolysis by inhibiting the intake of dietary triglyceride via the role of a pancreatic lipase based intervention is a promising approach to combat the obesity epidemic (Birari and Bhutani, 2007; Kumar and Dubey, 2015). Extensive research has demonstrated that lipase mediators which control lipid metabolism are effective in inhibiting the action of important digestive enzymes such as gastric and pancreatic lipase, by attenuating the uptake of gastrointestinal dietary lipids and subsequently, mimicking the influence of reduced food consumption (Mukherjee, 2003).

There are several natural lipase inhibitors including various types of marine alga, fungus, bacteria and plant species (Kumar and Dubey, 2015). For example, polyglucosamine (low-molecular weight chitosan mixed with ascorbic and tartaric acid) has shown inhibitory activity as it reduces the absorption of consumed lipid. It is also an effective agent of fat-binding (Cornelli *et al.*, 2017). Additionally, Lipstatin is a natural lipase inhibitor that acts on pancreatic triglyceride lipase; the hydrogenated form, tetrahydrolipstatin, is a known anti-obesity drug with the market name "Orlistat" (Hadvary *et al.*, 1988; Kumar and Dubey, 2015).

Orlistat is a pharmacological weight loss treatment. It acts as a lipase inhibitor and it is the only authorised anti-obesity medication in Europe (Centre for Public Health Excellence at NICE UK; National Collaborating Centre for Primary Care, 2006, de la Garza *et al.*, 2011). However, several unpleasant effects have been attributed to Orlistat administration including: fatty spotting, increased flatulence, urgent faecal excretion, steatorrhea, dyspepsia and some effects on the kidneys such as kidney stones formation and renal deterioration resulting in lack of compliance (Ferraz *et al.*, 2004; Birari and Bhutani, 2007; Drew *et al.*, 2007; Kopelman *et al.*, 2010; Richardson *et al.*, 2016). Therefore, there is a demand for anti-obesity medication which could assist in lowering the total amount of food consumed, varying metabolism systems and elevating the thermogenesis rate with no potential adverse effects (Bray, 2000; Schrauwen and Westerterp, 2000; Cornelli *et al.*, 2017; Blüher, 2019). As a result, the exploring of safe and active agents for anti-obesity and overweightness which can become an alternative drug is important (Bhutani *et al.*, 2007). Furthermore, naturally derived materials have been shown to inhibit lipase. For example, a grape seed extract has the potential as a weight loss treatment, according to an *in vitro* nutritional investigation which showed an inhibitory capacity against pancreatic lipase and lipoprotein lipase activity (Moreno *et al.*, 2003). Also, many food plant extracts have shown activity in terms of limiting dietary fat absorption and acting as an inhibitor of pancreatic lipase; these include tea polyphenols, some proteins from soybean (Gargouri *et al.*, 1984) and wheat bran (Lairon *et al.*, 1985). Wheat bran and wheat germ have also been shown to reduce cholesterol responses (Borel *et al.*, 1989).

Basic dietary fibres have a significant inhibitory effect on pancreatic lipase (Tsujita *et al.*, 2007). Interestingly, among those natural ingredients, over the past two decades recent

attention has focused on the potential of marine bioactive substances that are widely recognized as beneficial against obesogenic and diabetic activities (Huebbe *et al.*, 2017). Several brown algae extract such as alginates, fucoidans, and phenolic constituents have a profound influence on reducing the digestion of carbohydrate and protein. This is achieved through the inhibition of many digestive enzymes including: amylase, glucosidase, pepsin and pancreatin, as well as being fermented by the colonic microflora (Chater *et al.*, 2015b; Huebbe *et al.*, 2017). Also, they may suppress triglyceride hydrolysis and, thus, inhibit dietary fat absorption in the small intestinal tract (Chater *et al.*, 2015b; Nakazono *et al.*, 2016).

3.2 Dietary fibres and alginate's role in human physiological processes

Dietary fibres that are nutritionally beneficial and edible components extracted from fruits, vegetables, nuts, legumes and cereals have provided a protective barrier against a range of non-communicable diseases as well as enhancing gastrointestinal motility via reducing gastric emptying, appetite and extending transit time required for the delivery of nutrients into the small intestine (Gidley and Yakubov, 2019).

Additionally, they also play an important role in attenuating lipid hydrolysis in the small intestine which has profound effects on reducing the total uptake of dietary lipids and improving fatty faecal excretion. Moreover, viscous fibres have a role in slowing down the ingestion of dietary cholesterol and triacylglycerol, resulting in a reduction of plasma lipid levels and a rise in faecal lipid excretion (Tsujita *et al.*, 2007). Several dietary fibre polysaccharides including guar gum, pectin and alginates, have been shown to be a rich bulking tool and have an increased ability for gel formation (Birketvedt *et al.*, 2005). Many specific hydrocolloids have shown health benefits and physiological proprieties as presented in **Table 3.1**.

Physicochemical property	Dietary Sources	Physiological effect
Fermentation	Resistant starch, β-Glucans, Pectin, Guar	Energy source · increase in biomass Short chain fatty acid production · Reduction in pH of the colon (inhibition of 7-α-dehydroxylase), The anti-neoplastic activity of butyrate
Water Holding Capacity	The non-fermentable portion of hydrocolloids, for example, Cellulose, Arabinoxylans, Algal hydrocolloids	Increased stool bulk shorter gut transit times
Viscosity	Pectin, Guar, β-Glucans Psyllium Alginate	Delayed gastric emptying and slower transit time through small bowel, Glycemic control, cholesterol lowering
Gel Formation	Guar, Locust bean gum, Alginate	Reduced rate of nutrient absorption (for example, glucose, bile acids)
Binding of Organic Molecules	Hydrocolloids with an extensive hydrophobic surface area, forexample, β-Glucans, Arabinoxylans, Methyl-cellulose	Binding of bile acids, carcinogens, and mutagens
Large particles irritating the colon	Rough wheat bran	Stimulate water secretion, Laxative effect
Satiety	Modified starch	Thick and creamy mouth feel

Table 3.1 The physio-chemical properties of specific hydrocolloids, including alginate and their physiological effects, depending on their structure, chemicals and physical properties. This table was adapted from http://www1.lsbu.ac.uk/water/dietary_fiber.html

Alginates are marine-derived polysaccharides that are not capable of being digested in the human stomach and the upper digestive tract (Prosky, 2000; Champ *et al.*, 2003; Brownlee *et al.*, 2009). They are a potent reducer of digestive enzyme activity, particularly in the upper gastrointestinal tract and this reduction is down to their structure. Work from the Pearson Lab has shown that several alginates have the potential to significantly inhibit pancreatic lipase activity by up to 75% and 85%, and *in vitro* alginates can inhibit pepsin activity up to 80% (Wilcox *et al.*, 2014; Chater *et al.*, 2015a; Houghton *et al.*, 2015). Thus, inhibiting the lipolytic activity of pancreatic lipase using alginates can be considered a promising approach in reducing obesity.

3.2.1- Effects on gastrointestinal distension, satiation, and satiety

Alginates can form gels in acid or with calcium ions (Brownlee *et al.*, 2009). In a study where rats were fed a diet with and without 5 g of alginate (G-rich alginate), as well as with or without dietary calcium, for 4 weeks, it was observed that there was a decrease in the total food consumed and postprandial glycaemic levels with an alginate calcium diet compared to the other diet group. This was due to the viscosity of the alginate and gel formation in the stomach with the dietary calcium (Ohta *et al.*, 1997). Additionally, in a study involving healthy males and females who were given a novel preload beverage of sodium alginate that provided gastric gelation, it was shown that there was a significant reduction in the average protein, carbohydrate, fat and calories consumed during one week (Paxman *et al.*, 2008b). As described by (Guo *et al.*, 2019), rats fed with sodium alginate had increased stomach distension and an extended period of gastric emptying. This was associated with a reduction in the total nutrient intake. Moreover, in a study investigating the effect of milk including sodium alginate, a reduction in the feeling of hunger and increased postprandial fullness was reported (Hoad *et al.*, 2004). Another study revealed the potential effect of using a gel beverage containing alginate-pectin components twice a day in a group of overweight and obese women; it reported a significant decrease in total food and calorie intake associated with an increased feeling of satiety compared with a control beverage (Pelkman *et al.*, 2007). A previous study has also shown a reduction in postprandial energy intake and prior satiation following the consumption of low fibre cookies enriched with 5% gel forming alginate compared to cookies with guar gum, cellulose and the control cookies without fibre (Wanders *et al.*, 2013).

3.2.2-Effects on food intake regulation & nutrient digestion

The regulation of food intake depends on the gastrointestinal reaction to the digested food. This includes several signals such as stomach distension, gastric clearance, intestinal motility and gastrointestinal peptides (Phillips and Powley, 1996; Anderson *et al.*, 2006; El Khoury *et al.*, 2015). In a clinical trial where ileostomy patients ingested a sodium alginate supplementation with a lower dietary fibre diet, they demonstrated an increased level of fatty acid excretion (Sandberg *et al.*, 1994). Furthermore, a study conducted on diabetic men consuming sodium alginate supplement during breakfast, demonstrated a noticeable decrease in plasma glucose and insulin associated with slowed gastric clearance (Torsdottir *et al.*, 1991). However, further studies investigating the effect of alginate on the feeling of satiation, appetite regulation, gastrointestinal hormones and the gastric motility in overweight or obese subject must be carried out.

3.2.3- Effects on metabolic responses

A recent fat digestion study, designed as a double-blind, randomised, controlled cross-over carried out on 29 ileostomy participants determined the effect of two meals either a 100g alginate-incorporated toast with 20 g of butter or 100g control bread with the same amount of butter on lipid hydrolysis activity. The alginate bread included 13.3 g of fat for each 100g, and the control bread included 1.7 g of fat for each 100g of bread. The intake of an alginate bread meal showed a reduced level of plasma triglyceride compared with the intake of the control bread meal. This indicates that alginates can reduce dietary lipid absorption from an individual meal and it has the potential to be used as an anti-obesity drug through its ability to inhibit lipase activity (Houghton *et al.*, 2019).

In a single study involving 12 healthy individuals where they consumed beverages including alginate mixed with extracted soy protein, it was found that there was a significant reduction in the postprandial plasma glucose levels and peak insulin levels following administration of alginate and soy protein compared to beverages without alginate. This was due to the influence of intragastric gelation (Huang *et al.*, 2019). Another study providing an alginate enriched crispy bar illustrated impaired glycaemic outcome following the alginate crispy bar intake (Williams *et al.*, 2004).

3.3 Edible Lipid and Carbohydrate digestion

With respect to lipid-based foods, dietary lipids in the form of oils and fats are a key component in the human diet and are an essential constituent for high energy density macronutrients, enhancing palatability, providing desirable aroma, textural features and mouthfeel perception for foods as well as contributing to up to 38% of whole energy intake; as well as being a significant resource of nutraceutical lipids such as vitamins A, D, K, E, carotenoids and fatty acids. (Matsuo, 2004; McClements *et al.*, 2008; Pivk Kupirovič *et al.*, 2012; Mao and Miao, 2015; Qin *et al.*, 2016; Wang *et al.*, 2019). However, an elevated intake of dietary lipids has imposed a significant effect on excess adiposity which results in obesity and is causally linked to several chronic medical conditions(Amine E K et al, 2003; Aarak *et al.*, 2013).

In addition, fat and oils have a widespread utilization within food and beverage products as an essential food component, for instance, milkfat in cheese and yogurt, animal fat in processed meats, and oil-based vegetables like salad dressing. As well, they naturally form a liquid or solid food's surrounding medium such as nuts, seeds and diary milk (Guo *et al.*, 2017). The digestion of dietary lipids has been described in detail in Chapter One. Generally, dietary carbohydrates are considered an important source of energy consumed in the human diet. These include: starch, and glycogen (polysaccharides), as well as sucrose and lactose (disaccharides) (Gray, 1975). It is well known that the release of salivary alpha-amylase and pancreatic alpha-amylase in the small intestinal phase is an essential step in polysaccharide digestion to produce glucose sugars (Zaharudin *et al.*, 2018). Marine macro algae (seaweed) as a natural tool has been shown to be a safe, antioxidant agent, and an enzyme inhibitor. For example, phlorotannin-rich extracts from *Ascophyllum nodosum* brown algae could be an important inhibitor of α-amylase, and α-glucosidase (Zhang *et al.*, 2006; Nick Pantidos and McDougall, 2014). Moreover, palatable bioactive ingredients such as polyphenolics and alginates, have been shown to effectively inhibit the action of α-amylase by delaying the liberation of glucose (Zaharudin *et al.*, 2018). Therefore, controlling plasma glucose levels using alginates may be considered as a therapeutic approach to target hyperglycaemia and type 2 diabetes (Wu *et al.*, 2011).

As described in chapter 2, pork sausage has been chosen as a delivery vehicle of seaweed extract (alginate) in this study. Meat and meat products have a relatively high nutritional value as they are an important source of protein, energy and other significant micronutrients such as copper, magnesium, cobalt, phosphorus, chromium and nickel (Chan *et al.*, 1996; Givens and Gibbs, 2006; McAfee *et al.*, 2010a) .

In the UK and Ireland, meat is traditionally consumed and is considered an essential food group in the diet. Compared to several years ago, red meat and its food products have a lower fat content (Higgs, 2000; McAfee *et al.*, 2010b). The traditional food products such as sausages, pies and salami, have a rich fat content estimated to be up to 50%. However, due to a reduction in the fat content of red meat, many current food products such as ham and sausages now have reduced fat content also (Higgs, 2000). There was a noticeable reduction in fat contents in fresh red meat and processed meat products; it was around 22g of fat in 1991 and this was reduced to 14.5 g in 1998 according to the data obtained from the National Food Survey (NFS) (Salmon J 1991,Chaplin et al 1996) This chapter presents the findings from an acute clinical feeding study where alginate, a marine-derived polysaccharide, was incorporated into pork sausage. This could potentially provide a natural body weight-control tool through the administration of alginate into a daily diet. Additionally, the health benefits or health obstacles owing to the consumption of fresh and processed meat should not be problematic, if this meat intake is accompanied by a suitable healthy and balanced dietary pattern (Higgs, 2000).

3.4 Aims:

The aims of this acute randomised, double-blinded, placebo-controlled, two-way crossover intervention study were

1- Firstly, to address the hypothesis that sausage with 4% alginate would be an effective delivery vehicle to achieve a reduction in carbohydrate and fat digestion in this healthy population.
2- Secondly, to assess the acute metabolic effects of alginate enriched sausage on the postprandial whole blood glucose and triacylglycerol levels in the healthy subjects via finger prick blood samples using the Roche Accutrend testing system.
3- Finally, to evaluate the area under the curve (AUC) of the blood concentrations of glucose (measured at 2 hours) and triglycerides (measured at 4 hours) after the consumption of two pork sausage meals (one with control pork sausage and the second meal with alginate enriched pork sausage) in healthy individuals.

3.5 Methods:

3.5.1- Ethical procedure

All the study procedures were approved by Newcastle University's Research Ethical Committee (01615/7449), Faculty of Medical Science. The subjects were given their written consent form after having received a written information sheet about the study. All participants in this study had been allocated with a unique ID coding to identify their data. All the ethical profile is presented **Appendix 3.1.**

3.5.2- Participants recruitment:

Twenty-six volunteers were willing to participate in "The effect of alginate on carbohydrate and fat digestion in a mixed meal-A Pilot Trial". One subject was excluded due to the use of medication which affects metabolic function. This medication was Levothyroixe (75mg, daily). Seven subjects could not take part due to time constraints and four subjects did not respond and complete the study consent form. A total of 15 participants were recruited, of which 6 were females. The participants were considered healthy with no medication, no use of tobacco products, non-pregnant, non-lactating, aged over 18 years, and had no food allergies to the test meal (sausage sandwich). This number of participants although small was enough to shows a difference between the two study arms. This would then allow power calculations to be performed.

They were recruited from Newcastle University Medical School via poster advertisement displayed in various locations around the University campuses which include: an advertising board in the undergraduate study room, the second floor inside and outside the Institute for Biosciences, Medical School and in the ground and first floor of Cookson Building, Medical School at Newcastle University as well as via online newsletters on the university staff home page and posted on electronic forums via emails for all the previous participants, staff in the local area of the Medical School and friends.

3.5.3- Study design:

The study was designed as an acute randomised, double-blinded, placebo-controlled, two-way crossover intervention looking at carbohydrate and fat digestion via finger prick blood samples. All subjects participated in two test days at the Student resource room, The Medical School, Newcastle University, at a time which best suited them. The measurement of compliance did not take place in this pilot trail.

Each intervention day was separated by a two or more day's washout period between them as this was considered an appropriate amount of time to eliminate potential postponed effects. In the evening, after 8 pm prior to each test day the participants were instructed to refrain from alcohol, tea or coffee consumption. On the test day, the volunteers came in a fasting state between 8:30 am and 9 am, and the first finger prick was conducted at time point 0

minutes for both glucose and triglyceride test analysis. Each participant was instructed to consume all the food provided and was provided with both types of sausages as a test meal but in a random order, and without knowing which one was which.

After the subjects had finished the sausage meal, more finger-prick blood samples were taken at 0, 15, 30, 45, 60, 90, and 120 minutes for glucose test analysis and at 0, 30, 60, 120, 180, and 240 minutes for triglyceride test analysis. Subjects were then able to leave by approximately 1:30 pm. During the blood sampling period, the participants were instructed to sit quietly and perform a light activity such as reading, writing, or performing computer work. The study was conducted in a comfortable room with standardised natural lighting and air flow and computer facilities.

The research was funded by The Saudi Arabia Ministry of Education and the Biotechnology and Biosciences Research Council (BBSRC). In recognition of the subject's time commitment, they were given an honorarium of a £20 voucher per visit, upon completion of the study.

3.5.4- Composition of the test meals:

Both alginate and control pork sausages were provided by Ruitenberg Innovation Manufacturing, Ruitenberg Ingredients B.V., Griftstraat 8, Netherlands 0031-571270000 www.ruitenberg.com and were stored at -18˚C in sealed boxes until required.

Before the test day, the sausage was defrosted at room temperature (22–24 ºC) and freshly grilled on the early morning at 8:00 am on the test day. The study test meal consisted of 20 g of butter, two slices of medium Warburtons white bread (40g each), two pieces of sausage either alginate or control pork sausages (46.4g and 48.5g respectively), accompanied by a cup of water (250ml). This provides an energy content of 524.4 and 532.2kcal for the alginate and control sausage meal serving respectively. Overall macronutrient composition of fat, carbohydrate, protein, and fibre are presented in **Table 3.2**. This meal was served and provided randomly at each study day. The volunteers were instructed to ingest the meal within a 10-minute time frame. Finally, at the end of the sampling period, the volunteers were offered a light snack before leaving the facility.

Nutritional Information	Alginate sausage meal		Control sausage meal	
	Per alginate sausage (46.4g)	Per serving as a meal	Per control sausage (48.5 g)	Per serving as a meal
Energy (kJ)	395.8	3312	421	3522.9
Energy (kcal)	94.6	524.4	98.5	532.2
Carbohydrate (g)	0.5	38.5	0.5	38.4
Sugar (g)	0.05	2.6	0.05	2.6
Protein (g)	7.8	23.1	7.5	22.4
Fat (g)	6.9	23.9	6.6	30.2
of which saturates (g)	0	6.6	0	6.6
Fibre (g)	2.6	6.9	0.7	3.2
Salt (g)	0	1	0	0.2
Sodium (mg)	440	1274	240	874

Table 3.2: Approximate nutritional composition of the test meals including alginate and control pork sausages given to the participants during two separate visits.

3.5.5- Biochemical measurement (blood)

The Roche Accutrend testing system (Accutrend Plus meter) (Roche Products Limited, 6 Falcon Way Shire Park, Welwyn Garden City, AL7 1TW United Kingdom) was used to measure the blood glucose and triglyceride concentrations for each measurement at each time point.

3.5.6- Statistical analysis

All the statistical analyses were performed using GraphPad Prism Statistical software (version 7.0, GraphPad software Inc., San Diego, CA, USA) with significance set at $P < 0.05$. All the 15 participants completed the two intervention days and therefore, all of them were included in the analysis. A minimum of 10 subjects were required in order to detect a 10 % difference in fat and carbohydrate mean values to allow for a 10 % drop out rate, a total of 15 subjects were recruited to this pilot study and then a sample size calculation was conducted to power a full-scale trial. The sample size required was estimated to be about 270 participants.

The data were expressed as mean ± standard deviation (SD). The area under the curve (AUC) calculation to analyse the difference of the blood glucose responses between alginate and control sausage group was conducted via AUC analysis and the same assessment was used for triglyceride measurement. This metric measurement method presents the difference in average between the treatment arms of the study, i.e. the average concentration of blood glucose and triglyceride over the time interval. The AUC calculation used the volunteers as their own controls when comparing the mean serum triglycerides and plasma glucose between the two-sausage meals. In addition, iAUC applied in the calculation of Glycaemic index (GI) for food in nutritional research(Jenkins *et al.*, 1981). It involves healthy participants in an acute study to minimise the variation between the individuals' plasma glucose. Comparing the two interventions at individual blood sample time points for both glucose and triglyceride was performed using two-way ANOVA.

