DIGESTIVE ENZYME MODULATING PROPERTIES OF KUWAIT SEAWEED AND SEAWEED EXTRACTS

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Abstract

The primary aim of this research was to investigate the inhibitory impact of Kuwait seaweed and its aqueous (WE) and ethanolic extracts on Pancreatic Lipase (PL) utilising turbidity assay, Michaelis-Menten kinetic analysis, and a synthetic model of the gut system. Next, the total polyphenolic content (TPC) was measured. A total of seven seaweeds, belonging to various species, were collected from the Kuwait coastline between 2019 and 2022. The green seaweeds identified were *Cladophora sericioides* and *Codium papillatum*. The brown seaweeds identified were *Sirophysalis trinodis*, *Colpomenia sinuosa*, *Iyngaria stellata*, and *Padina boergesenii*. The red seaweed was *Gelidium Pusillum*. The brown seaweed *S. trinodis* was harvested during two distinct seasons, November 2020 (N) and April 2021 (A). Therefore, the temporal effect on the inhibitory action of *S. trinodis* was investigated.

It was found that all Kuwaiti seaweed homogenates, as well as four WE extracts and four ethanol pellets, showed significant inhibition of PL activity to varying extents. These samples were used for further research. The kinetic analysis revealed that Kuwaiti seaweed samples showed mixed-type inhibition of pancreatic lipase. The Kuwait seaweed samples did not inhibit fat digestion. It has been determined that Kuwaiti seaweeds contain varying concentrations of TPC. However, no correlation was found to be statistically significant between seaweed TPC and its PL inhibitory effect. The significant temporal effect on PL activity and TPC were noted in the WE extract from *S. trinodis*.

The observed variance in the results across seaweed species suggests that each seaweed is likely harbours species-specific compounds with distinctive structural features. The ani-lipase properties of the phytochemicals found in Kuwaiti seaweed samples are due to their additive and synergistic effects.

Dedication

I want to dedicate this thesis to a superwoman who has always been there by my side anytime, anywhere and in any situation. She has supported me from the beginning of my life to the completion of my PhD journey and the achievement of this milestone.

Therefore, this thesis is dedicated to Latifa Alshammari, my mother.

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I would like to thank people for their direct and indirect support in completing my PhD journey and achieving my aspiration. I would like to divide them into three groups: education, family, and friends. Finally, I would like to thank my Sponsor.

- **Education Group**

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Sponsor

Finally, I sincerely thank my home country, Kuwait, for providing a fully funded scholarship making my everlasting dream a reality in pursuing my higher studies. Hoping this research contributes meaningfully as a form of reciprocation to my country and benefits its economy as well as people's well-being.

Last but not least, I would like to thank everyone who has enriched my life with love, kindness, and respect.

Declaration

This thesis is submitted for the degree of Doctor of Philosophy at Newcastle University's Institute of Cell and Molecular Biosciences. I performed all the laboratory work and data analysis under the supervision of my supervisors.

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List of Abbreviations

ANOVA	Analysis of Variance
BMI	body Mass Index
CVD	Cardiovascular Disease
C. sericioides	*Cladophora sericioides*
C. papillatum	*Codium papillatum*
-COOH	Carboxylic Group
C. sinuosa	*Colpomenia sinuosa*
°C	Degrees Celsius
DD	Dasman Diabetes Institute
WE	Deionised water
FDA	Food and Drug Administration
E	Enzyme
EE	EE
EGCG	Epigallocatechin gallate
EP	EP
ES complex	Enzyme-substrate Complex
F. vesiculosus	*Fucus vesiculosus*
GIT	Gastrointestinal Tract
G. Pusillum	*Gelidium Pusillum*
g/l	Gram per Litter
GCC	Gulf Cooperation Council
HPLC	High-performance Liquid Chromatography
IC50	Half maximal inhibitory concentration
I	Inhibitor
IFSO	International Federation for the Surgery of Obesity and Metabolic Disorders
I. stellata	*Iyengaria stellata*
JPHC	Japan Public Health Centre
KEPA	Kuwait Environment Public Authority
KFAS	Kuwait Foundation for the Advancement of Sciences
KISR	Kuwait Institute for Scientific Research
K_m	Michaelis-Menten constant