3.6 Results:

3.6.1- Participants

A total of 15 subjects were seated in a special room and completed both the alginate and control sausage meal randomly at each test day without any adverse effects.

3.6.2- Macronutrient digestion (Fat & Carbohydrate Digestion)

The postprandial changes in glucose and triglyceride with consumption of the alginate and control sausage meals are presented in **Figures 3.1** and **3.2.**

3.6.3- Effect of alginate on serum triglyceride levels in healthy humans after oral administration of both alginate and control sausage

Several studies indicated that alginate inhibits pancreatic lipase. This study has illustrated that pork sausage including 4% of alginate was effective at reducing the overall serum triglyceride (TG) response and the postprandial peak of blood lipid throughout digestion in 15 participants compared to the control sausage meal.

As shown in **Figure 3.1**and **Table 3.3**, the serum triglyceride reached a maximum level 1.71±0.91 mmol/l before the administration of alginate sausage meal, whereas the highest serum triglyceride of the control sausage became 1.91±1.29 mmol/l prior to the intake of control sausage meal. The lowest TG serum level in the alginate sausage group reached 1.27±0.50 mmol/l at 30 minutes of digestion whilst the lowest TG serum level reached 1.45±0.49 mmol/l at 180 minutes following the control sausage meal consumed. With alginate sausage meal, a decreasing trend for the serum triglyceride was seen from 1.49±0.63 mmol/l, 1.47±0.54 mmol/l, and 1.43±0.43 mmol/l at 60, 120 and 240 minutes respectively, whereas there was an increase of TG serum at 180 minutes to became 1.66±0.72 mmol/l following the digestion. However, the serum triglyceride for the control sausage meal showed a gradual increase over the time from 1.81±1.20 mmol/l, 1.87±1.42 mmol/l and 1.90±0.73 mmol/l at 60, 120 and 240 minutes respectively, whilst the TG serum value showed a reduction 1.45±0.49 mmol/l at 180 minutes. After 4 hours of digestion, the serum triglyceride from the alginate sausage group at 240 minutes showed a reduction 1.43±0.43 mmol/l, however,

the control sausage group at the final point of digestion (240 minutes) demonstrated increased TG serum level 1.90±0.73 mmol/l.

The overall effect of alginate on the lipid absorption compared to the control was not statistically significant ($P > 0.05$) and the area under the curve for the two arms at baseline 0 min was 362.1 ± 48.8 for the alginate meal and 412.8 ± 84.1 for the control. The AUC using volunteers as their own controls showed that there was a noticeable reduction in the TG serum response when the alginate sausage meal was administrated compared to the control sausage. These results indicated that alginate enriched sausages inhibited dietary lipid absorption in the gastrointestinal tract after 4 hours of digestion. **Table 3.3** illustrates serum triglyceride at 0, 30, 60, 120, 180, and 240 minutes of alginate and control pork sausage meal digestion.

Time (min)	0	30	60	120	180	240
TG (mmol/L) from alginate sausage	1.71±0.91	1.27±0.50	1.49±0.63	1.47±0.54	1.66±0.72	1.43±0.43
TG (mmol/L) from control sausage	1.91±1.29	1.51±1.08	1.81±1.20	1.87±1.42	1.45±0.49	1.90±0.73

Table 3.3 Data are mean ± SD of serum triglyceride at 0, 30, 60, 120, 180, and 240 minutes of digestion for both of alginate and control pork sausage meal for 15 healthy volunteers.

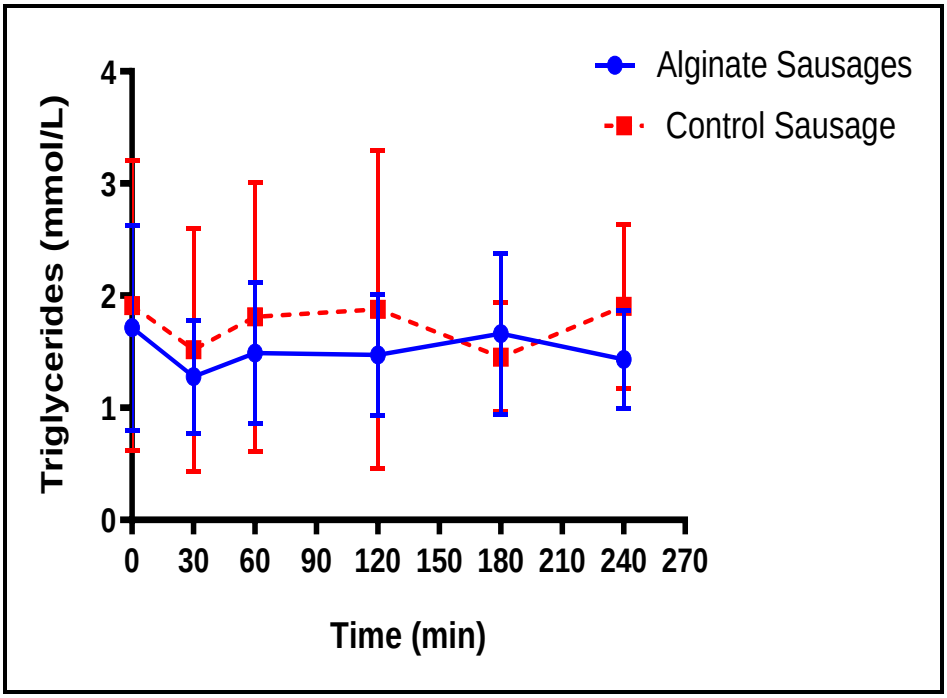


Figure 3.1: The effects of an alginate and control sausage meal on serum triglyceride concentration in 15 healthy participants before (at 0 min) and after administration of the meal. The AUC of blood TG before and after administration of alginate and control pork sausage meal in 15 healthy participants using volunteers as their own controls compared the mean serum triglycerides between the two-sausage meals. No statistical difference for the mean ± SD can be drawn from this figure due to the small population size.

3.6.4- Effect of alginate on plasma glucose levels in healthy humans after oral administration of alginate sausages

Regarding carbohydrate digestion, the plasma glucose levels were measured immediately before the meal at 0 minutes and after that at 15, 30, 45, 60, 90, and 120 minutes for the alginate and the control sausage meal. The normal fasting blood glucose concentration range between 3.9 mmol/l and 5.6 mmol/l for healthy people who are free from prediabetes and diabetes. **Figure 3.2** illustrates that the blood glucose reached the maximum level of 5.33±0.91 mmol/l at 45 minutes of the postprandial period and reached a lower level of 3.94±0.50 mmol/l at 120 minutes, the final time point of alginate meal digestion. The trend toward the postprandial peak of the circulating blood glucose was 4.88±1.11 mmol/l one hour after ingestion of the control sausage meal and the minimum blood glucose level was obtained as 4.1±0.68 mmol/l at 120 minutes of the postprandial period.

The incremental area under the curve (iAUC) was 534.8 ± 32.6 for alginate and 549.2±38.5 for control ($p > 0.05$). There was no significant difference in plasma glucose concentration when using the volunteers as their own controls and when they consumed both types of sausage meal. This result indicated that, although the average blood glucose at 120 minutes of alginate was lower than control, alginate-enriched sausage meal did not affect the postprandial glucose concentrations in the healthy subjects after a mixed meal. **Table 3.4** represent blood glucose response from 0 min until 120 min of the carbohydrate digestion.

Time (min)	0	15	30	45	60	90	120
Glucose (mmol/L) from alginate sausage	4.03±1.05	4.26±0.70	5.01±0.63	5.33±0.91	4.33±0.87	4.29±1.09	3.94±0.50
Glucose (mmol/L) from control sausage	4.44±0.81	4.19±0.80	4.76±1.03	4.87±1.34	4.88±1.11	4.57±1.16	4.1±0.68

Table 3.4 Data are mean ± SD of blood glucose concentration during digestion of both alginate and control pork sausage meals for 15 healthy volunteers.

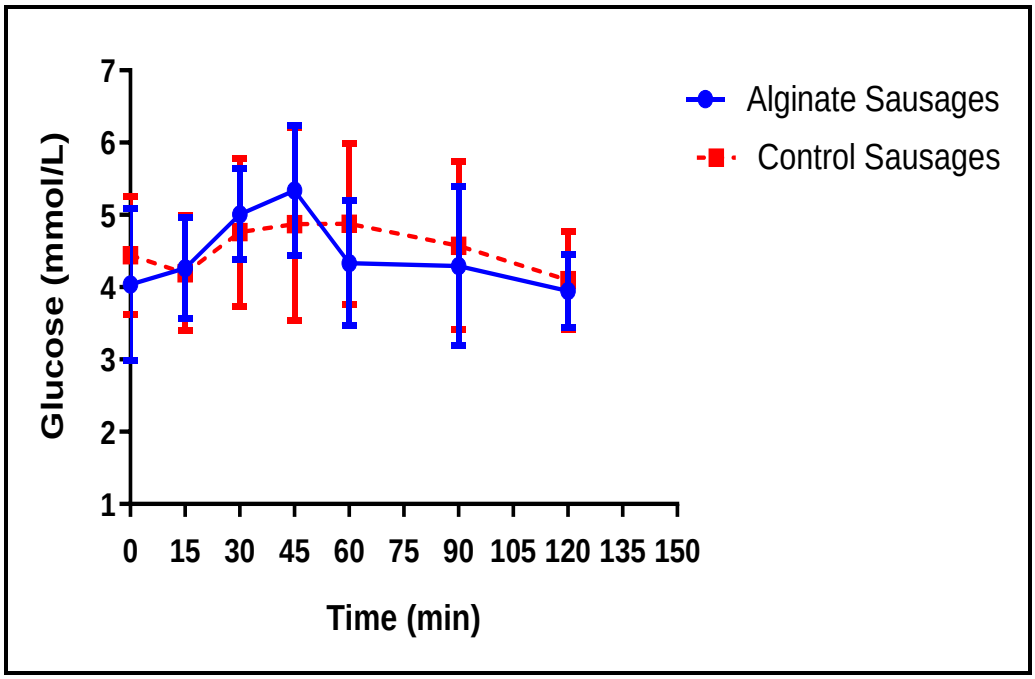


Figure 3.2: Plasma glucose concentration in 15 healthy participants before and after administration of the sausage meal. The iAUC of blood glucose before and after the administration of alginate and control pork sausage meal in 15 healthy participants using volunteers as their own controls compared the mean plasma glucose between the two-sausage meals. No statistical difference can be drawn from this figure due to the small population size.

3.7 Discussion:

In this acute feeding research, the hypothesis that 4% alginate within the pork sausage meal has the potential of reducing the calories ingested from the pork sausage meal including alginate to lower the level of fat and carbohydrate digestion in a healthy volunteer.

This intervention could be effective in lowering serum lipids by reducing the intestinal uptake of ingested lipids. There are several types of polysaccharides extracted from seaweed and they are estimated to form up to 30-71% of their dry weight such as fucoidan, cellulose, agar, carrageenan and alginate (O'Sullivan *et al.*, 2010; Fleurence *et al.*, 2012).

A unique aspect of alginate is the ability to form a viscous gel in order to slow down the intestinal uptake of lipids and glucose as well as by causing a reduction in postprandial blood glucose, insulin and fat levels (Huebbe *et al.*, 2017).

The finding from this acute randomised double blinded placebo crossover study that investigated the fat and carbohydrate digestibility via finger prick blood sample in 15 healthy participants showed a larger research group, estimated to be at least 270 individuals, to increase statistical power is needed to ensure that any significant difference can be detected between the study arms.

The minimum sample size required to detect the true proportion of the population with e = 5% margin of error and 90% confidence level and Z=1.65 A Z-score is a numerical measurement that describes a value's relationship to the mean of a group of values. Z-score is measured in terms of standard deviations from the mean

p = standard deviation and n = population size, **Equation 3.1.**

$$\text{Sample Size (n)} = \frac{Z^2 \; p\,(1 - p)}{e^2}$$

$$n = [(1.65)^2 * 0.5.\ (1- 0.5)] / (0.05)^2$$

n = 272 individuals required.

The equation was obtained from (Al-Subaihi, 2003).

The results obtained from this pilot trial showed the consumption of an alginate sausage meal could be associated with more favourable metabolic outcomes compared to the control sausage meal. This could potentially provide evidence of the effectiveness of alginate to be used for attenuating dietary lipid digestibility. In particular, it could provide some evidence of a modulation of fat digestion by alginate incorporated sausage.

In addition, the findings from this result regarding fat digestion showed a trend toward reduction in the uptake of TG following alginate intake, however it was not significant compared to the control sausage meal over the selected time points of digestion. This relates to the fact that, from the small population size used as described above, it will not be possible to detect a significance level between the two arms of the study. Interestingly, the AUC showed a measurable difference between the acute metabolic effects on the blood concentrations of glucose and triglycerides after the consumption of alginate and control pork sausage meals among 15 health individuals.

The AUC was improved after alginate meal administration compared to the control meal intake. 6.9g of dietary fibre included in the alginate sausage meal could have showed an inhibitory effect on pancreatic lipase which was demonstrated by the lower amount of TG in the blood compared to the control sausage meal which contained just 3.2 g of fibre ingested Data from this lab has shown that a specific alginate inhibited the pancreatic lipase activity up to 75% *in vitro* (Wilcox *et al.*, 2014). It has been reported that soluble dietary fibres could decelerate nutrient absorption in rats (Georg Jensen *et al.*, 2012). As alginate is a soluble dietary fibre that has a role in forming a gel matrix in a stomach, this increases small intestinal viscosity and lowers the digestion of the nutrients, thus reducing their absorption rate and interaction with digestive enzymes (Ohta *et al.*, 1997; Paxman *et al.*, 2008a; Guo *et al.*, 2019).

A recent study has evaluated the effect of alginate oligomers, including alginate products from a Japanese company containing *Durvillaea* and *Lessonia nigrescens* (AO) and the same two alginates digested with bacterial alginate lyase (E-AO) on high fat diet mice (oral intake of corn oil) for 14 days. The study illustrated that the mice group fed with E-AO showed a noticeable reduction in pancreatic lipase activity, serum TG levels and an attenuated uptake of intestinal lipids. This was associated with a significant reduction in adipose tissue accumulation compared to the mice given AO (Nakazono *et al.*, 2016).

Interestingly, the outcome of the acute feeding of an alginate sausage meal is in the line with the previous study that investigated the effect of 4% alginate enriched bread on fat digestion. The AUC reported no significant change of serum triglycerides between alginate and control bread; although alginate reduced the triglycerides uptake, this reduction was not significant (Houghton *et al.*, 2019). This finding was similar to previous research that reported no significant difference on the

plasma triglyceride between the intake of an alginate enriched beverage when compared to the control beverage (Paxman *et al.*, 2008a).

Regarding carbohydrate digestion, although the iAUC for plasma glucose response were reduced after alginate compared to the control administration meal, the iAUC of blood glucose concentrations has not shown a difference between the alginate and control sausage meal. Therefore, it could be assumed that no differences between the alginate and control sausage meal was attributable to alginate having no effect on carbohydrate digestion.

Surprisingly, there was no one from the 15 participants who had a postprandial blood glucose concentration higher than the fasting blood glucose concentration after the two hours from the alginate and control sausage meal consumption. Thus, an important indication could be that, this group of participants had an optimal insulin response. The data showed that as presented in **Figure 3.2**, the postprandial blood glucose levels were lower than the fasting blood glucose concentration that has normal range rating between (3.9 mmol/l and 5.6 mmol/l) after alginate and control sausage meal digestion which indicated the participants were healthy with no risk of diabetes diagnosed. When the fasting blood glucose level is ranging between 5.6 and 6.9 mmol/l an intervention to controlling glycemia is required and if the fasting blood glucose concentration becomes 7 mmol/l or increased, diabetes is detected (WHO). In light of this, the finding suggests that, people with impaired fasting plasma glucose and diabetes should be targeted in this research, as it assumed that they are more likely to show an effect in terms of blood glucose concentration over two hours of carbohydrate digestion.

In addition, Oral glucose tolerance test (OGTT) should be applied to assign participants to appropriate groups. This test involves 10 -12 hours overnight fasting, fasting blood glucose sample (baseline) and after two hours of glucose post-ingestion (Chen *et al.*, 2018). If the participants' show impaired fasting plasma glucose after the OGTT test, they will be targeted in this research. A further important point that should be considered is that the majority of those participants were students and adults who are young adults and their aged range between 18 and 22 years old. This could explain that no participant had a postprandial blood glucose level higher than fasting blood glucose concentration. A previous study reported that in a comparison study of basal metabolic rate (BMR) rate between two age groups, 98 subjects aged between 20 – 30 years (young age group) and 39 subjects aged ranging between 50 – 65 years (middle- age group), it was indicated

that the BMR for the middle- age group was significantly lower than for young people groups (Klausen *et al.*, 1997).

3.8 Study limitations:

There are several limitations to this acute feeding study. A limitation of the present intervention is that the metabolic outcomes related to fat and carbohydrate digestion were only provided by a small population size (15 participants) and therefore did not represent an optimal measure of the effect of alginate sausage meal on the fat digestion in the required size of population. In relation to this, a larger sample size is required as presented in **Equation 3.1**.

A further limitation is that this study only assessed the effect of alginate pork sausage meal in a short period of time, and this could be considered not enough to observe a marked effect in such acute feeding studies. Thus, further longitudinal investigation using a larger population is required. Moreover, consuming one meal that include one sausage sandwich containing 20g of butter and two pieces of sausage after more than 8 hours of fasting would seem not enough.

3.9 Conclusion:

Inhibiting the absorption of some dietary elements such as dietary lipids and carbohydrates, is one of the major methods for combating obesity which could also lower the risk of T2DM. This pilot study aimed to determine the effect of alginate incorporated pork sausage on the physiological function of specific nutrient uptake that is carbohydrate and lipid and has shown a trend toward the potential attenuation of the dietary lipid's breakdown and uptake owing to alginate functionality. Although the results of this intervention managed to show the possibility for the comparison between the control and intervention arms, it was not possible to show a significant level of difference between the study arms either with fat or carbohydrate digestion due to the small study population. Moreover, this data is potentially capable of suggesting the appropriate study group such as people with T2DM which could provide a benefit for them in terms of reduced lipids digestibility.

Chapter Four: Digestion via the Model Gut including fat digestion and alginate determination

4.1 Artificial digestion

Digestion is a complex chemical, enzymatic and mechanical operation that can break down dietary macronutrients and release these molecules throughout the upper GI tract. These molecules can have beneficial or adverse impact on health and disease prevention (Dupont *et al.*, 2019). Human nutritional research plays an important role as a 'gold standard' for investigating questions concerning food (Minekus *et al.*, 2014).

Increasing attention has focused on the development of a structural model of food-based delivery devices to encapsulate, protect and release bioactive ingredients to improve human wellbeing (McClements *et al.*, 2008). In addition, understanding the incorporation of food properties, food digestion and absorption would benefit food manufacturers and enhance food's nutritional properties, as well as preserve bioactive components throughout storage, delivery, deployment and their release into particular regions of the gastrointestinal tract (Bauer *et al.*, 2005; Sanguansri and Augustin, 2006).

Therefore, *in vitro* digestive models play an increasingly important role in investigating the structural and chemical variations that are associated with food ingredients under physiologically simulated GI relevant settings (Hur *et al.*, 2011).

The *in vitro* Model Gut System (MGS), developed in Newcastle University, at the Institute of Cell and Molecular Bioscience and Institute of Bioscience, is a model that represents the physiological, chemical and enzymatic conditions required to mimic the human gut with inclusion of some physical processes of digestion by using stirring.

As such, this model is a unique system for simulating the digestive processes of the compartments of the gastrointestinal tract (GI), starting from mouth, to stomach and to the terminal small intestine to accurately mimic the human digestive process. Moreover, this model has been validated to study the chemical and enzymatic digestion of food and nutraceutical components including carbohydrates, protein and fat hydrolysis. This model has also demonstrated the efficacy of the bioactive compound, alginate, as a novel lipase inhibitor for anti-obesity treatment (Wilcox *et al.*,

2014). Furthermore, the usage of MGS is beneficial as it reduces cost, saves time, and it is high throughput; this provides an accurate, repeatable method to utilise and control the digestive process (Minekus *et al.*, 2014). Importantly, determining food digestion by MGS is an ethical alternative to *in vivo* research in either human or animal studies which are expensive, difficult to control and have ethical considerations which need to be taken into account (Dupont *et al.*, 2019).

Many aspects of simulated digestion *in vitro* have been utilised as a powerful experimental tool that distinguishes digestibility of allergens (Wickham *et al.*, 2009), the control of release of bioactive components in terms of burst release or extending the release at an exact location in the GI (Hur *et al.*, 2011), the amount of drug released through drug delivery (Kuentz, 2011), the measurement of food mycotoxins (Versantvoort *et al.*, 2005), in addition to assessing macronutrient digestibility and acceptability including lipids (Lorrain *et al.*, 2012), proteins (Kopf-Bolanz *et al.*, 2012), carbohydrates (Hasjim *et al.*, 2010) and food matrix release of minerals and trace elements (Miller *et al.*, 1981).

Brown seaweed enriched and alginate enriched foods including bread, cheese and pork sausage have been digested by this model of digestion as seen in Figure 4.1. Following this digestion, two experimental approaches to analyse carbohydrate and fat digestion took place in this PhD research; measuring polysaccharides including alginate and other materials positive in the PAS assay as well as the amount of glycerol released after *in vitro* digestion.

4.2 Fat structure, digestion and absorption

Obesity is related to several non-communicable diseases and scientific evidence shows that the treatment for obesity and overweightness relies on reducing energy intake and dietary lipids to limit an excess accumulation of body fat. Therefore, a reduction of 5 -10% of original body weight has been associated with a decrease in the risk of developing type 2 diabetes mellitus and reduce the risk of metabolic syndrome through the decrease of fat mass, serum insulin, fasting plasma glucose and lower the risk of cardiovascular disease (Lapointe *et al.*, 2010; Hall *et al.*, 2012; Awang *et al.*, 2014).

Therefore, fibre enriched foods have the potential to lower the risk of obesity and the related metabolic problems. Edible lipids consist of a substantial amount of triacylglycerols (TAGs),

phospholipids, and cholesterol esters. They are digested by the actions of the lipolytic enzymes gastric and pancreatic lipase (Carriere *et al.*, 1993b; Manson and Weaver, 1997; Porsgaard *et al.*, 2005). The digestion of dietary lipids in the stomach and small intestinal environments is influenced by several factors, such as the activity of lipase in many parts of the alimentary canal, the emulsified droplets' volumes, and the interfacial activity among the emulsion droplets. In addition, further factors play a role in fat digestion, for example the surface region of digested foods and the type and abundance of the micronutrient compounds alongside the presence of biological elements such as acids and enzymes (McClements *et al.*, 2008; Shen *et al.*, 2011).

4.3 Fat digestion and alginate determination

This chapter presents the synthetic gut model used as a valid system to simulate the conditions of the human gastrointestinal channel from mouth, stomach and the upper small intestine. Moreover, this *in vitro* method was used to assess the digestion of the fat within the study's food vehicles which are foods rich in fat. This was used to test the hypothesis that food containing 4 % seaweed and alginate may have the potential to inhibit the hydrolysis of lipids. The release of alginate from these foods during digestion was also quantified.

As alginates are polymer polysaccharides, they can be detected in a colorimetric assay. The Periodic acid Schiff's (PAS) assay works by breaking down complex carbohydrate molecules. (Kilcoyne *et al.*, 2011). This method has been adapted from Mantle and Allen (1978). Previous work conducted on the quantification of alginate released from 5.2 g of alginate bread following *in vitro* digestion has shown that the PAS assay was sensitive enough to screen a smaller amount of alginate – 208 mg – after the digestion, providing good linearity that could be easily repeated (Houghton *et al.*, 2014). Thus, the Periodic acid Schiff's (PAS) assay is a suitable method for quantifying alginate release in this study.

4.4 Aims

The aims of this chapter were to:

1- To validate the MGS with the *in vitro* digestion of a variety of food products including bread, sausage and cheese.
2- To take samples at separate time points at T_0, T_{15}, T_{30}, T_{60}, T_{61}, T_{75}, T_{90}, T_{120}, T_{150}, and T_{180} minutes which represents the mouth, stomach and the upper intestine phase of the artificial digestion.
3- To investigate the impact of seaweed and alginate incorporated cheese, bread and pork sausage on the lipid hydrolysis activity *in vitro*, measuring the amount of glycerol released after the digestion.
4- To estimate the release rate of alginate from the cheese, bread and sausage vehicles by using the Periodic acid Schiff's (PAS) assay throughout the three digestive phases in a model gut.

4.5 Material and Methods

A) Model gut system (MGS)

4.5.1- Materials

Most chemicals and reagents were obtained from Sigma-Aldrich (Poole, UK). All digestive enzymes including: α-amylase from hog pancreas, pepsin from gastric mucosa, and pancreatin from porcine pancreas were obtained from Sigma, whereas gastric lipase involved 400 U/L gastric like lipase from bacteria and this was obtained from Amano Enzyme, Inc AP12. Pig bile was freshly collected from a local slaughterhouse. The brown seaweed *Ascophyllum nodsum* and some alginate such as DMB and GHB were kindly donated by FMC BioPolymer and Technostics Ltd. CC01 alginate was provide d by Coca-Cola, UK.

4.5.2- Preparation of Synthetic GI Fluids

These synthetic GI fluids have been designed to imitate the pH variations of the human gastrointestinal tract and were prepared and kept in a suitable glass container at room temperature (~20 ˚C) as a stock solution. Both digestive enzymes and porcine bile were added freshly for each replicate run.

4.5.2.1 Synthetic Saliva

The artificial saliva was prepared from 62 mM sodium di-hydrogen phosphate (NaH_2PO_4), 6 mM di-potassium hydrogen phosphate (K_2HPO_4), 15 mM sodium chloride (NaCl), 6.4 mM potassium chloride (KCl), 3 mM calcium chloride ($CaCl_2$) titrated with 1 M NaOH to pH 7.4 and 5µl of 3mg/ml α-amylase.

4.5.2.2 Synthetic Gastric Juice

The gastric fluid was composed of 49.6 mM sodium chloride (NaCl), 9.4 mM potassium chloride (KCl), 2 mM potassium di-hydrogen phosphate (KH_2PO_4), and 5mM Urea titrated with 1 M HCL to pH 2.0. In addition, 0.5mg/ml porcine pepsin and 400 µ/L of bacterial gastric lipase were added.

4.5.2.3 Synthetic Pancreatic Juice

This was prepared by adding 110 mM sodium bicarbonate ($NaHCO_3$), 2.5 mM di-potassium hydrogen phosphate (K_2HPO_4), 54.9 mM sodium chloride (NaCl), 1 mM calcium chloride ($CaCl_2$) and 1.67mM Urea titrated with 1 M NaOH to pH 8. For each run, 21g of pancreatin from porcine pancreas was added to 300 ml of pancreatic juice on a shaker and then filtered twice using glass wool to remove any insoluble material.

4.5.2.4 Fresh Bile

Fresh porcine bile was stored frozen at –20˚C after collection from a local slaughterhouse. 25 ml of bile was required, and this was added to each replicate. The stock of bile was used for several projects. So it was replaced periodically with fresh bile. Therefore it was not possible to always use the same stock.

4.5.3- Preparation of Food Samples

Food samples used in this research, digested in the MGS, include alginate and control bread, seaweed, alginate, and control cheese as well as alginate and control pork sausage. All food samples were prepared via the same procedure. All food samples followed the same method of preparation before MG digestion as described in sections 4.5.33 and 4.5.4

4.5.3.1 Alginate and control bread

Bread was chosen due to its popularity as a food product in Western diets. 4% CC01 alginate was used in this bread. The CC01 was supplied by Coca Cola. This alginate includes ratio M/G as 0.81 fraction of guluronate (**F_G**) and fraction of mannuronate (**F_M**) (Albalawi, 2020). The alginate bread was made at home with 548g white flour, 20g Oil, 360g Milk, 15g Sugar, 10g Salt, 7g Yeast, and 40g Alginate. The control bread had the same recipe without the alginate. 5.2g of bread was digested at each run.

4.5.3.2 Seaweed and alginate cheese

The alginate and seaweed cheeses were produced by the Northumberland Cheese Company. The alginate (GHB) Manugel GHB alginate (M/G ratio = 0.6) with strong gel ability (Enobakhare *et al.*, 2001) and seaweed (*Ascophyllum nodsum*) were supplied by FMC Biopolymer and Technostics Ltd (Hull, UK) and incorporated into the cheese. The control cheese was made from the same type of cheese i.e. full fat hard cow's cheese with nettle herb added. 20 g of cheese was used for each run of digestion.

4.5.3.3 Alginate and control pork sausage

The pork sausages were produced by Ruitenberg Ltd, The Netherlands. The nutritional ingredients have been previously described in chapter 2. Ruitenberg alginate was used within the sausage. For each run, either one control sausage 46.4g or alginate sausage 48.5g cut into small pieces was used.

4.5.4-Model Gut (MG) procedure

The digestion process in this *in vitro* model of simulated digestion was monitored over a period of three hours and represents the three stages of mouth, stomach, and the upper intestinal tract. Each type of food was run in triplicate. This model, as presented in **Figure 4.1**, consists of three 500 ml glass outer vessels with two vessels contain food samples. The third vessel has no sample; this is a model gut solution (MGS) that used as a background control during MG *in vitro* digestion at each experiment. The three vessels with two food samples and one with MGS were run simultaneously. Each vessel is connected to an overhead stirrer to mimic the stomach and SI (small intestinal) motility and with a peristaltic pump set at 0.5ml/min to pump the gastric juice for 60 min and at 0.25ml/min of pancreatic fluid for 120 min. The vessels were placed in a water bath set at 37 ˚C, throughout MG digestion.

First stage – The experiment started at 0 min with two food samples broken up into small pieces to simulate the mouth's mastication. Food samples included 20g of alginate (Alg ch), seaweed (Sw ch) and control cheese (C ch), or one piece of approximately 47g of alginate pork sausage (Alg Ps) or control sausage (C Ps), or 5.2g of alginate bread (AB) or control bread (CB). 5ml of synthetic saliva, 10 ml of deionised H_2O and 5µl of 3mg/ml α-amylase were added into all vessels and mixed for 30 seconds.

Second stage – 50ml of gastric juice including pepsin and gastric lipase were added to the three vessels and mixed. The remaining gastric juice (150 ml) was pumped via a peristaltic pump at 0.5 ml/min for 60 min to each vessel.

Third stage – At 61 min of digestion, 25 ml of porcine bile was added, and the filtered 300ml pancreatic juice was pumped at 0.25ml/min for 120 min with continual mixing.

During the three phases of digestion, 500µl of sample were collected at T_0, T_{15}, T_{30}, T_{60}, T_{61}, T_{75}, T_{90}, T_{120}, T_{150}, and T_{180} minutes.

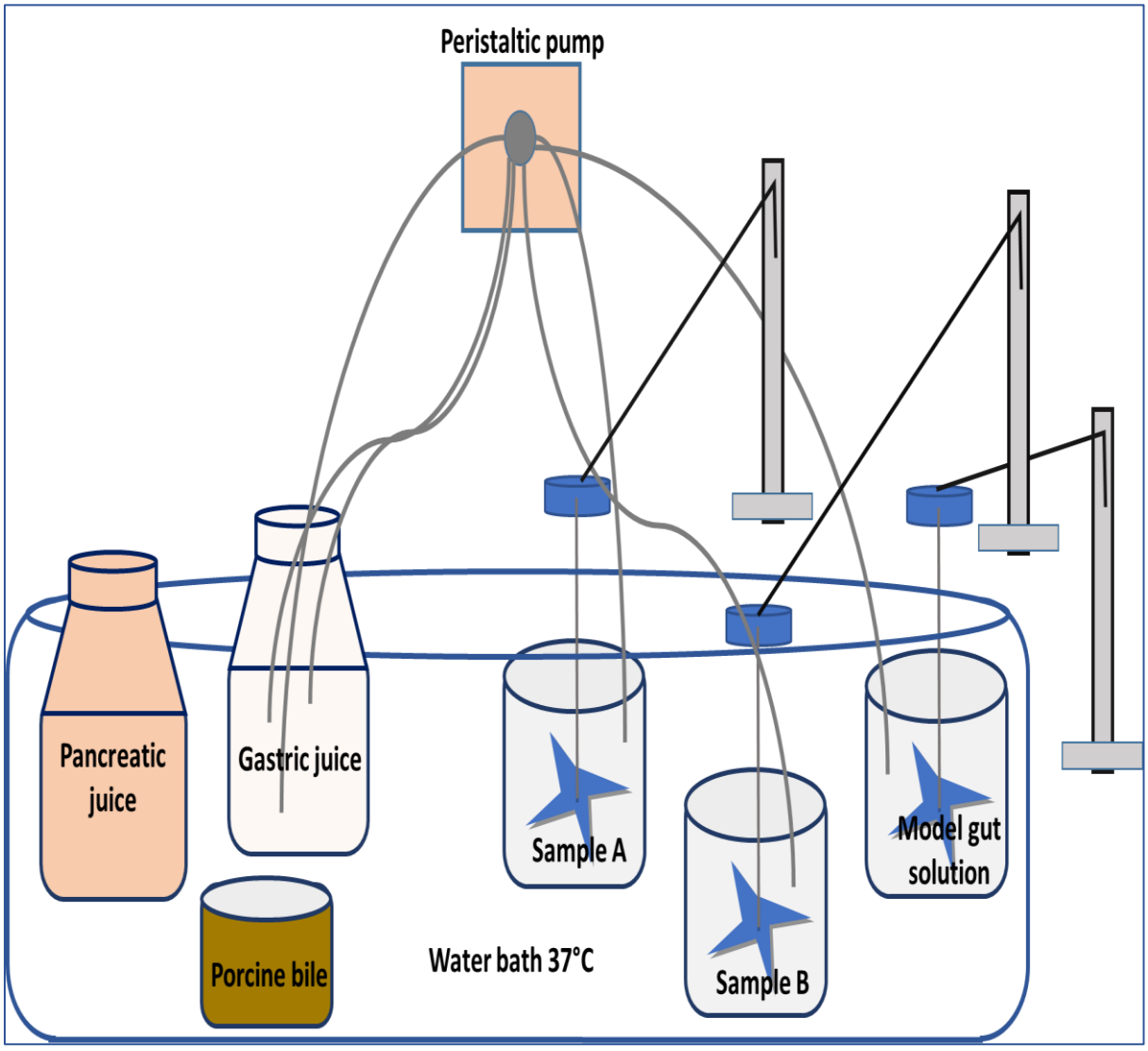


Figure 4.1 – Equipment set up for MG *in vitro* digestion. Sample A represents a food sample of cheese, bread or sausage as a control, alginate or seaweed sample. Sample B represents the same selected sample for sample A at each run of the experiment. MGS acts as a background control for the experiment. The digestion starts with the addition of synthetic saliva into each vessel at 0-59 s. At 1-60 min, 50 ml of synthetic gastric juice is added to each vessel and 150 ml of gastric juice is pumped through the peristaltic pump into each sample. At 61-180 min, 25ml of bile is added and 300ml of synthetic pancreatic juice is pumped into each vessel.

B) Fat Digestion (Glycerol Assay)

4.5.5- Chemicals and reagents

All the material used in the synthetic model gut were the same as previously described in detail in chapter three. Free Glycerol Reagent and all the chemicals needed were purchased from Sigma-Aldrich, Inc (Poole, UK). Alginates and seaweeds were kindly supplied by FMC Biopolymer, Technostics Ltd (Hull, UK) and Ruitenberg Innovation Manufacturing.

4.5.6- Sample preparation

The food products included: cheeses produced by the Northumberland Cheese Company, England, and pork sausages obtained from Ruitenberg Ingredients B.V, The Netherlands. These food vehicles contained a high fat content. The seaweed and alginate cheese contained 8.9g of fat for each 30g of cheese, and the control cheese had 9.3g of fat for each 30g of cheese. The one piece of control sausage (48.5 g) had 6.6 g of fat and alginate sausage (46.4g) had 6.9 g of fat.

4.5.7- Glycerol Detection

The free Glycerol assay is considered a simple method for quantifying the total glycerol content after digestion. To prepare a standard curve, 40 ml of deionized water was added into glycerol powder and stored in an amber bottle in the fridge. A glycerol standard curve was produced using 2.5 mg/ ml DH_2O as a stock solution for serial dilution down to 0.08 mg/ml for each experiment and 5µl of each glycerol concentration was added in duplicate. All the study samples produced from model gut digestion previously were defrosted at room temperature. The samples were centrifuged at 10,000 rpm (9.3 ×1000g) for 10 minutes, and then 5µl of the supernatants were added into each well of a 96 well plate. Following this, 80µl of free glycerol reagent was added to each sample well and all the samples were tested in triplicate. All the plates were incubated at room temperature for 20 minutes and were mixed on a shaker. The 96-well microplate reader (TECAN infinite M200 PRO) and computer

(Microsoft Excel 2013) were used. Absorbance was measured at 550 nm to determine the total glycerol content after the fat digestion of bread, cheese and sausage samples.

4.5.8-Statistical Analysis

Statistical analysis comparing alginate, seaweed, and control cheese along with the comparison of alginate and control pork sausage were performed using GraphPad Prism Statistical software version 7. The results were presented as means and SD, and the differences in the amount of glycerol release between alginate, seaweed, and control cheese as well as between alginate and control pork sausage were considered significant when the *P* value was ≤ 0.05.

C) Periodic Acid Schiff's (PAS) Assay

4.5.9- Periodic acid Schiff's (PAS) assay concept

The PAS assay was described by Mantle and Allen (1978). It is a method employed to detect a wide range of carbohydrate containing molecules including mucin, polysaccharides, glycolipids, and glycoproteins through the periodate oxidation of carbohydrates. This assay relies on two steps; firstly, the periodate oxidation of carbohydrates (hydroxyl groups) to form an aldehyde group. The second step involves the reaction between the Schiff's reagent chemicals and the aldehydes formed in a solution resulting in a purple to red colour development (Kilcoyne *et al.*, 2011; Randrianjatovo-Gbalou *et al.*, 2016).

4.510- Chemicals, materials, and reagents

Porcine mucin was isolated from gastric mucus and was prepared to a concentration of 1mg/ml in DH_2O. All chemicals including sodium metabisulphate, Schiffs fuchsin-sulphite reagent, periodic acid at 50%, acetic acid and methanol were obtained from Sigma-Aldrich Co Ltd, Dorset, UK. Artificial saliva, gastric juice, pancreatic juice and all the digestive enzymes were previously detailed in section 4.5.2 in this chapter.

4.5.11- Samples preparation

4.5.11.1 Preparation of the Schiff reagent

16.7mg of sodium metabisulphate ($Na_2S_2O_5$) was dissolved in 10 ml of the Schiff fuchsin-sulphate reagent and incubated for one hour at 37 degrees centigrade.

4.5.11.2 Preparing the periodic acid solution

20 µl of 50% of periodic acid was added to 10 ml of 7% acetic acid and mixed.

4.5.12- Mucin, and Alginate Standard Curves

The isolated porcine mucin solution was prepared to a concentration of 1mg/ml in DH_2O and was diluted in DH_2O to create a mucin standard curve with mucin concentration of 0, 0.1, 0.2, 0.3, 0.4, 0.5 mg/ml; this was used as a positive control during all the PAS assay experiments. In addition, several varying concentrations of alginates such as CC01, GHB and DMB alginate solution were used ranging between 0.5 and 2 mg/ml to produce standard curves. Alginate standard solutions were made up in either DH_2O or dissolved in a solution collected at the last phase of gastric digestion (at 60 minutes), and at the end stage of digestion (at 180 minutes).

4.5.13- PAS assay procedure in gastric and pancreatic phase

4.5.13.1 Gastric phase

All the digested samples including bread, cheese and sausage taken at 0 – 60 minutes were adjusted to pH between 6 and 7 using 1M NaOH and then were centrifuged at 13,200 rpm (16.1x1000g) for 10 minutes. After centrifugation, 500 µl of each sample's supernatant were mixed with 500 µl of DH_2O and vortexed for 30 seconds. 200 µl of each sample was pipetted into a 96 well plate in triplicate.

4.5.13.2 Pancreatic phase

500 µl of all the digested samples from 61 – 180 minutes were mixed with 500 µl of methanol i.e., a 1:1 dilution. All the samples were then incubated at -20 ˚C for 30 minutes before they were centrifuged between 4,100 (1.5x1000g) and 12,000 rpm (11.4x1000g) for 20 minutes at 4 ˚C for complete separation. Supernatants were removed and pellets resuspended in 4 ml of DH_2O. Assuming the amount of material contains alginate at 1 mg for each ml, this results in a concentration of 0.25 mg/ ml as a 1: 4 dilution. Further dilution was carried out on all samples to achieve a 1:8 dilution in order to bring values onto the standard curve. In addition, 20 µl of the periodic acid solution was added to all samples in the 96 well plate. The samples were then incubated at 37 ˚C for one hour. After that, 20 µl of the Schiff's reagent was added to the samples and then left at room temperature for 30 minutes for the colour to develop. The absorbance was then measured at 550 nm.

4.5.14- pH of model gut system

The pH measurement for all samples including bread, cheese and pork sausage were achieved using a Martini Mi150 pH meter at room temperature after calibration with a buffer solution of pH 4 and then pH 7. pH measurements were taken at several time points of *in vitro* digestion starting from T_0 until T_{180} minutes.

4.5.15- Statistical Analysis

All statistical analyses were performed using GraphPad Prism Statistical software version 7 and were presented as means and SD. Statistical significance was taken as $P \leq 0.05$. An independent t-test was undertaken to compare the mean release of alginate and the PAS assay positive material from the alginate bread and the control bread. The same approach was used to determine the difference between alginate sausage and control sausage and between alginate and control cheese. Analysis of variance (ANOVA) was performed to determine the differences between the alginate and the PAS assay positive material between the alginate, and control cheese vehicle at 0, 60, and 180 minutes of digestion. This was also used for the alginate and control sausage, and alginate and control bread.

4.6 Results

4.6.1- Digestion in the model gut

Cheese digesta images are shown in figures 4.2, 4.3 and 4.4, and pork sausage digesta images are illustrated in figure 4.5 and 4.6. This is to demonstrate that the *in vitro* digestion carried out within the MGS mimics the mouth, stomach, and small intestinal phases.