Km^2	Square Kilometre
K_1	Rate Constant for ES Formation
K_{-1}	Rate Constant for the Dissociation of ES Complex
K_2	Rate Constant for the Dissociation of ES Complex to E+P
K_{ia}	Inhibition Constant for the Free E
K_{ib}	Inhibition Constant for the ES Complex
MS	Mass Spectrometry
µl	Microliter
µU/ml	Micro-units per Millilitre
mg	Milligram
mg/dL	Milligrams per Decilitre
ml	Millilitre
mM	Millimole
nm	Nanometre
pNPB	p-Nitrophenyl Butyrate
pNPL	4-Nitrophenyl Laurate
-OH	Hydroxyl Groups
OD	Optical Density
P. boergesenii	*Padina boergesenii*
PGE	Phloroglucinol Equivalent
PUFAs	Polyunsaturated Fatty Acids
pH	Potential of Hydrogen
PSU	practical Salinity Units
P	Products
S. trinodis	*Sirophysalis trinodis*
SD	Standard Deviation
S	Substrate
TPC	Total Polyphenol Content
UV	Ultraviolet
V_{max}	Maximum Reaction Rate
WE	Deionised Water Extract
WHO	World Health Organisation

COVID-19 Statement

The COVID-19 pandemic has significantly impacted my PhD research progress. As my research mainly depends on lab work, most of my work has been delayed during the COVID-19 lockdown, kids' homeschooling and quarantines. The pandemic was an enigmatic period that affected everyone's wellbeing, psychology and physiology, especially being an international student. That period was very difficult in all aspects of my personal life as well as my family. Therefore, I will shed light on the most important issues that have affected my studies, leaving other details to avoid renewing infuriating feelings.

My research studies were at a halt during the lockdown. Most importantly, a delay in the delivery of Kuwait seaweeds, which were the core of my research studies, has occurred due to Kuwait being also under lockdown causing limited access to staff at KISR (the Kuwaiti institute that I have been working with the responsible for gathering the seaweeds, the core requirement for my research lab). Consequently, I contacted another institute in Kuwait, hoping to acquire some seaweed samples. Fortunately, a delivery was found and delivered through Kuwait University in August 2022.

On the other hand, Newcastle University, after the lockdown, has significantly reduced access times and imposed a 30% limitation on the maximum capacity for staff in labs.

On top of that, I'm a mother of six children, two of them have been born during my studies (Latifa in March 2020 and Ibrahim in November 2021), which required a significant amount of attending. Furthermore, during that period, I spent a significant time home-schooling my children and maintaining their health and overall well-being. This has made it extremely difficult to make any reasonable progress on my research, especially being an international student with no external support from an immediate family to help in these matters. However, the Kuwait Cultural Office and Newcastle University compensated me for the unavoidable lost time and interruptions that severely affected my lab work, so I could finally achieve my goal.

Chapter 1 Introduction

Improving the physical and mental health and well-being of individuals is of vital importance and necessitates a conscientious recognition of the social determinants impacting their health. This imperative extends beyond an individual's personal well-being to encompass significant public health implications, requiring substantial costs and placing considerable demands on healthcare services. Despite various health concerns, obesity stands out as a pivotal factor linked to escalated morbidity and mortality rates. Although awareness efforts and public health initiatives to tackle obesity, the epidemic persists unabated. The World Health Organisation (WHO) has recognised obesity as a global health crisis due to its substantial association with significant morbidity and mortality.