4.6.1.1 Cheese digesta

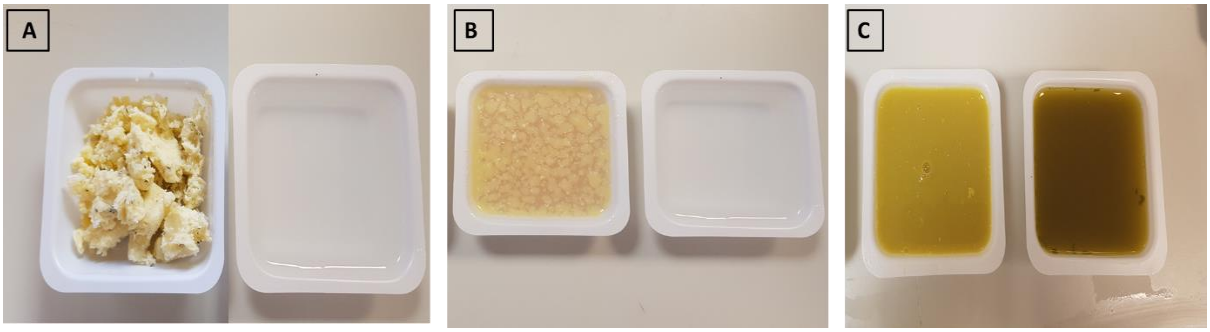


Figure 4.2 Images of the alginate cheese digesta obtained in the model gut. Images were after (A) 0 min (mouth, cheese left hand panel, MGS, right hand panel), (B) 60 min (stomach) and (C) 180 min (small intestine).

Figure 4.3 Images of the control cheese digesta obtained in the model gut. Images were after (A) 0 min (mouth, cheese left hand panel, MGS, right hand panel), (B) 60 min (stomach) and (C) 180 min (small intestine).

Figure 4.4 Images of the seaweed cheese digesta obtained in the model gut. Images were after (A) 0 min (mouth, cheese left hand panel, MGS, right hand panel), (B) 60 min (stomach) and (C) 180 min (small intestine).

4.6.1.2 Pork sausage digesta

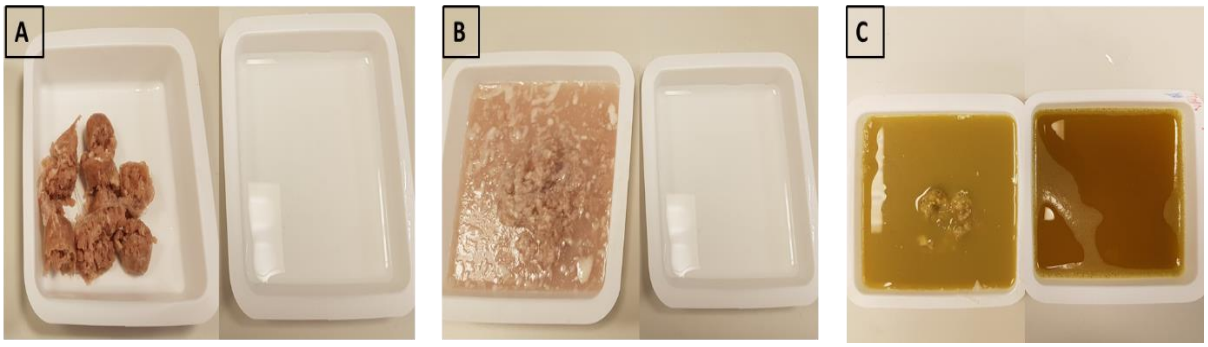


Figure 4.5 Images of the alginate pork sausage digesta obtained in the model gut. Images were after (A) 0 min (mouth, sausage left hand panel, MGS, right hand panel), (B) 60 min (stomach) and (C) 180 min (small intestine).

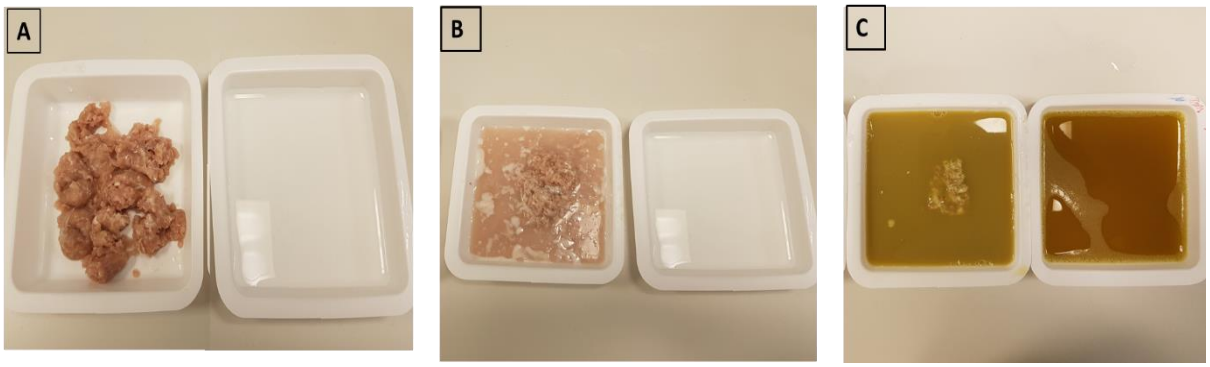


Figure 4.6 Images of the control pork sausage digesta obtained in the model gut. Images were after (A) 0 min (mouth, sausage left hand panel, MGS, right hand panel), (B) 60 min (stomach) and (C) 180 min (small intestine).

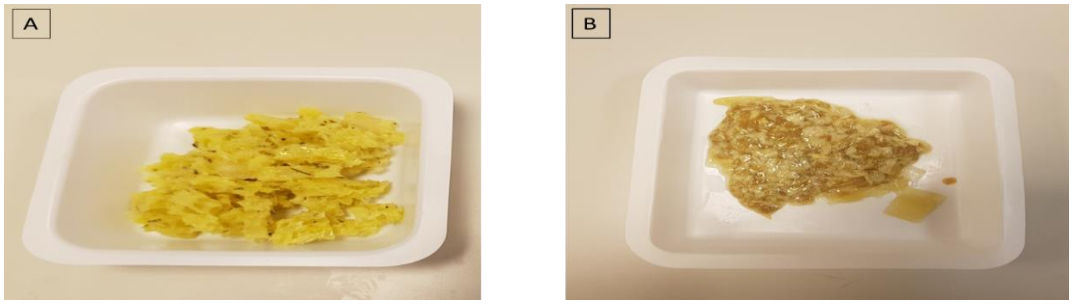


Figure 4.7A Image of material found at the end point of the MGS digestion from alginate cheese. **Figure 4.7 B** material found at the end point of the MGS digestion from control pork sausage.

4.6.2- Glycerol standard curve

The release of free glycerol was measured by the glycerol standard curve in DH_2O with the usage of the free glycerol reagent as seen in **Figure 4.8**. This assay has the ability to quantify the free glycerol liberated and provides an increased absorbance at 550 nm ranging between 0 – 0.6 OD with increasing glycerol content, with good linearity (R^2= 0.99), (n = 6).

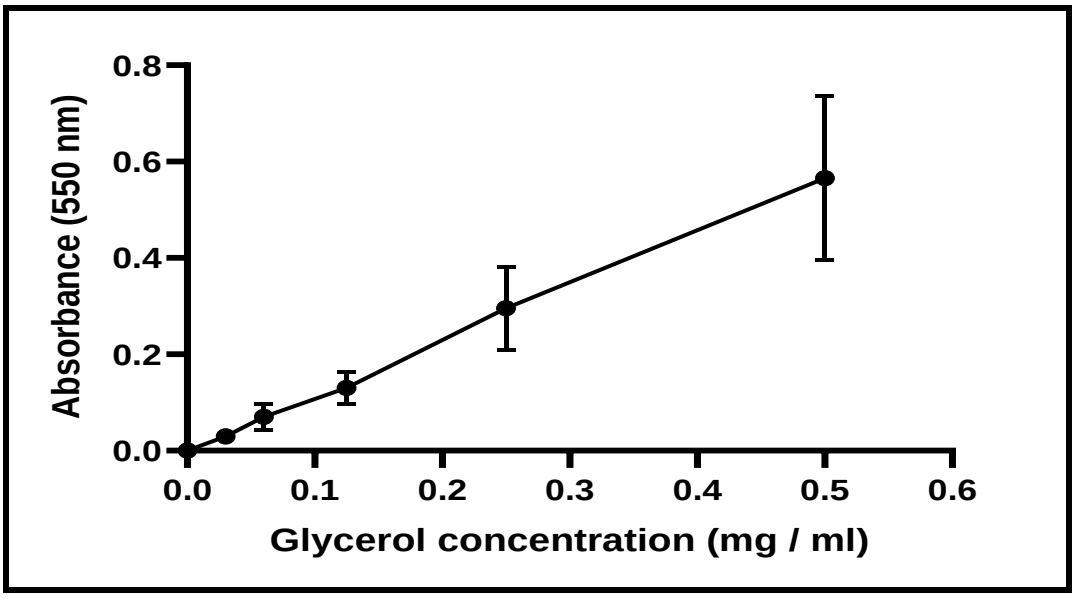


Figure 4.8 The glycerol standard curve (mean± SD) in DH_2O shows the absorbance across the glycerol concentration range (n = 6).

4.6.3- The effect of alginate and seaweed on fat digestion

4.6.3.1 Cheese

As can be seen from **Figure 4.9**, the fat digestion for all cheese samples took place in the mouth phase (0 min) starting with 19 (± 20), 31.7 (±17) , 26.5 (±10) mg of glycerol released from control, alginate and seaweed respectively; this is due to the presence of free glycerol in the cheeses. At the gastric phase over an hour, the cheese was digested in the presence of gastric lipase. At 15 min, there was an increased release of glycerol 22.6 (± 24), 70.6 (± 18), and 66 (± 33) mg for control, alginate and seaweed onwards until the end of gastric phase with values of 82.7(±15) and 73.5(±40) mg for alginate and seaweed respectively at 60minutes. The control cheese showed a lower glycerol production 31.5(± 39) mg compared with alginate and seaweed cheeses. At the beginning of the small intestinal phase, where the majority of fat digestion occurs, there was a sharp increase in the glycerol release at 61 min from control cheese 21.4(± 34) mg which reached 190.5(± 16) mg at 180 min. Alginate and seaweed cheese showed a gradual increase in the amounts of glycerol released from 61 min as 104.3(± 67.5) and 62 (± 63) mg to become 184(± 3)and 95.6(± 17) as recorded at the last point of the simulated digestion. At 120 min, the average glycerol produced from seaweed cheese, 68.3(± 7.5) mg was significantly lower than the amount liberated from alginate cheese 120.3(±12) mg at the same time in the digestive process (P = 0.01). The glycerol released from seaweed cheese at the end of the digestion was significantly lower than the glycerol produced by alginate (P = 0.01) and control cheese (P = 0.00). The incorporation of seaweed within cheese shows a potent inhibition of fat digestion.

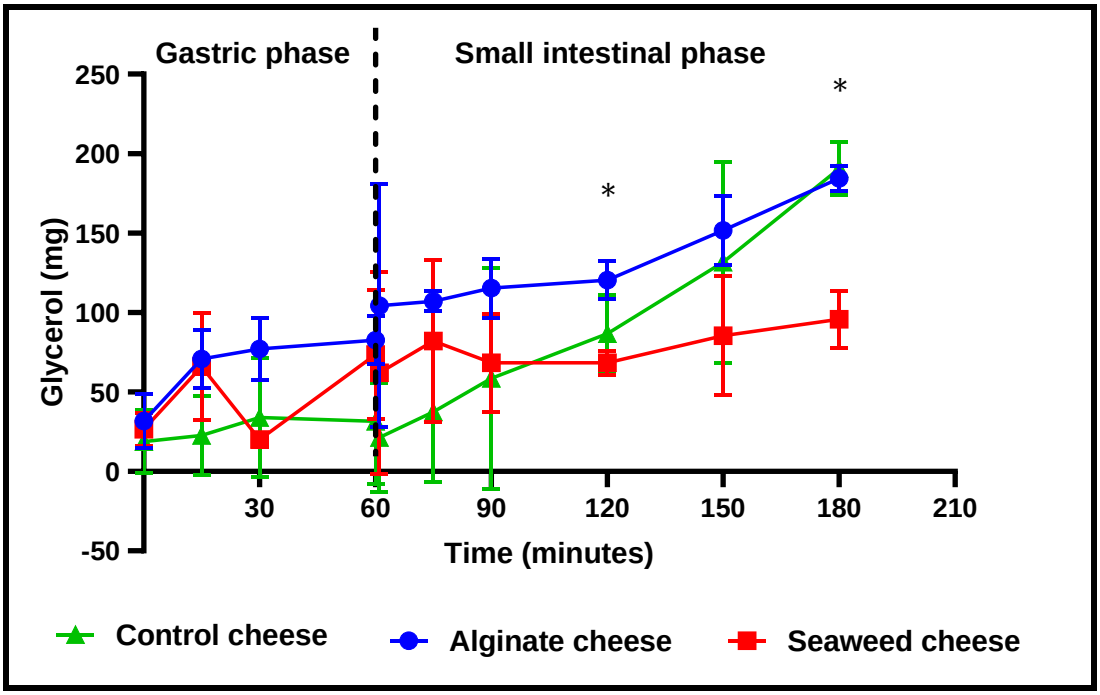


Figure 4.9 Glycerol digestion in the mouth, gastric and small intestinal phases of the model gut system. The figure presents total glycerol recovered from MG of the alginate, seaweed and control cheese after the addition of TCA (trichloroacetic acid) to stop the enzymatic activity. There are three replicates and errors are presented as standard deviation. During the mouth and gastric phases, no significant difference in glycerol release was observed between all the cheeses. At 120 and 180 min, the total released glycerol significantly differed between alginate and seaweed cheese (P = 0.011) and (P = 0.011) respectively and between control and seaweed at 180 min (P = 0.005). The figure represents (n = 4) with total of 36 samples.

4.6.3.2 Pork sausage

In regard to alginate and control sausage digestion, the fat digestibility showed a similar pattern compared to the cheese. As the digestion started from mouth phase, glycerol release was 56.5(±21) and 36.7(±20) mg for alginate and control sausage respectively. At timepoints 15, 30 and 60 min of gastric phase digestion, there was less glycerol production by the alginate sausage as shown by 54(±19.9), 47.8(±21.9) and 33.3(±12.6) mg of glycerol respectively. However, the control sausage at the same timepoints showed a slight increase with a minor difference in the glycerol release with 33.8(±23.2), 36(±25.6), and 38(± 30.6) mg of glycerol respectively which was not significant. Within the SI phase of digestion, both the alginate and control sausage showed an increased trend

towards the total release of glycerol after 15 min of digestion. At 75, 90, 120 and 150 min, 46.8(±29), 84.5(±44.5), 132.3(±57.7) and 168.7(±96.7) mg of glycerol respectively was released from the alginate sausage. The same trend was shown in control sausage with 36.1(±25), 45.3(±14.9), 95.6(±62.8) and 114.8(± 88.8) mg of glycerol being released at the same timepoints respectively. At 180 min i.e., 120 min into simulated small intestinal digestion phase the glycerol liberated from alginate sausage was reduced (but not significantly) from 168.7 (±96) mg at 150 min to 163.5(±81) mg at 180 min. However, by the final timepoint of control sausage digestion, the glycerol release increased to 191.6 (±98) mg compared to 114(±8) mg of glycerol at 150 min. This could suggest that alginate slightly inhibited the fat digestion in alginate sausage compared to control sausage at 180 min of digestion, but not significant ($P > 0.05$)

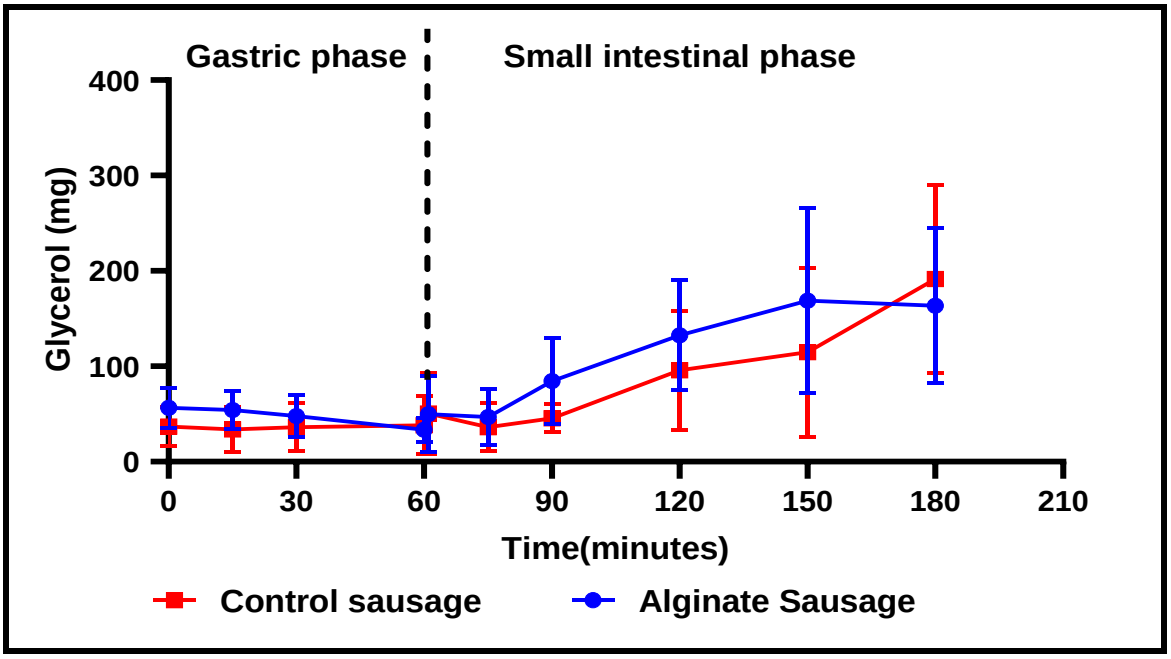


Figure 4.10 Glycerol digestion in mouth, gastric and small intestinal phase of the model gut system. The graph shows total glycerol produced from alginate and control pork sausage digested via MG after the addition of TCA (trichloroacetic acid) to stop the enzymatic activity. They were tested in triplicate; error bars show standard deviation.

4.6.4- Mucin Standard Curve

Mucin was used as a positive control for all PAS assay experiments. The mucin standard curve was produced by dissolving porcine gastric mucin in the DH_2O at 1mg/1ml with perfect linearity ($R^2 = 0.99$) and with an absorbance range between 0 – 3.5 OD as shown in **Figure 4.11**.

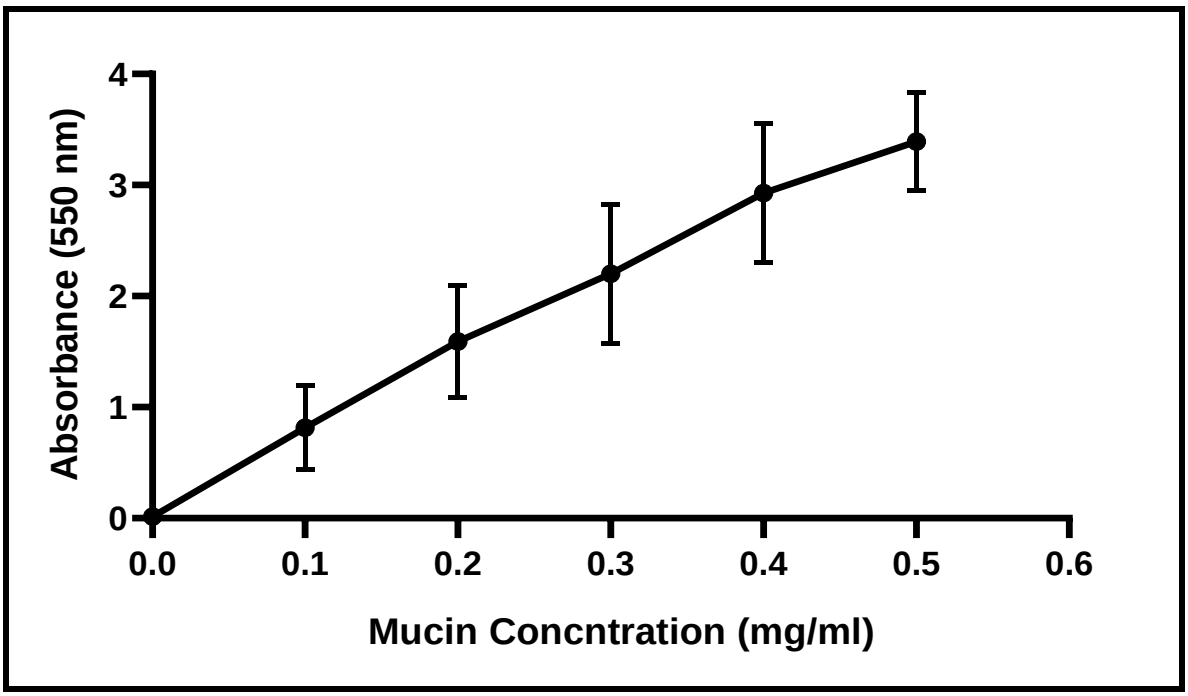


Figure 4.11 Mucin standard curve with a starting solution of 1mg/ml mucin in DH_2O. Results are shown as the mean (± S.D). This was used as a positive control for the PAS assay, (n = 12) with good linearity (R^2= 0.99).

4.6.5- Alginate Standard Curve for Gastric and Small Intestinal Solution

4.6.5.1- GHB Alginate standard curve

The GHB alginate standard curve created from MGS during the gastric phase (end at 60 min) and the small intestinal phase (end at 180 min) are shown in figure 4.12 respectively. The absorbance of GHB alginate in the simulated gastric phase ranged between 0.0 up to 1.2 OD for 0.0 – 0.5 mg/ml. The range of absorbance was 0.0 – 0.3 OD for 0.0 – 0.20 mg/ml for GHB alginate in the model gut solution of the intestinal phase.

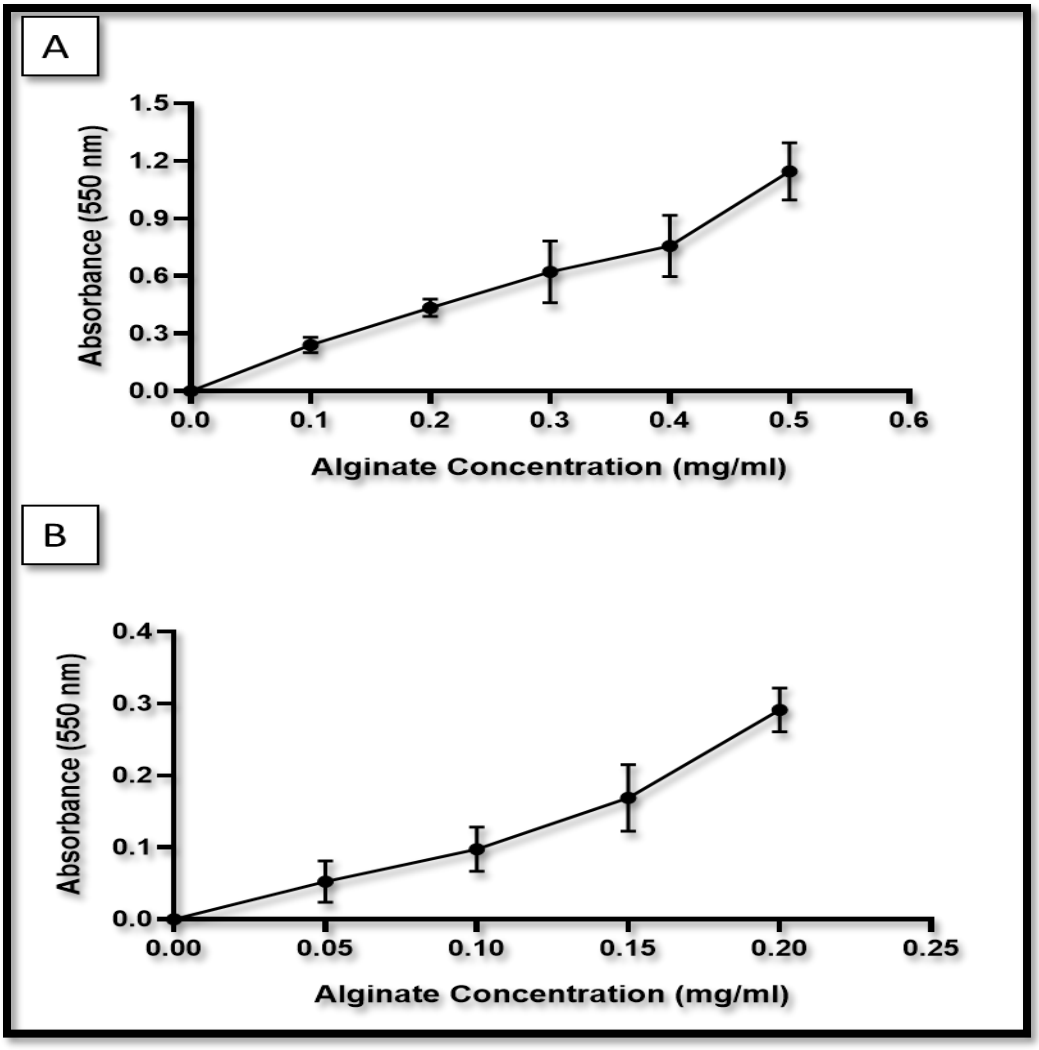


Figure 4.12 GHB alginate standard curve in the gastric and intestinal phases of the model gut using the PAS assay. Panel A shows GHB alginate in gastric juice taken at 60 min which represent the last phase of stomach digestion, n= 5 (R^2= 0.97). Panel B shows GHB alginate in the small intestinal juice taken at 180 min which is the final stage of digestion in the MGS, (n = 6) with (R^2 = 0.95).

4.6.5.2- CC01 alginate standard curve

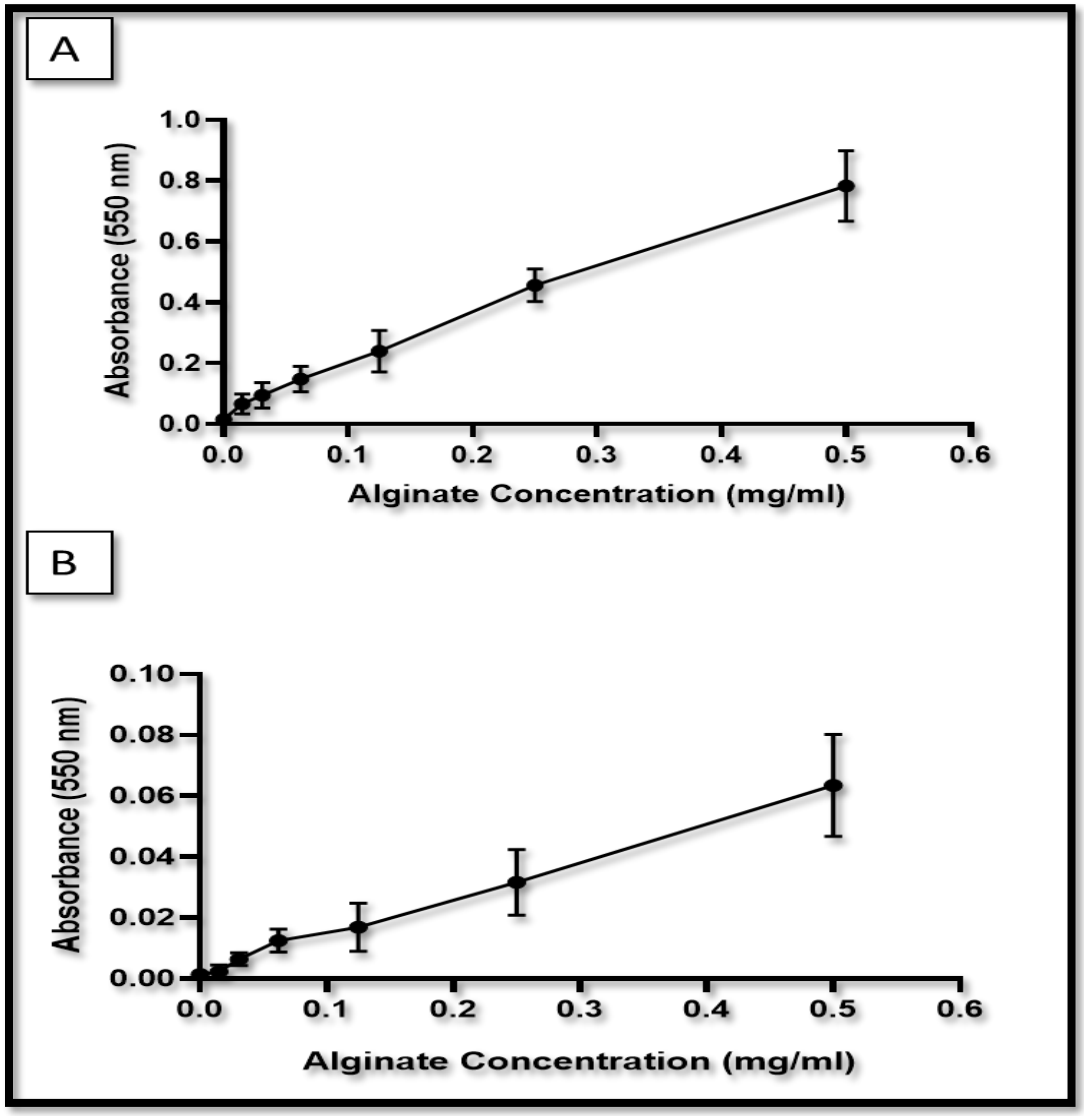


Figure 4.13 CC01 alginate standard curve in the gastric and intestinal phase using the PAS assay. Figure A shows CC01 alginate in gastric juice taken at 60 min which represents the last phase of stomach digestion, n= 6 (R^2= 0.99). Figure B shows CC01 alginate in small intestinal juice taken at 180 min, the final stage of digestion in MGS, (n = 6) with (R^2 = 0.99).

4.6.6- pH measurements during food digestion

pH in the model gut should mimic those of *in vivo* digestion. pH measurements were carried out for all cheese, bread and sausage samples. Regarding bread digestion, at 0 min during the mouth phase, the pH was 7.1(±0.05), 7.1(±0.06), and 7.2 (±0.06) for MGS, AB, and CB respectively and the pH was not significantly different between all ($P > 0.05$). After that, during the gastric phase at 15 min of the digestion, there was a massive reduction in the pH to 1.3(±0.08), 1.6(±0.04), and 1.3(±0.03) for MGS, AB, and CB respectively with no statistical difference between all of them ($P > 0.05$). At the beginning of the pancreatic phase of the digestion (61 min), the pH was raised to 2.8(±1.15), 3.7(±0.4), and 3.3(±0.7) for MGS, AB, and CB. This increase resulted in a significant difference between MGS and AB $P< (0.05)$, MGS and CB $P< (0.05)$, and between AB and CB $P< (0.05)$. Finally, at the end of digestion (180 min), the pH had gradually increased to reach 6.9(±0.4), 6.7(±0.3), and 6.6(±0.3) for MGS, AB, and CB respectively with a significant difference between MGS and CB $P< (0.05)$ and between MGS and AB $P< (0.05)$ with no significant difference between AB and CB $P > 0.05$. **Figure 4.14** shows the average pH (± SD) of the bread digestion through the model gut system.

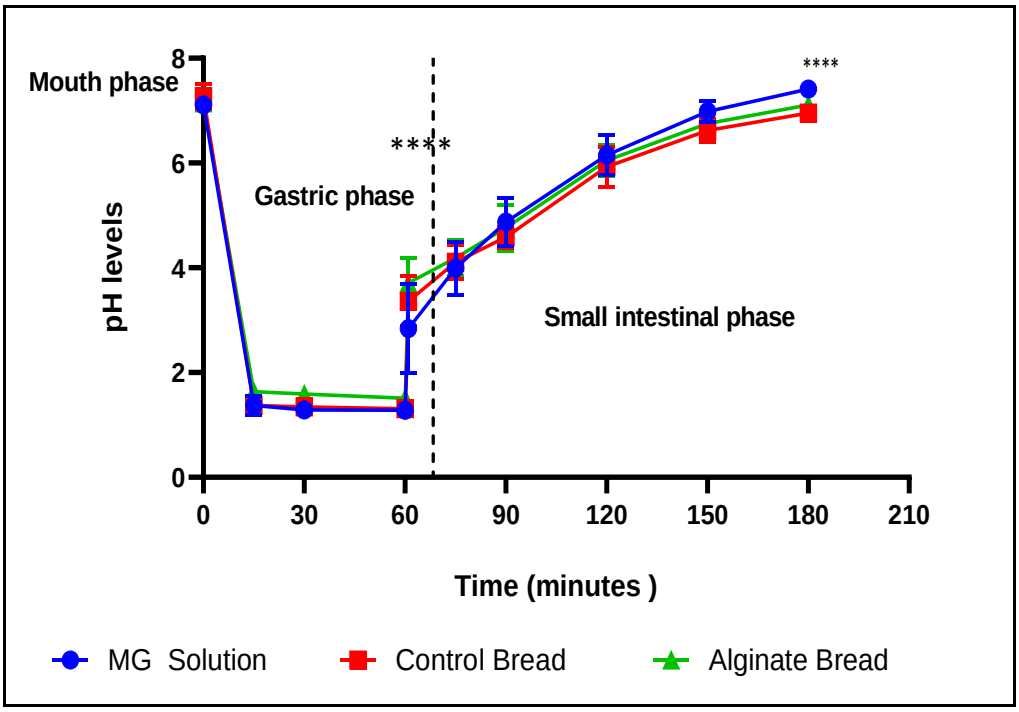


Figure 4.14 pH measurements for bread digestion. pH levels mean (± S.D) of MG, control, and alginate bread (n= 4). The figure compares between the pH levels of mouth phase (0 min), gastric phase (60 min) and the small intestinal phase (180 min) for MG, Control bread and Alginate bread. There were significant differences of pH levels for MG between the mouth and gastric phase ($P < 0.05$) and between the mouth and the small intestinal phase ($P < 0.05$) and between gastric and the small intestinal phase ($P < 0.05$). There were significant differences of pH levels for Control bread between the mouth and gastric phase ($P < 0.05$) and between the mouth and the small intestinal phase ($P < 0.05$) and between gastric and the small intestinal phase ($P < 0.05$). There were significant differences of pH levels for Alginate bread between the mouth and gastric phase ($P < 0.05$) and between gastric and the small intestinal phase ($P < 0.05$).

The pH measurements during the cheese digestion are shown in **Figure 4.15.** At the mouth phase, the pH at 0 min was 7.1(±0.2), 5.9(±0.2), 6.1(±0.3), and 5.3(±0.2) for MGS, alginate, seaweed and control cheeses respectively. The pH level of MG was significantly different to that of Alg ch, and C ch ($P < 0.05$) but not significant with Sw ch ($P > 0.05$). The same trend was observed between MGS and Alg ch, Sw ch, and C ch ($P < 0.05$) after 15 minutes of digestion. A reduction in the pH level was seen after the addition of gastric juice at 15 min; 1.8 (±0.2), 5.3(±0.2), 6.0(±0.3), and 5.3(±0.2) for MGS, Alg ch, Sw ch, and C ch respectively suggesting the cheese was buffering the gastric juice. After 60 min of digestion, with the addition of pancreatic secretions representing the duodenal phase, at 61 min the pH slightly increased and was 4.8 (±0.2), 6.0(±0.2), 6.1(±0.3), and 5.6(±0.2) for MGS, Alg ch, Sw ch, and C ch with a significant difference in the pH values ($P < 0.05$) of MGS and between Alg ch, Sw ch, and C ch. At the last point of the *in vitro* digestion, 180 min, the pH was 7.3(±0.2), 6.7(±0.2), 6.8(±0.3), and 6.2(±0.2) for MGS, Alg ch, Sw ch, and C ch respectively with no significant difference between MGS and Alg ch and Sw ch. There was significant difference in the pH between the MGS and C ch. At the small intestinal phase, there was a significant difference of the pH levels between MGS and Control cheese.

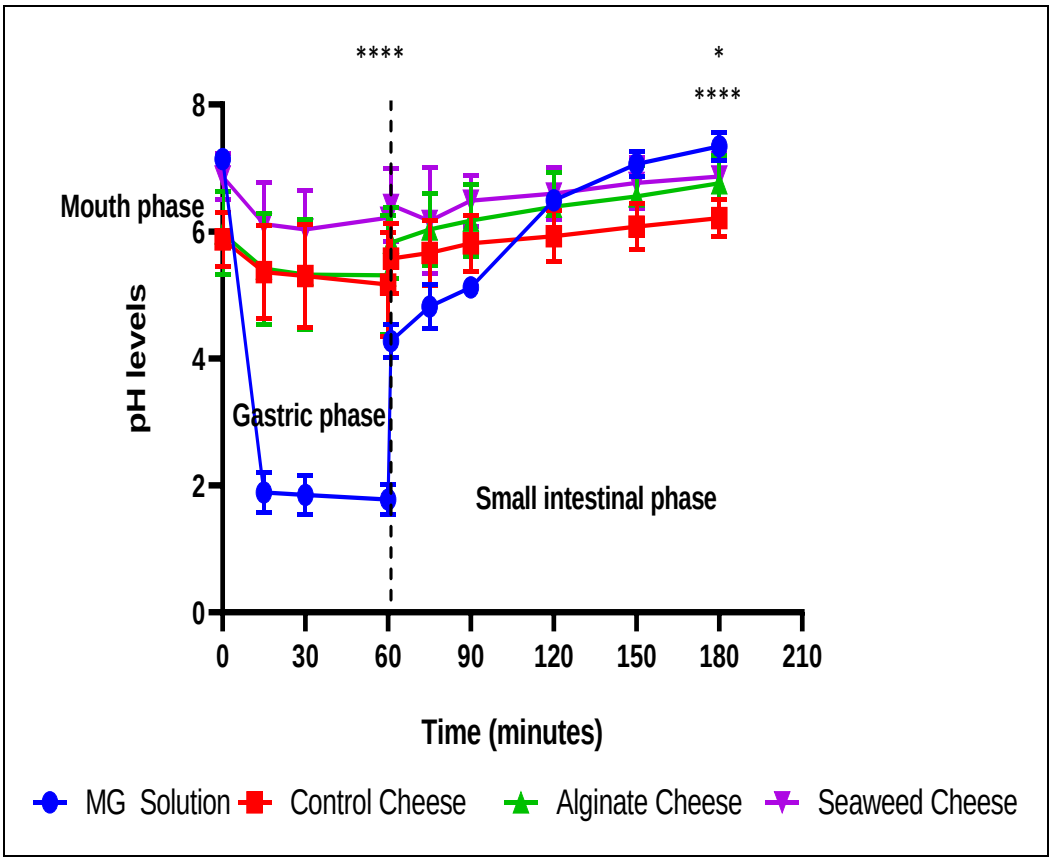


Figure 4.15 pH measurements for cheese digestion. The pH of the model gut solution, control, alginate, and seaweed cheeses of the model gut system at 0, 60 and 180 min (n= 4). The figure compares between the pH levels of mouth phase (0 min), gastric phase (60 min) and the small intestinal phase (180 min) for MG, Control, Alginate and Seaweed cheese. There was a significant difference between the pH levels of MG and Alginate cheese, between MG and Control cheese, and between MG and Seaweed cheese during the gastric phase ($P < 0.00$) and the small intestinal phase ($P < 0.00$). There was a significant difference between MG and Control cheese ($P < 0.00$) at the end of digestion (180 min).

The pH measurements for sausage digestion are shown in **Figure 4.16**. During the salivary phase at 0 min, the pH was 7(±0.0), 5.9 (±0.09), and 6.4(±0.0) for MGS, Alg PS and C PS respectively and significantly different between all the three samples ($P < 0.00$). Following that, during the gastric phase at 15 min, there was a massive reduction in the pH of MGS 1.5 (±0.0), and a slight decrease of the pH for Alg PS which was 5 (±0.0) and C PS which was 4.2(±0.0) Suggesting the sausages were buffering the gastric juice. A significant difference in the pH values was observed during all the stomach phase of digestion until 60 min ($P < 0.00$) for all samples. At the beginning of the pancreatic phase, the pH began to increase to 2.8(±0.0), 5.6(±0.0), and 5.1(±0.0) for MGS, Alg PS and C PS respectively and showed a significant difference at 61 min until 120 min of digestion among all of MGS, Alg PS and C PS. There was a significant difference between the pH levels from the gastric phase compared to small intestinal phase. There was significant difference among all the sample at 150 min ($P < 0.00$), expect between Alg PS and C PS at 150 min. At last time point of digestion (180 min), there was an increase in pH to 7.3(±0.0), 6.4(±0.0), and 6.2(±0.1) for MGS, Alg PS and C PS respectively and this increase was significant between MGS and Alg PS ($P < 0.00$) and between MGS and C PS ($P < 0.00$). However, this increase did not reach the level of significance ($P > 0.05$) between Alg PS and C PS at 180 min.