1.1 Obesity

Obesity, a recognised metabolic disorder, can be defined as an abnormal accumulation of fat resulting from an imbalance between energy intake, in the form of food, and energy expenditure, in the form of physical activity and/or exercise (Bray, Kim and Wilding, 2017). Excessive fat deposition has been linked with a myriad of diseases, including diabetes, hypertension, cardiovascular disease, musculoskeletal disorders, asthma, sleep apnea, gastroesophageal reflux, reproductive system disorders and various cancers (Bray, Kim and Wilding, 2017). Furthermore, maternal obesity significantly correlates with gestational diabetes, pre-eclampsia and an increased risk of caesarean section and post-term pregnancy complications (Heslehurst *et al.*, 2017). Recent studies have shown a strong association between elevated fat levels and increased risk and complications of COVID-19 (Simonnet *et al.*, 2020). In addition to its impact on physical health, there is strong evidence linking obesity to poor mental health, including depression and anxiety (Seyler *et al.*, 2025).

Obesity is commonly assessed using body mass index (BMI), which is calculated by dividing an individual's weight over their height squared, with the WHO categorising obesity as a BMI >29.9 kg/m^2 (WHO, 2020). Globally, the prevalence of obesity has risen dramatically since the 1980s, with an estimated 670 million adults and 120 million children classified as being obese in 2016. Despite public health efforts, obesity rates

among adults are projected to continue escalating by over 150 million by 2025 (Mohammed *et al.*, 2018), as illustrated in Figure 1. 1A, which delineates global obesity prevalence in 2022, with darker shades indicating a higher prevalence of obesity (WHO, 2022). While there are numerous contributing factors, the Covid-19 pandemic is likely to impeded public health initiatives and exacerbated obesity rates beyond initial projections (Alfaris, 2021; Myers and Broyles, 2020).