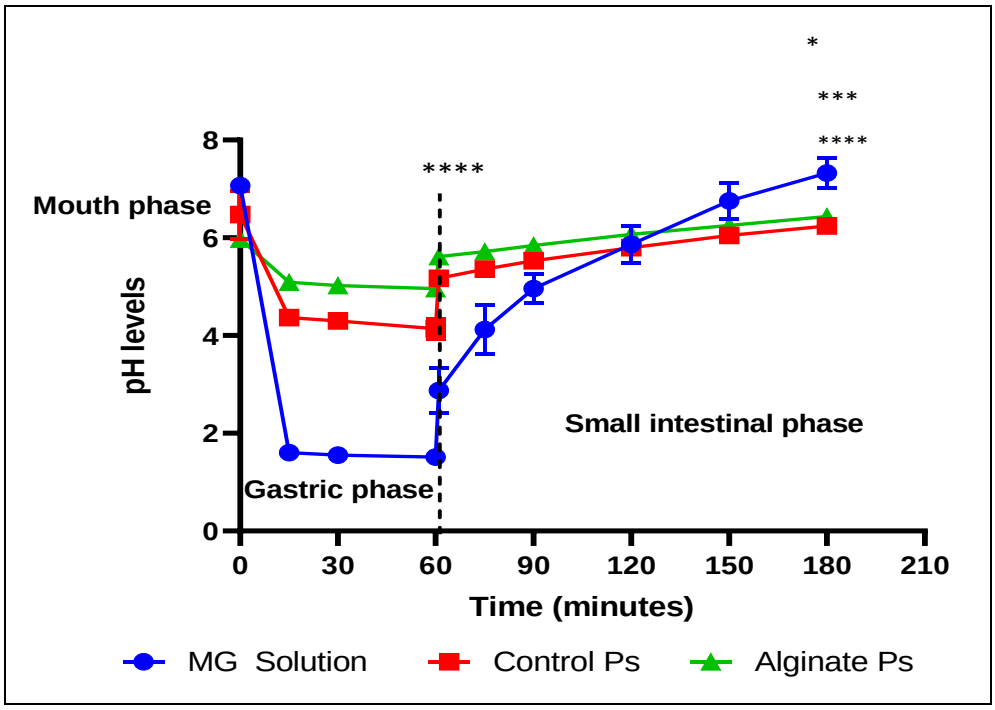


Figure 4.16 pH measurements for pork sausage digestion. The pH of the model gut solution, control, and alginate sausages of the model gut system at 0, 60 and 180 min (n= 5). The figure compares between the pH levels of mouth phase (0 min), gastric phase (60 min) and the small intestinal phase (180 min) for MG, Control and Alginate pork sausage. There was a significant difference between the pH levels of MG and Alginate pork sausage and between MG and Control pork sausage during the gastric phase ($P < 0.00$) and the small intestinal phase ($P < 0.00$).

4.6.7- Quantification of alginate released from the study's food vehicles

Measurement of alginate that has been released during the *in vitro* digestion process of the model gut system was carried out using the modified PAS assay and the levels obtained from the alginate standard curve. The study foods which include bread, cheeses and pork sausage were completely digested. Therefore, the digested samples were taken out at different time intervals starting from the mouth phase at T_0 min, and then T_{15}, T_{30}, T_{60} min for the gastric phase, and then for the Small intestine phase at T_{61}, T_{75}, T_{90}, T_{120}, T_{150}, T_{180} minutes of digestion.

4.6.7.1 Alginate release from bread

Regarding alginate bread, the mucin standard was used as a positive control and was used to detect the alginate released from the digested bread, as presented in **Figure** 4.17. 4% of CC01 was used to produce the AB. As a result, each 100g of bread contained 4 g of alginate, and 5.2g of bread was used as a mimic of bread masticate in the human mouth. Based on this calculation, **Equation 4.1**: (5.2x4)/100 = 0.208g x 1000 = 208 mg at 180 minutes (the final point of model gut digestion). According to that, 5.2g of AB will produce 0.208g (208mg) of alginate at the final point of the bread digestion. **Figure** 4.17 shows a significant effect of time at 0.5. 60 and 180min on the release of alginate within MG digestion ($P < .05$).

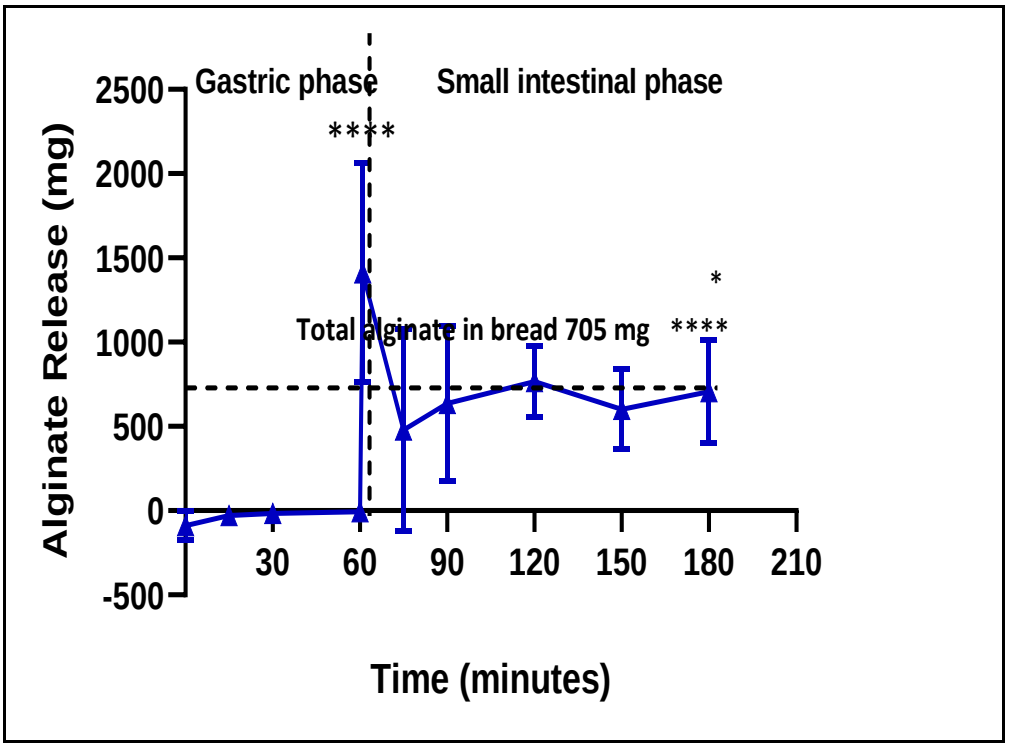


Figure 4.17 Alginate released in the stomach and small intestinal phase of MG digestion for alginate bread. Values are mean (± SE) (n =4), P values <0.05 are represented by * and P values <0.0001 are represented by **** and indicate significant difference in alginate production between 0, 61 and 180 min. There was a significant release of alginate between 0 min and 61 min ($P < 0.05$), and between 0 min and 180 min ($P < 0.05$) and between 61 min and 180 min ($P < 0.05$). The horizontal dotted line at 705 mg shows total amount of CC01 alginate present within the bread.

4.6.7.2 Alginate release from cheese

In regard to alginate cheese, the same procedure has been used to determine the amount of alginate release based on the GHB alginate standard curve. 4% of GHB alginate was used to produce the Alginate cheese. As a result, each 100g of cheese contained 4 g alginate and 20g Alginate cheese was digested and used as mimic of cheese masticate in the human mouth. This should release 800mg of alginate at the end of digestion. Later, 30g of cheese was digested instead of 20g in order to replicate the daily amount of cheese consumed by the participants in the earlier study (chapter 2, cheese acceptability study). For 30g of cheese, there should be a total of 1200mg of alginate released at the end of the simulated digestion. Based on this calculation, **Equation 4.2**:

(30x4)/100 = 1.2g x 1000 = 1200mg at 180 minutes (the final point of model gut digestion). The main release of alginate occurred in the small intestinal phase of digestions (Figure 4.18). There was a significant difference in the release rate of alginate from cheese between 0 and 61 min ($P < 0.05$), and between 0 and 180 min ($P < 0.05$), and between 61 and 180 min ($P < 0.05$). The expected release rate of alginate was calculated according to the equation 4.2. The 75mg of alginate indicated by the Y axis from alginate release rate. However only a small percentage of the alginate appears to have been released.

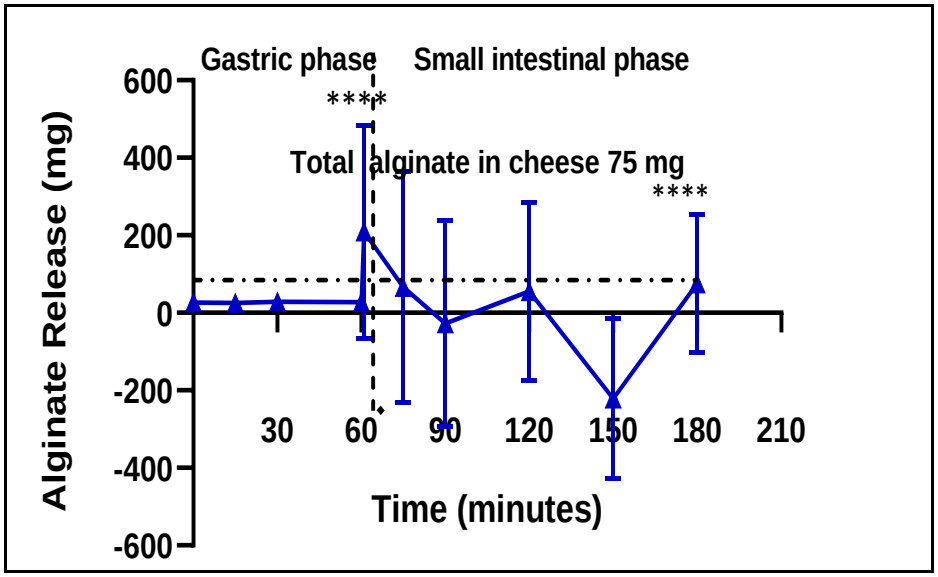


Figure 4.18 Alginate released in the stomach and small intestinal phase of MG digestion for alginate cheese. Values are mean (± SE) (n =4), P values <0.0001 are represented by **** and indicate significant difference in alginate production between 0, 61 and 180 min. There was a significant release of alginate 0 min and 61 min, and between 0 min and 180 min and between 61 min and 180 min. The horizontal dotted line at 75 mg shows total amount of GHB alginate present within the cheese.

4.6.7.3 Alginate release from pork sausage

The same procedure has been used to determine if any alginate had been released from the simulated digestion of alginate-containing pork sausage. The CC01 alginate standard curve was used to measure the release of alginate, however, different alginate could have different PAS positivity. 4% of alginate was incorporated in the sausage which results in 4g of alginate for each 100g of the sausage dough. One alginate sausage is 48.5g and this is the amount of sausage added to the model gut. Based on this calculation, **Equation 4.3**:

(48.5x4)/100 = 1.94g x 1000 = 1940mg this should represent the maximum alginate release at 180 minutes (the final point of model gut digestion). The majority of alginate is released from the sausage in the small intestinal phase of the simulate digestions, Figure 4.19. There was a significant difference in alginate release over the MG digestion ($P < .05$). There was a significant difference

in the release rate of alginate between 0 and 61 min ($P < 0.05$), and between 0 and 180 min ($P < 0.05$), and between 61 and 180 min ($P < 0.05$).

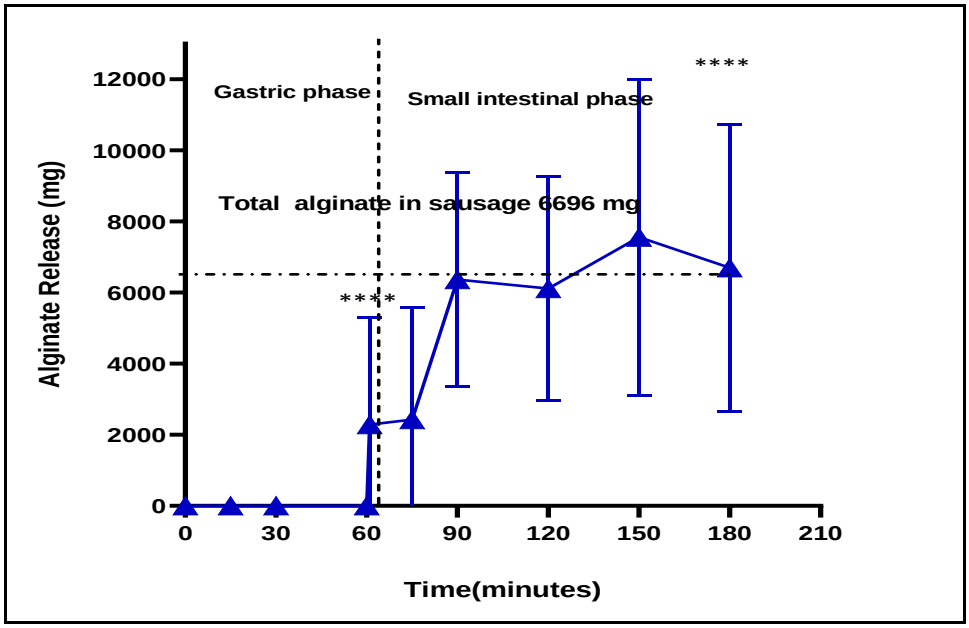


Figure 4.19 Alginate released in the stomach and small intestinal phase of MG digestion for alginate pork sausage. Values are mean (± SE) (n =5), P values <0.0001 are represented by **** and indicate significant difference in alginate production between 0, 61 and 180 min. There was a significant release of alginate at 0 min and 61 min, and between 0 min and 180 min and between 61 min and 180 min and the P values are $P = 0.0001$. The horizontal dotted line at 6696 mg shows total amount of alginate present within the pork sausage.

4.7 Discussion

The *in vitro* digestibility models continues to play an increasingly important role in the investigation of the gastrointestinal aspects of food and pharmacological components; this system is effective with respect to the imitation of *in vivo* digestion in humans, with cost effectiveness as well as by providing a quick result (Minekus *et al.*, 2014). In the present chapter, the digestive process took place within the novel model gut system to simulate the condition of macronutrient digestion for a variety of food products including alginate fibre. This *in vitro* digestion, investigated via an experimental model, represents the mouth, stomach and duodenum tract. This synthetic MG effectively mimics the *in vivo* response that takes place in the stomach and the small intestine.

4.7.1 Fat digestion in the simulated model gut (MG)

An essential part of this PhD project was aimed at investigating the effect of whole brown seaweed incorporated within seaweed cheese and the seaweed extract (alginate) incorporated in alginate cheese and alginate pork sausage; alongside their controls within the artificial model gut system, this allows the determination of their potential to inhibit the free glycerol release from *in vitro* fat digestion and to quantify how much is released through the *in vitro* digestion. This aim is not only for fat digestion, but also to further support the development of these functional food products and maintain the algal food quality. Based on the data presented here, the fat contained in these foods with whole brown seaweed and alginate was digested at varying rates; this may be related to fat chain length and the level of saturation, evidenced by the significant release of free glycerol at the last point of the *in vitro* digestion. In addition, the ability of seaweed to attenuate the dietary fat digestibility from the cheese has also been demonstrated. As with all cheese types, the alginate and control sausages were digested in a similar manner to human digestion via the synthetic model gut *in vitro* including the oral, gastric and small intestinal phase of digestion. *In vivo,* all these food products were acceptable in terms of a lack of unpleasant gastrointestinal side effects, flavour and without a compliance issue (all data presented in chapter two). Thus, it was apparent from the cheese and sausage acceptability studies that it could be advantageous to produce these functional food products with natural seaweed extracts with the aim of controlling obesity.

Previous research has suggested various means of modulating the digestibility of dietary lipid such as delaying dietary fat digestion until the latest parts of the small intestine (Corstens *et al.*, 2018). As such this approach can attenuate the digestion of food consumed, control appetite and enhance the feeling of satiety (van Avesaat *et al.*, 2015). Moreover, another approach is that brown seaweed extracts such as alginate can significantly inhibit pancreatic lipase activity *in vitro* by as much as 72% and therefore has the potential to influence lipolysis activity resulting in the reduced absorption of dietary lipids (Wilcox *et al.*, 2014). In addition, as previously examined and evidenced from the data presented in chapter three, alginate delivered from a pork sausage meal reduced lipid digestion in healthy humans and was shown to lower postprandial circulating serum triglycerides levels following ingestion when compared to the control meal. Therefore, it is for these reasons, the addition of functional hydrocolloids such as alginate could be a useful food-based approach that might potentially control food lipolysis. Further to this, this study is also important in increasing our understanding of how these foods behave in simulated digestion when hydrocolloids such as alginate, are added. It is apparent that foods are complex compounds with a combination of nutritional characteristics which are susceptible to change when these compounds are mixed. As a result, it is important to understand the interaction between these food elements in order to predict how they may behave during digestion (Ramírez *et al.*, 2015). In this research, 4% of whole brown seaweed and 4% of sodium alginate were added to Cheddar cheese and pork sausage in order to detect significant effects on the attenuation of fat digestion which has not been reported previously.

Measuring the total free glycerol liberated from the triglyceride molecules is an indication of the occurrence of fat digestion. Based on the result obtained from the *in vitro* digestion of cheese and pork sausage, the fat digestion was considered in relation to the release of glycerol at T_0, T_{15}, T_{30}, T_{60}, T_{61}, T_{75}, T_{90}, T_{120}, T_{150}, and T_{180} minutes of digestion within the synthetic model gut system. In regards to human fat digestion, within the small-intestinal lumen, three main factors are believed to play an essential role in lipid digestibility: These are the presence of pancreatic lipase, colipase and bile acids which have been reported to control dietary triglyceride digestion (Brownlee *et al.*, 2010). The digestion of fat takes place in the small intestinal gut, however, a significant amount of intragastric lipolysis occurs in the stomach. About 10-30% of dietary triacylglycerols undergo lipolysis to free fatty acids and di and mono acylglycerols before the duodenal phase (Hamosh and Scow, 1973; Liao *et al.*, 1983; Hamosh, 1990); at this stage, they assist in fat emulsification and

improve dietary TAGs solubilisation (Hamosh *et al.*, 1975). This enables both pancreatic lipase and colipase to interact with the lipid substrates (Borgström, 1980; Mu and Høy, 2004). Gastric lipase has an optimal activity level at pH of ~5.4 which contributes to intragastric digestibility (Carriere *et al.*, 1993a). In this data, both of alginate and control cheese fitted the optimal level of pH of lipase activity as it has the activity level at pH of ~ 5.4, however, seaweed cheese had no lipase activity due to increased pH value. The optimal lipolysis activity of gastric lipase in the pH range of 4.5 and 5.5 (DeNigris et al., 1985).

In this data, the total glycerol released after the artificial digestion of the MG was an indication of degree of lipolysis in these foods. The main results showed that a considerable amount of the glycerol was released during the small intestinal phase from alginate, seaweed and control cheese as presented in **Figure** 4.9 as well as from alginate and control sausage as shown in **Figure** 4.10.

The release of glycerol was higher during this stage than during the gastric phase of *in vitro* digestion for these food products. The pH measurements for both alginate and control cheese were reported as 5.5, 5.4 and 5.0 at 15 min, 30 min and 60 min respectively and slightly higher for seaweed cheese at 6.3, 6.1 and 6.2 at 15 min, 30 min and 60 min respectively, as seen from **Figure** 4.15 and 4.16. This pH level could therefore be considered sufficient for gastric lipolysis activity during the gastric phase of digestion for cheeses and pork sausage digestion.

4.7.1.1 Fat digestion with cheese

In regard to cheese digestion, the total amount of glycerol release was significantly higher in the small intestinal phase compared with the gastric phase. The glycerol contents in seaweed cheese digesta was the lowest, whereas both of alginate and control cheeses showed no significant difference in the amount of glycerol released. These data presented here provides evidence that within the small intestinal phase at 120 min, seaweed had significantly reduced glycerol release compared to the alginate ($P < 0.05$). At 180 min, the average glycerol released from seaweed cheese was also significantly lower than glycerol release from alginate cheese ($P < 0.05$) as well as from the control cheese ($P < 0.05$). However, no further significant differences were observed

during the gastric and intestinal periods of the simulated digestion of the three cheeses. Inhibition of fat hydrolysis during the *in vitro* digestion was influenced more by seaweed and then alginate incorporated food. This finding suggests that the ingestion of seaweed incorporated foods could effectively reduce lipids hydrolysis and thus be beneficial in terms of body weight reduction. It is possible that the inhibition of glycerol liberation from seaweed cheese compared to the alginate cheese may be due to other seaweed compounds. The bioactive compounds present in brown seaweed include polyphenols, fucoidans, laminarin and alginate, any of which could act alone or in combination as strong inhibitors of pancreatic lipase activity with potential regulation of lipid digestibility (Chater *et al.*, 2016). A recent study reported that a mixture of phenolic compounds such as phlorotannins, and a water-soluble polysaccharide such as alginate, from *Ascophyllum* markedly enhanced the inhibition of lipase activity *in vitro* (Austin *et al.*, 2018). However, from this data, alginate has not showed a potent effect on the digestion of fat which can be explained to some extent by the gelling effect. When alginate was added to the cheese, it formed a gel ball of varying size and mixed with the cheese curd. During the artificial digestion of the alginate cheese in the MG, although all cheese was fully broken down, there was undigested material remaining at the end point of the MG digestion (180 min) which looked like yellow sponge or yellow plastic leaves (as can be seen in Figure 4.7 A); this was not found with seaweed or either type of the control cheese. This remaining material could be insoluble alginate which would explain the lack of lipase inhibition as to inhibit the alginate would need to be in solution. There are several potential explanations for the varied findings of fat digestion effects. First, it is important to mention that alginate mixture was added to the cheese cured and mixed manually. Although it was mixed carefully to ensure homogeneity, it cannot be guaranteed that each 30g of digested alginate cheese had an equal amount of alginate. As a result, it could be that each individual run of alginate cheese in the MG may have contained a different amount of alginate. This could explain why there is no significant difference in regard to glycerol release between alginate and control cheese whereas seaweed cheese showed a significant difference. Increase the runs of repeats could help in estimates the expected amount of alginate in the food matrix. The important point is that alginate should be hydrated and then added to cheese this could lower the alginate's ability to bind with Ca2+ ions producing a strong gel. In my experiments, there was not enough alginate in MG solution so no effect on fat digestion.

An additional point to consider is that several soluble dietary fibres with gel formation action such as alginate, have been shown to bind with bile acids and thus could account for any interference with their uptake in the ileum or result in a reduction in lipase activity due to lack of fat emulsification. Interference with dietary lipid transportation and absorption in the small intestine (Furda, 1990; Yoshie *et al.*, 1995). In this data, alginate incorporated cheese did show a slightly lower amount of glycerol released in the small intestinal phase of digestion, however, not a significant level compared to the control cheese. Although it was assumed that the addition of alginate as a pancreatic lipase inhibitor could reduce the lipolysis activity, this has been shown to be less effective in the cheese matrix which could be due to the presence of Ca^{2+} ions producing a strong alginate gel. Also, in dairy products such as cheddar cheese, the lipolysis of cheese relies heavily on the activity of microbial lipases. Moreover, several factors have an influence on the rate of lipid digestion. The presence of calcium has been found to increase the level of lipid hydrolysis (Ayala-Bribiesca *et al.*, 2016). Concerning alginate cheese, a recent study demonstrated the association between varying levels of calcium enriched cheddar cheese in terms of texture properties during the ripening time and their influence on *in vitro* digestibility. It was found that increased calcium content resulted in a more rapid lipolysis process for enriched calcium cheese compared to the control cheese; this was as a result of interactions between free fatty acids and calcium which produced a calcium soap in the presence of neutral pH. This "soap" precipitated under intestinal pH, thus liberating lipids from the water, thereby, facilitating the access of lipase to its substrate (Devraj *et al.*, 2013; Ayala-Bribiesca *et al.*, 2016). Thus, Ca^{2+} has the potential to reduce the ability of alginate as a pancreatic lipase inhibitor. A previous study reported that in the presence of Ca^{2+}, pancreatic lipase was able to bind more easily to the substrate interface, stimulate the lipolysis of short, medium and long chain fatty acids and facilitate the mixture of micelle-lipase compounds (Alvarez and Stella, 1989). It is evident that Ca^{2+} can contribute to the level of lipolysis activity. The gallbladder bile is abundant in Ca^{2+} which also stimulates lipase activity (Palmer, 1973). As the gastrointestinal tract contains a lower concentration of Ca^{2+} than is required for the lipase activity, it is reliant on the calcium within the diet (Mansbach *et al.*, 1975). In this regard, the cheese matrix is rich in Ca^{2+} and this could explain the lower ability of alginate to inhibit fat lipolysis during *in vitro* digestion. In addition, it is worth mentioning that TAGs are the main component of milk fat accounting for 97 – 98% of overall lipid content (Vieitez *et al.*, 2016). Owing to the data presented for glycerol digestion in all cheese include alginate, seaweed and

control, the increased rate of fat digestion in the gastric phase could be explained by an early study which reported that milk lipids were widely hydrolysed to diglycerides and fatty acids in the stomachs of suckling rats (Helander and Olivecrona, 1970), however, this MG replicates adult human physiology, which may have less lipolitic activity than infant. Since the cheese fat originally comes from milk fat, involved partly in intragastric lipolysis, this might explain the increase in glycerol released during the gastric phase of the cheese digestion. Moreover, an earlier study investigated the role of lingual lipase within dietary fat digestion and reported that lingual lipase acts as a primer stimulation of dietary triglyceride lipolysis in the stomach (Hamosh and Scow, 1973).

4.7.1.2 Fat digestion with pork sausage

The same digestive procedure in the simulated model gut which included the salivary, gastric and small intestinal phase was also used for alginate and control sausage. Measuring the free glycerol released from TAGs molecules was a sign of complete fat digestion. The highest percentages of released glycerol were found in the intestinal phase. In relation to alginate sausage, there was early lipolysis activity during the salivary phase and then under the gastric conditions of digestion but with no significant release of glycerol. At the intestinal phase, further glycerol release took place and increased gradually to reach the highest amount released during the second hour of duodenal digestion. After this time point, less glycerol production was seen towards the end point of the digestive process. The control sausage had the same trends toward fat digestion, however, there was an increase in the release of glycerol until the end of digestion 180 min without reaching significant difference ($P > 0.05$). The alginate from alginate incorporated sausage had no significant effects on the fat digestion of sausage during the three phases of MG digestion. It was noted that the amount of glycerol released from alginate sausage is lower than control sausage at the last point of digestion (180min) with total released glycerol around 150mg and 180mg for alginate and control sausage respectively. This suggests that alginate reduced the fat digestion compared to the control sausage but with no significant difference. These data agree with the results obtained from the alginate cheese, as both alginate cheese and alginate sausage showed a

slight reduction at the last point of the intestinal digestion but this was not significant and with Alginate cheese some undigested material remained as shown in Figure 4.7A.

Considering all these aspects, the fat content reflected the content of TAGs within the ingredients used to produce these food products. These food vehicles of cheese or the pork sausage are rich in TAG; 100g of cheese includes 29.8g fat and 100g of pork sausage consisting of 14.4g of fat. This is a higher amount of dietary fat compared to the alginate bread which consisted of 13.3g of fat for 100g of bread. Based on this knowledge, 4% alginate (i.e. 4g of alginate added to 100g of cheese or sausage) appears to be too low to allow detection of any significant inhibitory effects alginate may have on fat digestion in these foods. However solubility of the alginate could be very important A further interpretation could be that during the gastric phase of digestion for both cheese and pork sausage, the pH measurement was less acidic and close to a neutral pH with values of around 5.8 and 5.3 for alginate cheese and sausage respectively (Figure 4. 15 and 4.16) with cheeses and sausages potentially buffering the gastric juice. The gastric phase for alginate bread had a pH of around 1.8 which is significantly more acidic (Figure 4.14) than compared with the cheese and sausage. A previous report demonstrated that gastric lipase is an active intragastric enzyme (Abrams *et al.*, 1988). It has been noted to have optimal lipolysis activity in the pH range of 4.5 and 5.5 (DeNigris *et al.*, 1985). The answer fit with the previous comment which indicate the lipase activity for alginate and control cheese with no activity for the seaweed cheese.

4.7.2 Quantifying alginate and determining its release (PAS assay)

The importance of this modified method is its ability to measure the alginate that is released from the food vehicle in digestive solutions with different pH values and containing components from the upper intestinal tract such as porcine bile and the pancreatic juice. The presence of bile and pancreatic juice change the digestive solution from a transparent colour to a strong brown-green colour in the small intestinal phase of digestion. As it is very clear that dark coloured solutions affect the OD of the colourmetric measurement techniques (Houghton *et al.*, 2014), it is important to have a way of measuring which is not affected by this. In addition, the results presented in this chapter indicate that the PAS assay technique is a simple method which can be used to detect the alginate released even a tiny amount from the different food vehicles that have been used. For example, CC01 alginate standard curve in gastric phase detect the lowest value as 0.006 mg/ml

and 0.002 mg/ml in MGS whereas, GHB alginate curve in gastric phase detect the lowest value as 0.24 mg/ml and 0.05 mg/ml in MGS, as presented in Figure 4.13 and Figure 4.12 respectively.

The present data has shown that the bread, cheese and sausage was digested *in vitro* via the MG, however there was some undigested material for alginate cheese as presented in **Figure** 4.7. This model which simulates human digestion has been important to study the gastro-intestinal behaviour of food and to investigate the matrix release of micronutrients. It is clear that the simulated digestion method mimics *in vivo* digestion of humans and takes into consideration the role of digestive enzymes, their concentrations, pH conditions and the required time of digestion (Minekus *et al.*, 2014). In this research, the model gut has been used to study the release of bioactive alginate from these food products. It has demonstrated several advantages including speed, lower cost and it allows samples to be taken at several time points throughout digestion. In addition, the quantification of the bioactive alginate released from these food matrixes is considered important in determining the effectiveness of including alginate in food products for the control of lipid digestion and ultimately as a method of reducing obesity.

The data here implies that the PAS assay was an effective method to detect the release of alginate from *in vitro* digestion of the food matrix. If the correct standard curves with the correct alginate can be used alginate was still released from foods subjected to high temperature during production such as the high temperatures needed to bake bread. Additionally, the study foods were well digested by the MG. The bread mainly consisted of carbohydrates which are ingested in the form of starch in the small intestinal tract (Ellis *et al.*, 1981). Also, cheese mainly consists of dairy fat and protein. In a study investigating the effect of the cheese matrix on simulated fat digestion, it was found that lipids and protein were released at a similar rate during the small intestinal phase of *in vitro* digestion of Cheddar cheese (Lamothe *et al.*, 2012). Lastly, pork sausage mainly consists of protein and all these nutrients were completely digested. Following digestion, alginate can be measured as it is considered an indigestible fibre and soluble, therefore it can be quantified.

In regard to the PAS assay, the mucin standard curve in DH_2O demonstrates a perfect relationship between the concentration and absorbance of mucin (glycoprotein); this can be used as a positive control to detect carbohydrate within polysaccharides as presented in **Figure** 4.11. An alginate

standard curve either in gastric and small intestinal solution taken at the end of each phase, showed excellent linearity with an R^2 value of 0.99 across a wide range of absorbance values (**Figures** 4.12, 4.13). This finding is in agreement with the previous quantification study of alginate bread (Houghton *et al.*, 2014). It was apparent from this data that the PAS assay was suitable for screening a small amount of alginate in samples taken during the gastric phase and in the small intestinal phase. In addition, it is a good technique for assaying a considerable number of fractions involving carbohydrate, mucin and glycoprotein (Kilcoyne *et al.*, 2011). The data presented from the PAS assay clearly demonstrates alginate release from the digestive mixture of bread, cheese and sausage took place in the upper digestive tract. However, less release was seen in the gastric phase as presented in **Figures** 4. 17 A & B, 4.18, 4.19 respectively. It is therefore possible that alginate may be directly binding to some upper gastrointestinal compounds such as bile acids and salts, which could result in a considerable restriction on the amount of alginate released. However, solubility of the alginate maybe the biggest factor governing its detection. Moreover, from the bread and sausage digestion, there was a higher estimated alginate production in the small intestinal phase, whereas alginate cheese digestion shows a lower estimated value of alginate. This could be consistent with the fact that 2 hours of *in vitro* digestion of the cheese (which is rich in fat content) in the upper tract may not be enough time to complete the digestion and therefore to release alginate from the digested matrix. As evident from this chapter, the presence of Ca^{2+} can contribute to gel formation which again could affect solubility; it has reported that alginate is able to create a physical hydrogel in the presence of divalent calcium ions (Hori *et al.*, 2009).

4.8 Limitations

These experiments have some limitations. Firstly, determining the release of alginate from the seaweed cheese content via the alginate standard curve was not possible. This is probably due to the brown seaweed content of several hydrocolloids such as alginate, fucoidan and laminarin. The complexity of the hydrocolloid content within the brown seaweed would certainly affect the accurate measurement of the alginate content from the seaweed as multiple compounds would be contained within the cheese. The quantification of saccharides matrix will include the other hydrocolloids alongside the alginate content. This will affect the accuracy of the results as these polysaccharides oxidized by periodate.

A further limitation is that the model gut system used in these *in vitro* investigations is a "closed system". For example, a lipid-based mixture is subjected to enzyme-mediated hydrolysis which results in an increase in the material released during lipid digestion such as fatty acids (FAs) and monoglyceride (MG) in the absence of absorption process (compared to *in vivo*). As these digested products accumulate on the surface of an oil droplet, this leads to the attenuation of the interaction with pancreatic lipase and colipase and thus, could restrict further digestion (Devraj *et al.*, 2013).

Furthermore, it could be that cheese is an unsuitable food matrix for alginate addition. A study reported that enzymatic activity levels change on exposure to differing pH values and calcium concentration (Minekus *et al.*, 2014). This could explain the reduced ability of alginate to inhibit pancreatic lipase during small intestinal digestion.

4.9 Conclusion

Overall, this finding supports the idea that modulating dietary fat digestibility and finding the appropriate amount of seaweed bioactives required to be added to enriched foodstuffs to modulate this could be promising in the development of functional foods enhancing health and well-being. The digestive model that has been used in these experiments (MG) represents the oral, stomach and small intestinal phase of human digestion; it is capable of giving valid, repeatable outcomes as all types of cheese, sausage and bread were digested and provide the possibility for comparison between different samples.

In addition, it was clear that this simulated *in vitro* digestion provides the possibility to measure the rate of fat digestion via the presence of glycerol in the synthetic model gut solutions through all phases of upper GI digestion and allowed all the digested food samples (bread, cheese and pork sausage) to be sampled at several time intervals during the simulated digestion without affecting the final result of the digestion. By this method, it was possible to detect the effect of alginate and seaweed incorporation in foods on the rate of fat digestion. Seaweed cheese showed the lowest glycerol release through the *in vitro* digestion. However, the presence of Ca^{2+} in the cheese matrix could play a major role in lowering the ability of alginate to inhibit pancreatic lipase.

The modified PAS assay quantification method is one of the most commonly used techniques for estimating the level of polysaccharides and glycoprotein in a sample. In this research, the PAS was assay used to estimate the marine algae alginate content as a functional ingredient in various food products. It was regarded to be a suitable approach to quantify the release of small amounts of alginate in the MGS across different pH values. This *in vitro* data shows promising evidence of the effectiveness of seaweed-enriched foodstuffs in reducing fat digestion. However, further human intervention studies would be required to illustrate a reduction in bodyweight, and also to demonstrate the acceptability of seaweed incorporated food products including alginate. Other seaweed bioactive components could also possess potent inhibitory activity on fat digestion, alongside the alginate-rich functional fibre.

Finally, in this research, the data suggests that the inhibition of fat digestion via seaweed bioactive components from food contain a considerable amount of dietary fat could be useful for controlling obesity. This approach seems particularly relevant given that it is providing a functional food with improved nutritional value (Hell et al., 2018). For example, an increase in amino acids and fatty acids (Prabhasankar et al., 2009). In addition, the synthetic digestion via this model gut has provided a means to understand how these bioactive components could modulate the digestion of fat substrates and at the same time, test the efficacy of digestion of a novel food product. As such, this MG provides an effective simulation of the complex mixture of physiological and physicochemical functions that takes place in the gastrointestinal tract of human beings (Hur et al., 2011; Aguirre-Calvo et al., 2020).

Chapter 5: Overall discussion

Recent statistics have indicated that in the United Kingdom, one out of four adults are classified as obese and by 2050, it is expected that 50% of women, 60% of men and 25% of children will be obese (Grant *et al.*, 2007). This is an increasingly common problem and is related to many health issues as reviewed in Chapter One.

In this PhD thesis, the main scope of interest was to use several traditional food products which are traditionally consumed by the UK public. These food products should be reasonably priced and readily available on the market such as bread, cheese, and sausage. These food products have been enriched with a functional marine algae-based component extracted from a species of brown seaweed; thus, part of the focus was on alginate as a dietary fibre functional polysaccharide. Therefore, this research offers a novel approach which seeks to improve the commercial production of the food products as well as provide an avenue for enhancing health and wellbeing. Evidence demonstrates a considerable interest in the utilization of seaweed-based food due to several possible reasons. It was reported that marine algae has been traditionally consumed in Asian cuisine for a long period of time but gradually, the algal foods have gained a more widespread interest within the Western world (Jiménez-Escrig and Sánchez-Muniz, 2000). Moreover, with the increase in veganism, seaweed extracts have gained growing interest by food manufacturers as an alternative to animal-derived food products such as gelatine (Marshall, 2020). Furthermore, edible seaweeds normally consists of a considerable amount of dietary fibre – about 23.5% to 64% of dry weight – and these amounts exceed the fibre content in wheat bran (Benjama and Masniyom, 2012). Therefore, they can be a good source of dietary fibre in a population like the UK which does not consume the recommended level.

As previously reviewed in Chapter One, *Ascophyllum nodosum* brown seaweed is abundant in hydrocolloids including alginate, fucoidan, and laminarin, with a rich amount of proteins, iodine (Jaspars and Folmer, 2013), mannitol (sugar-free sweetener) and vitamin E which contributes to increased satiation and nourishment (Ngo *et al.*, 2011). In addition, alginate is a dietary fibre (Brownlee *et al.*, 2005); several types of carbohydrates with a low digestibility such as fibres, provide potential health benefits, for example, they tend to have a lower dietary caloric content

and can attenuate plasma glucose levels. This is mainly due to their inability to be digested by human digestive enzymes and the resulting reduced absorption. However, a considerable intake could be related to gastrointestinal side effects and may influence the well-being and the acceptability of foods incorporating these fibres (Grabitske and Slavin, 2009). Therefore, *Ascophyllum nodosum* brown seaweed, as a fibre-rich seaweed with alginate and alginate as a functional polysaccharide were used in this research.

5.1 Acceptability of cheese and pork sausage

This part of the thesis aimed to fill a gap in the knowledge of the behaviour of algal food components in the human gut. In addition, it aimed to provide evidence for the manufacture of algal-derived food products with the intention of improving health and well-being. Furthermore, an increase in the daily intake of dietary fibre would be even more beneficial in tandem with a potential and safe solution to treat obesity. Previous data from this lab has shown up to 72.2% *in vitro* reduction in pancreatic lipase activity for a specific alginate (Mannuronan (treated with epimerase), with F_G 0.8) (Wilcox *et al.*, 2014). This finding demonstrates how alginate could help in decreasing the intestinal uptake of dietary lipids. Also, an alginate-based product study has shown the ability of alginate to increase satiety, reduce further food intake and decrease hunger (Georg Jensen *et al.*, 2012). This scientific principle has been investigated in popular stable food products incorporated with seaweed bioactive ingredients as either whole seaweed or extracted compounds such as alginate. It has been presented in Chapter Two that *Ascophyllum nodosum* brown seaweed and alginate are effective and safe agents for incorporation in a novel cheese and pork sausage product for human subjects. During the entire duration of the study for both cheese and sausage, the brown seaweed and alginate were not associated with serious adverse GI effects. Both products were considered acceptable and several participants expressed interest in including these food products containing seaweed bioactive compounds in their habitual diet. The findings from this study are in agreement with a previous study investigating the addition of 4% alginate on the acceptability of alginate-enriched bread (Houghton *et al.*, 2019). Importantly, the study outcomes have shown that there was a very low dropout rate during the seaweed cheese week compared to the alginate and control cheese, which indicates tolerability and acceptability of the usage of marine bioactive ingredients such as brown seaweeds and their extracts (alginates) in food products. Furthermore, data from the acceptability studies of cheese and pork sausage have indicated that the ingestion of whole brown seaweed and seaweed extracts in the form of alginate

within these foods had no implications on the organoleptic acceptability and had no unpleasant effects associated with the GI or general wellbeing. However, a few participants reported the unpleasant taste of seaweed cheese and others for alginate cheese. This emphasises the importance of determining an acceptable quantity of whole seaweed or seaweed extracts which can be consumed as part of an individual's normal diet. An interesting point is, despite the aforementioned complaint, none of the participants returned their seaweed, alginate cheese or alginate sausage or dropped out of the study due to the taste. This present finding contrasts with the finding from another study using alginate-based beverages, as participants preferred the control products (Georg Jensen *et al.*, 2012). Furthermore, in a cereal bar enriched with alginate, the participants reported the poor taste of alginate and guar gum breakfast bar compared with the control bar (Mattes, 2007). This is likely due to the ability of alginate to form a gel in the mouth and this viscous texture results in a lower level of organoleptic acceptability of these foods (Wolf *et al.*, 2002). A participant's decision to complete or not complete the study relies on their choice of food and the flavour of the product; this could explain the variability in the acceptance of the study food provided (seaweed cheese, alginate cheese or alginate sausage). For example, one male participant stated that he prefers not to eat cheese daily during the summer (i.e. exam period), but he completed the study and did not complain about the taste of the cheese. This finding supports the assumption that these foods (cheese and sausage) are a reasonable method for the delivery of seaweed bioactive components including alginate into an ordinary diet. Alongside that, it is an effective means to improve the nutritional value of an individual's diet, particularly via the increase in daily fibre intake among the participants which is important to enhance wellbeing.

5.2 Acute effects of alginate-enriched sausage on fat and carbohydrate digestion

Alginate has recently been investigated in human trials as a potential treatment for nutritional disorders such as obesity (Houghton *et al.*, 2019). Pork sausage was used to demonstrate that alginate can be included in a food and not affect its acceptability. Identifying an effective food matrix/food vehicle to deliver alginate which could be used to reduce plasma triglyceride and plasma glucose concentration is an important requirement. Therefore, it was important to consider the process of digestion and metabolism of the pork sausage in the human system.

As discussed in chapter 3, several viscous fibres and marine polysaccharides have shown a beneficial effect on attenuating lipid hydrolysis in the small intestine in humans and improving physiological processes including effects on satiation, modulation of nutrient digestion and their effects on metabolic responses.

Therefore, many researchers have been investigating the physiological and bioactive effects of seaweed incorporated food products on several health-related signs such as levels of serum lipids and blood glucose levels in a male and female population with an increased incidence of T2DM and a high BMI (Brownlee *et al.*, 2009). It was stated that alginate as a dietary fibre was able to decrease the rate of intestinal nutrient uptake and enhance satiety. This could controlling T2DM and managing obesity. As reviewed in Brownlee et al, 2005 on the effect of alginate on intestinal uptake, the inclusion of alginate in diets may has a potential health benefits in reducing some conditions such as obesity and Type II diabetes (Brownlee *et al.*, 2005).