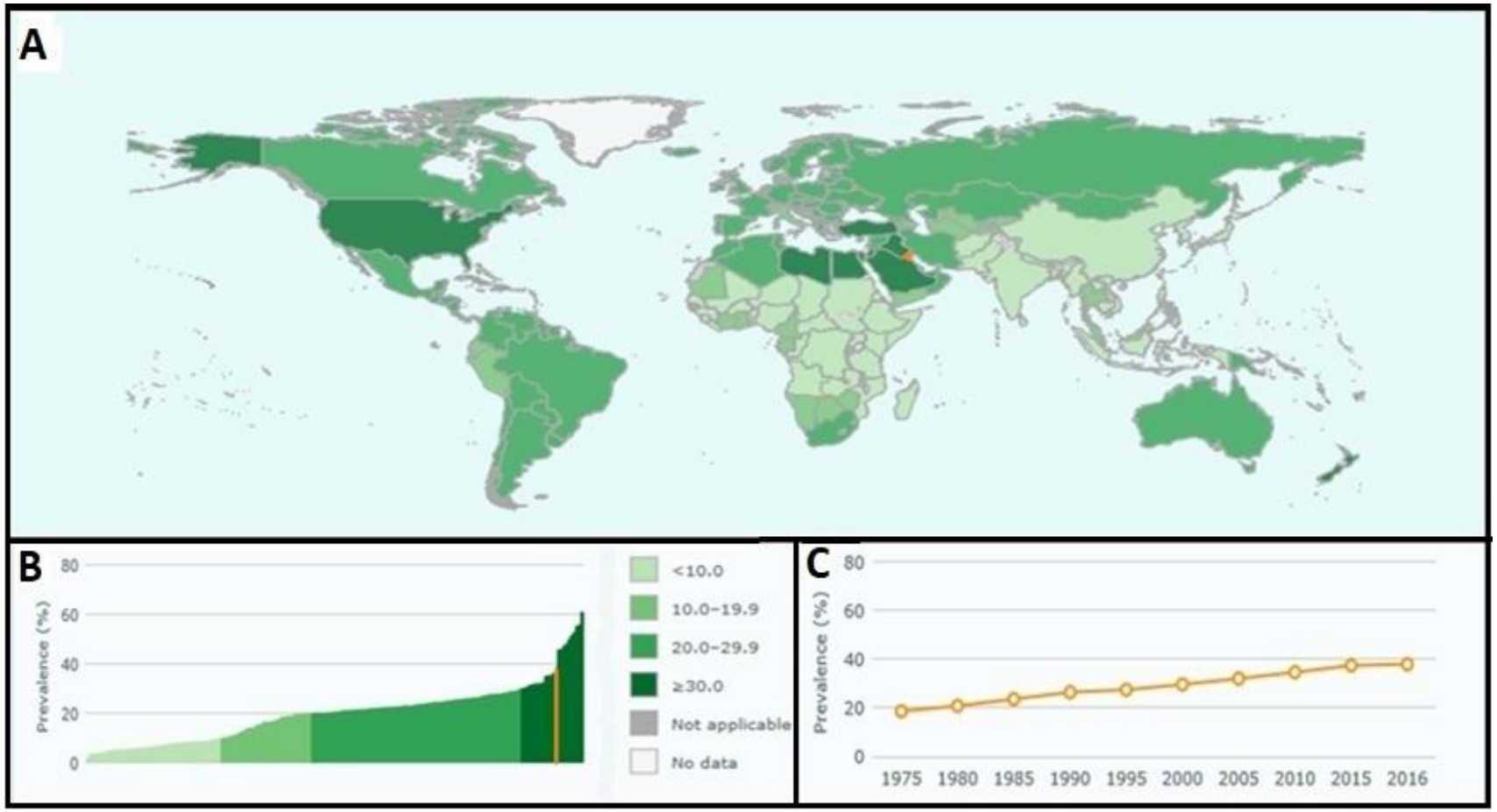

Figure 1. 1 Global obesity prevalence in adults according to the World Health Organization (WHO).
Darker shades of green illustrate higher levels of obesity. Figure 1. 1A depicts the global prevalence of obesity in 2022, and the orange colour is Kuwait. Figure 1. 1B denotes the levels of obesity in association with the different shades of green. The orange line represents the prevalence of obesity in Kuwait, estimated at 37.9%. Figure 1. 1C illustrates the increased levels of obesity in Kuwait from 1975 to 2016. Image adapted from (WHO, 2016; WHO, 2022).

1.2 Obesity in Kuwait

While Western countries have traditionally exhibited the highest rates of obesity, there has been a notable rise in the prevalence of obesity worldwide in recent years. Particularly in the Arabian Gulf region, obesity rates among men and women have increased significantly from 8 and 17% to 36 and 48%, respectively (AL Nohair, 2014). Kuwait, a small size (17.818 Km^2) located in western Asia, has been recognised among the top ten countries globally for obesity (Figure 1. 1A and 1B) (Samara, Andersen and Aro, 2019; WHO, 2016; WHO, 2020).

Kuwait has been identified as one of the countries with the highest obesity rates globally (Weiderpass *et al.*, 2019). The prevalence of obesity among Kuwait adults has doubled between 1975 to 2016 (Figure 1. 1C) with estimates reaching as high as 46 and 33% in females and males, respectively (Samara, Andersen and Aro, 2019; WHO, 2016). Notably, Kuwait also exhibits the highest prevalence of teenage and childhood obesity levels among its neighbouring countries (Elkum *et al.*, 2016; Musaiger *et al.*, 2016). The high incidence of obesity, particularly among teenagers and adults, is believed to have contributed significantly to the increased rates of coronary heart disease (Abdelaziz *et al.*, 2025), diabetes (Alkandari *et al.*, 2020), osteoarthritis (Yao *et al.*, 2023), and various forms of cancer in Kuwait (Gaskell *et al.*, 2024).