Further data from *in vitro* experiments in this thesis demonstrate that seaweed/alginate-incorporated products could have the potential to inhibit the ingestion of dietary lipids and therefore, might be a useful agent for controlling body weight and reducing the incidence of obesity. However, a considerable number of dietary and clinical research projects to investigate long term effects are still needed. A dietary intervention able to lower dietary energy intake and reduce the intestinal uptake of dietary lipids continues to be an effective option in weight reduction (Grube *et al.*, 2013). Data from the acute feeding study in Chapter three has shown a reduction in the serum triglyceride concentration and plasma glucose levels, suggesting a reduced nutrient uptake following the ingestion of an alginate pork sausage meal compared to the control meal. The result has shown the supplementation of brown seaweed extracts such as alginate into sausage given to healthy volunteers consuming a high fat meal has beneficial effects. This advantage may be accounted for by the role of alginate in pancreatic lipase inhibition. Furthermore, considering that sausage is a low fibre food, consuming this product will help in increasing the daily fibre intake. In this short study, the daily intake of alginate sausage increased the intake of fibre. This result could generate some advantages in terms of improving the nutritional values especially for people who prefer to eat the sausage daily. The further development of such food products is worthwhile as this results in food products with improved nutritional value. To this end, seaweed bioactive substances including polysaccharides, pigments, fatty acids, polyphenols and peptides

have been explored for their potential inclusion in functional food products. Despite this, extensive human investigation and nutritional intervention studies are still required.

In this acute feeding study, although the result reported that alginate was able to demonstrate *in vivo* inhibition of dietary lipids and has no effect on carbohydrate digestion in healthy participants, a longitudinal research study with a larger group of healthy participants is required to confirm any possible health benefits such as reducing obesity.

5.3 Application of seaweed and alginate foods in *in vitro* digestion on fat digestion and alginate release

Generally, the *in vitro* studies in this thesis aimed to add further knowledge about the behavior of seaweed and alginate foods *in vitro* via simulated digestion. The simulated model gut system, developed in Newcastle University, is a valuable resource which is time and effort saving, free of ethical requirements and less costly compared with animal and human experiments. All these experiments were carried out using this simulated gastrointestinal tract model (MG). This research focused on modulating the lipid digestion of cheese and pork sausage via the role of seaweed bioactives. Chapter four illustrates evidence of the possibility of modulating dietary fat digestion via bioactive seaweed and alginate in cheese and sausage products. The result showed that the addition of Manugel GHB alginate in a cheese or sausage matrix in the MG digestion resulted in a slight reduction in the glycerol recovered from the small intestinal phase at the final time point of digestion (180min). Compared to the control cheese and sausage, this was not significant, however, an effect was observed for seaweed cheese. The seaweed cheese showed a significant reduction in glycerol release in the small intestinal phase of digestion. This finding is in agreement with a recent study conducted on marine-derived compounds (polyphenols and polysaccharides) from *Ascophyllum nodosum* seaweed; this study showed the potent inhibition of pancreatic lipase activity *in vitro* by these compounds (Austin *et al.*, 2018).

Regarding the modified PAS assay, it is essential to measure the alginate release from these digested foods in order to know the maximum amount released as this is important in determining the suitability of alginate in foods aimed at reducing fat digestion. The *in-vitro* work from this thesis has demonstrated that this assay can be used to quantify the amount of alginate in a sample of both DH_2O and the model gut solutions including a range of concentrations from 0.1 to 2mg/ml. This method removes any possible interference which could affect the OD and shows good linearity (R^2= 0.99). Alginate was released within the small intestinal phase of digestion and was

determined for the bread, cheese and pork sausage vehicles. It was not possible to quantify the seaweed release from the digested seaweed cheese because seaweed contains non- carbohydrate components and other carbohydrates that are not periodic acid oxidisable. Seaweed consists of several phycolloids such as agars, alginate and carrageenans (Organization, 2014; Wan-Loy and Siew-Moi, 2016). Therefore, it would be difficult to isolate alginate from the other polysaccharides.

5.4 Areas of future research

The development of functional foods via the addition of several edible seaweeds, for instance brown algal and their bioactive materials such as alginate, could certainly be a popular and common option in the future. It could enhance the nutritional value of food, thus, having a beneficial impact on overall health and wellbeing. This thesis supports the use of marine bioactive substances in this application.

In regard to the nutritional and functional value of the marine seaweeds, another potential benefit comes from the usage of seaweed minerals as a unique alternative to excess salt in foods. By way of illustration, the addition of *Saccharina longicruris* brown seaweed and *Palmaria palmata* red seaweed into Camembert-type cheeses increased the fibre, minerals, carbohydrate, and protein content compared to these cheeses. This provides an increased fibre content and improves the functional value of the food (Hell *et al.*, 2018). This would be particularly beneficial to people who prefer to eat cheese regularly as it would provide them with a healthier choice with important nutrients. Although the addition of GHB alginate as a sodium alginate into cheese has resulted in big gel pieces, it would be beneficial to hydrate this alginate in water first and then add to the cheese. Another suggestion is that, within cheese, the usage of calcium or potassium alginate as both are insoluble could be helpful in producing smaller gel pieces and may be able to become the soluble in stomach. **Figure** 5.1 shows functional Camembert cheese enriched with brown and red seaweed. As both seaweeds are rich with functional polysaccharides, this could be an effective means of delivering important nutrients to individuals.

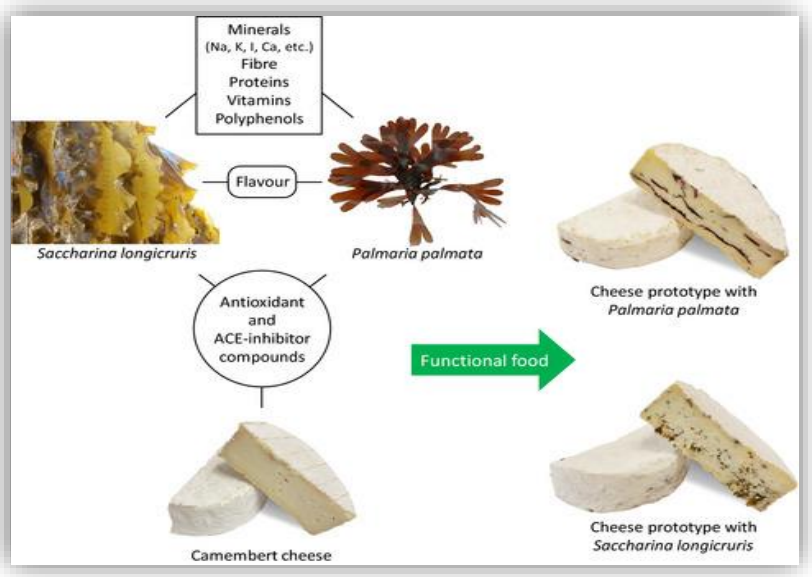


Figure 5.1 the functional Camembert cheese with incorporation of both *Saccharina longicruris* brown seaweed and *Palmaria palmata* red seaweed. These seaweeds are rich in fibre, minerals, carbohydrates, and proteins (Hell *et al.*, 2018). This figure has been adapted from *Hell et al., 2018*.

It would be beneficial to gradually increase the volume of algae and the algal-derived food products incorporated into various foodstuffs. In this research, 4% *Ascophyllum nodosum* in cheese was considered acceptable as well as 4% alginate in cheese and pork sausage. An acceptability study was conducted using wholemeal bread (400g) enriched with 4% whole *Ascophyllum nodosum* and this caused a significant reduction in calories consumed and the seaweed bread was acceptable (Hall *et al.*, 2012).

Thus, for the future, a series of experiments are conducted to address the effects of adding 5%, 7%, 10% and 12% of brown algae and its extract (alginate) into the same products in terms of their sensory properties such as aroma, texture, appearance and importantly taste. Many participants welcomed the idea of incorporation of seaweed and their extracts into their ordinary diet. Several studies have demonstrated that adding algal-derived alginate to bread and other baked goods gives positive results including activity as a satiating agent, salt replacer and microbial preventing agent. However, to date, the impact of incorporation of whole seaweed into bread product has not been comprehensively examined (Gupta and Abu-Ghannam, 2011; Hall *et al.*, 2012). Initial observations investigating the addition of brown seaweed (Wakame) as a powder in pasta dough

with several different concentrations from 0% as a control, 5%, 10%, 20% and 30% of semolina/Wakame (w/w) have shown that 10% wakame had a more favourable sensory analysis score than the 20%. The wakame pasta had improved pasta quality and a significant increase in the amino acid, fatty acid and fibre content (Prabhasankar *et al.*, 2009). There is substantial evidence acknowledging the beneficial usage of whole seaweeds and algal-derived food products that are rich in positive physiological benefits in Western societies. It is important to conduct such studies with a wider population to assist in the design of suitable seaweed and alginate foods that could help in the management of overweightness and obesity as well as controlling T2DM.

5.5 Conclusion

In summary, the overarching aim of this thesis was to elucidate the effects of a combination of *Ascophyllum nodosum* brown seaweed and its extract, alginate, in food vehicles on overall acceptability of the product, as well as the effects on plasma lipids and glucose in healthy subjects. Additionally, the *in vitro* behaviour of seaweed and alginate incorporated foods was investigated.

Data from this research showed several attractive features of seaweed and alginate cheese and alginate pork sausage. They were deemed acceptable for ingestion and did not change the total macronutrients intake of the participants recorded in their food diary for each day and week of the interventions. Furthermore, the daily intake of alginate-containing food assisted the participants in increasing their daily fibre intake. Bearing this in mind, this would be an effective nutritional approach to increase overall dietary fibre consumption. Importantly, these foods were safe to consume, and the participants did not report serious gastrointestinal symptoms such as flatulence, nausea, bloatedness, change to bowel habit and urgency to pass stool, over the duration of the cheese and pork sausage studies.

Additionally, these outcomes further support the fact that alginate is a safe agent. The acute feeding trial has demonstrated that alginate reduces serum triglycerides following ingestion of an alginate sausage meal compared to consuming the control sausage meal. This finding shows that alginate is a promising agent to inhibit dietary lipid digestion and assist in reducing the amount of fat absorbed, thus, leading to reduced caloric intake, potentially reducing obesity. Producing such food as those explored in this study would encourage the consumption of functional foods with

the potential to enhance health and wellbeing as well as providing several benefits for the weight loss market and for people with diabetes and other nutritional disorders.

Furthermore, using the MG for *in vitro* digestion, it was possible to quantify the glycerol release from the food products as a measurement of lipid digestion. In this data, alginate food did not demonstrate a significant reduction in the glycerol released. However, seaweed food in the form of seaweed cheese resulted in a significant reduction in the glycerol released at the last time point of digestion. This suggests that seaweed containing polyphenols and other functional polysaccharides have a stronger inhibitory activity on pancreatic lipase. However, alginate solubility problems must be addressed. According to alginate, alginate food such as alginate cheese has shown less effect on pancreatic lipase inhibition. When alginate solution was added to the cheese matrix, it produced a jelly balls in a various sizes distributed into the cheese matrix. This cause unequal distribution of alginate into cheese as well as almost of this jelly balls did not dissolved in a MG solution during in vitro digestion, therefore, less alginate can be detected and measured. It could be useful if alginate was soluble in warm or hot water and then added to the cheese to insure better distribution and avoid gel formation.

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Appendices

Appendix 2.1- Pork sausage nutrients analysis.

eurofins

Matthew Wilcox
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NE2 4HH

PO Number **96019**

AR-18-UD-462272-01

Reported on **23/11/2018**
Reported by **Nicholas Cockburn, Analytical Services Manager**

Certificate Of Analysis

Sample number	**400-2018-20141757**	Received on	**12/11/2018**
Your sample reference	**Pork Sausage Control with alginate skin**	Your sample code	**Newcastle University Control Sausage**

Test Code	Analyte	Result		SOP No.
Nutrition				
UD006	Moisture	65.1	g/100 g	Q/005 H/007
UD001	Crude Protein (Nx6.25) (Dumas)	16.1	g/100 g	Z/001
UD007	Ash	2.2	g/100 g	Q/001
UD017	Carbohydrates (available)	< 1.0	g/100 g	Q/035
UD08W	Fructose	<0.1	g/100 g	CHROM/344
UD08W	Galactose	<0.1	g/100 g	CHROM/344
UD08W	Glucose	<0.1	g/100 g	CHROM/344
UD08W	Lactose	<0.1	g/100 g	CHROM/344
UD08W	Maltose	<0.1	g/100 g	CHROM/344
UD08W	Sucrose	<0.1	g/100 g	CHROM/344
UD08W	Total sugars	<0.1	g/100 g	CHROM/344
UD003	Total fat	14.2	g/100 g	Q/002
B7039	Total Dietary Fibre (AOAC 991.43)	1.5	g/100 g	H/085
UD771	Energy value (kcal)	195	kcal/100 g	Q/035
UD771	Energy value (kJ)	811	kJ/100 g	Q/035
		Energy has been calculated to include the contribution from fibre, as required by Regulation (EU) No 1169/2011		
UD815	Salt (via sodium x 2.5)	1.31	g/100 g	
Fatty Acids				
UDFB1	Monounsaturated fatty acids	6.02	g/100 g	CHROM/215
UDFB1	Polyunsaturated fatty acids	2.37	g/100 g	CHROM/215
UDFB1	Saturated fatty acids	5.10	g/100 g	CHROM/215
UDFB1	Trans Fatty Acids	< 0.1	g/100 g	CHROM/215
Elements				
UD015	Sodium	0.525	g/100 g	ICP/003

Unless stated, all results are expressed on a sample as received basis.
† Indicates that this test was subcontracted

* Indicates that this parameter is not included in the UKAS accreditation schedule for the laboratory.
Opinions and/or interpretations within this report are outside our accreditation scope.

Key: cfu colony forming units
< denotes less than
> denotes greater than
~ estimated value

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Appendix 2.2- Cheese acceptability study (poster advert, consent form and debrief sheet).

- Poster advert

- Consent form

CONSENT BY VOLUNTEER TO PARTICIPATE IN A NUTRITIONAL STUDY

The acceptability of alginate based foods

Name of Lead Investigator: Prof Jeff Pearson

Volunteer Identification Number: _____

Please initial box

1. I confirm that I have read and understood the Participant Information Sheet dated 03FEB16 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 and my data will be withdrawn from the study, until the study is complete, after which data will be anonymised. ☐
3. I agree, in principal, to fill out the questionnaires and the food diaries provided to me. ☐
4. I understand that all stored questionnaire data will be coded and will not be traceable to me. ☐
5. I agree to take part in the above study. ☐

Name of Volunteer *(Please print)*	Date	Signature
Name of Research Team Member *(Please print)*	Date	Signature

- Debrief sheet

Participant debrief sheet

The acceptability of seaweed extracts in cheeses

Thank you for participating in our acceptability study. We hope that the information you have provided will help us to assess whether the inclusion of seaweed extracts in cheese products causes any changes in general well-being or gut function. Your data has been anonymised and cannot be traced back to you personally. We aim to publish the results of this study in a peer reviewed journal. Please let a member of the study team know if you would like to receive a copy of the final published article.

Please ensure you collect your gift voucher from a member of our team and sign on the line below.

Sign……..

Print……..

Date……..

If you have any further questions regarding this study please contact:

Muna Fallatah: m.k.o.fallatah@ncl.ac.uk , 0191 208 5013

Jeff Pearson: jeffrey.pearson@ncl.ac.uk, 0191 208 6996

If you would be willing to be contacted regarding further similar studies please tick the box below and return this sheet to a member of our team.

Yes, I would like to be contacted regarding further studies ☐

Name: ……..

Address: ……

Telephone Number: ……..

Email: ……

Appendix 2.3- Pork sausage acceptability study (poster advert, consent form and debrief sheet).

- Poster advert

Newcastle University

WOULD YOU LIKE TO TRY A NEW FOOD PRODUCT?

Contact us NOW to help us investigate the acceptability of pork sausages products containing the seaweed extract (Alginate)

We are looking for:

- Men or women over 18 years old, non-smokers
- No current ill health and not taking medication
- Willing to record what they eat over6 weeks
- Willing to include novel alginate based foods within their normal diet over 4 weeks

For more information, please contact

Muna Fallatah m.k.o.fallatah@ncl.ac.uk
0191 208 5013

You will be reimbursed for your participation, and travel expenses

- Consent form

CONSENT BY VOLUNTEER TO PARTICIPATE IN A NUTRITIONAL STUDY

The acceptability of alginate based foods

Name of Lead Investigator: Prof Jeff Pearson

Volunteer Identification Number: _____

Please initial box

1. I confirm that I have read and understood the Participant Information Sheet dated 03FEB16 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 and my data will be withdrawn from the study, until the study is complete, after which data will be anonymised. ☐
3. I agree, in principal, to fill out the questionnaires and the food diaries provided to me. ☐
4. I understand that all stored questionnaire data will be coded and will not be traceable to me. ☐
5. I agree to take part in the above study. ☐

_____	_____	_____
Name of Volunteer *(Please print)*	Date	Signature
_____	_____	_____
Name of Research Team Member *(Please print)*	Date	Signature

- Debrief sheet

Participant debrief sheet

The acceptability of seaweed extracts in pork sausages

Thank you for participating in our acceptability study. We hope that the information you have provided will help us to assess whether the inclusion of seaweed extracts in pork sausages products causes any changes in general well-being or gut function. Your data has been anonymised and cannot be traced back to you personally. We aim to publish the results of this study in a peer reviewed journal. Please let a member of the study team know if you would like to receive a copy of the final published article.

Please ensure you collect your gift voucher from a member of our team and sign on the line below.

Sign.....

Print.....

Date.....

If you have any further questions regarding this study please contact:

Muna Fallatah: m.k.o.fallatah@ncl.ac.uk , 0191 208 5013

Jeff Pearson: jeffrey.pearson@ncl.ac.uk, 0191 208 6996

If you would be willing to be contacted regarding further similar studies please tick the box below and return this sheet to a member of our team.

Yes, I would like to be contacted regarding further studies ☐

Name: .....

Address: .....

Telephone Number: .....

Email: .....

Appendix 3.1- The effect of alginate on carbohydrate and fat digestion in a mixed meal (poster advert, consent form and debrief sheet).

- Poster advert

Newcastle University

WOULD YOU LIKE TO TRY A NEW FOOD PRODUCT?

Contact us NOW to help us investigate fat digestion of pork sausages products containing alginate

We are looking for:

- Men or women over 18 years old, non-smokers
- No current ill health and not taking medication
- Willing to visit the NU-food facility in the Agriculture Building in Newcastle University twice
- Willing to include novel alginate based foods within their normal diet over 2 weeks and have no gluten intolerance.

For more information, please contact

Muna Fallatah m.k.o.fallatah@ncl.ac.uk
0191 208 5013

You will be reimbursed for your participation, and travel expenses

- Consent form

Volunteer Number __ _____

The effect of alginate on carbohydrate and fat digestion in a mixed meal- A Pilot Trial

CONSENT BY VOLUNTEER TO PARTICIPATE IN A NUTRITIONAL STUDY

me of Lead Investigator: Prof. Jeff Pearson

	Please initial box
I confirm that I have read and understood **the alginate pork sausage study** Information Sheet' dated 3 OCT18, version 1 for the above study and have had the opportunity to ask questions.	☐
I understand that my participation is voluntary and that I am free to withdraw at any time.	☐
I understand that finger-prick blood samples, taken as part of the protocol of this study, will only be used for glucose and triacylglycerol analysis and will not be stored.	☐
I understand that all data collected will be kept confidential and that all data will be coded so that they are anonymous and will not be traceable to me.	☐
I confirm that I have no known allergies to the test food including gluten.	☐
I agree to take part in the above study.	☐

Name of Volunteer *(Please print)*	Date	Signature
_____	_____	_____

Name of Research Team member *(Please print)*	Date	Signature
_____	_____	_____

3 copies required, 1 to be kept by the participant, 1 by the researcher and 1 in the site file

- Debrief sheet

Participant debrief sheet

The effect of alginate on carbohydrate and fat digestion in a mixed meal - A Pilot Trial

Thank you for participating in our study. We hope that the information you have provided will help us to assess whether the alginate sausage can causes any changes in blood glucose and fat after a meal. Your data have been anonymised and cannot be traced back to you personally. We aim to publish the results of this study in a peer reviewed journal. Please let a member of the study team know if you would like to receive a copy of the final published article.

Please ensure you collect your gift voucher from a member of our team and sign on the line below.

Sign……..……..

Print……..……..

Date……..……..

Value…….

If you have any further questions regarding this study please contact:

Muna Fallatah: m.k.o.fallatah@ncl.ac.uk, 0191 208 5013

Jeff Pearson: jeffrey.pearson@ncl.ac.uk, 0191 208 6996

If you would be willing to be contacted regarding further similar studies please tick the box below and return this sheet to a member of our team.

Yes, I would like to be contacted regarding further studies ☐

Name: ……..

Address: …….

Telephone Number: ……..

Email: ……