Identifying a single factor responsible for the alarming rates of obesity among adults and children in Kuwait is challenging and likely multifaceted. One significant feature is the social and economic transformations following the discovery of oil in the region. Additionally, the proliferation of fast-food outlets following the Second Gulf War has played a crucial role. These establishments offer cheap, calorie-dense foods readily accessible to the Kuwait population, compounding the impact of traditional high-fat dietary habits (Burki, 2016). Moreover, Kuwait's climate, characterised by deserts, high temperatures and humid conditions, poses challenges to physical activity and exercise, fostering a sedentary lifestyle and contributing to obesity rates (Burki, 2016; Gaskell *et al.*, 2024). Furthermore, there exists an association between genetics and obesity in the Kuwait population, with the region's demographic profile exacerbating the risk of obesity (Hebbar *et al.*, 2018). In combination, these factors underscore the

complexity of addressing the rising obesity rates in Kuwait and present significant challenges for public health initiatives targeting this issue.

The Kuwaiti government is aware of the escalating rates of obesity and associated comorbidities, taking proactive measures as early as 2006 with the establishment of the Dasman Diabetes Institute (DDI) under the patronage of the Amir of Kuwait and the Kuwait Foundation for the Advancement of Sciences (KFAS). The primary objective of the DDI is to effectively address and manage the occurrence and consequences of diabetes, including its root causes such as obesity and related conditions in Kuwait (DDI, 2006; WHO, 2014). This DDI institute specialises in the diagnosis, treatment, and follow-up care for patients, aiming to promote a healthy lifestyle. Equipped with state-of-the-art clinical expertise, imaging, and information technologies, the DDI facilitates both patient care and research endeavours (DDI, 2006).

1.3 Current Treatment

Lifestyle interventions incorporating physical activity and/or exercise, alongside adopting a healthy diet, remain the cornerstone of obesity management and mitigating its associated health risks (Fenton *et al.*, 2021). In addition to lifestyle interventions, pharmacological approaches offer an alternative for individuals facing challenges in weight reduction through lifestyle modifications (May, Schindler and Engeli, 2020). Orlistat, a globally utilised pancreatic lipase inhibitor, stands as one of the most recognised medications for obesity treatment (Heck, Yanovski and Calis, 2000). Additionally, Sibutramine and Rimonabant, acting as a serotonin-noradrenalin reuptake inhibitor, and a selective central cannabinoid (CB1) receptor antagonist respectively, have exhibited some promise in combatting obesity (Kushner, 2018). With the advent of off-label medication use and novel pharmacological therapies under development, there is hope for future pharmacological interventions.

Another alternative to lifestyle modifications and pharmacotherapies is surgery, with bariatric surgery emerging as the predominant surgical option for obese patients. The primary objective of bariatric surgery is to reduce stomach size, thereby limiting food intake and digestion capacity. Demonstrating effectiveness in weight loss and

reduction of obesity-related comorbidities, bariatric surgery is the most effective approach to obesity management (Kushner, 2014). Adopting a patient-centred approach is crucial in selecting the most suitable surgical procedure, whether it be gastric bypass, sleeve gastrectomy, gastric banding, or gastric balloon placement. All of these surgical modalities have demonstrated efficiency in reducing food consumption and inducing significant weight loss within a relatively short timeframe (Angrisani *et al.*, 2017). The escalation of obesity in Kuwait is paralleled by a significant increase in weight loss surgeries, as indicated by The International Federation for the Surgery of Obesity and Metabolic Disorders (IFSO) worldwide survey (Almarri *et al.*, 2017).

1.4 Side Effects of Current Treatment

The safest manner in which to lose weight is through physical activity and/or exercise and a controlled diet. Typically, weight loss of 5 to 10% has been demonstrated in patients with specialist support and follow-ups (Wadden *et al.*, 2012). However, there is a scarcity of research surrounding lifestyle modifications in Kuwait, which may be in part due to the climate and traditional lifestyles in Kuwait that make lifestyle modification challenging (Burki, 2016).

It is important to acknowledge that no weight loss therapies are free from adverse effects. Even weight loss through diet may develop into body dysmorphia and eating disorders. It is, therefore, important to manage patients effectively who are undergoing weight loss treatments to address any serious and sometimes fatal side effects that may require urgent treatment. Orlistat, the most well-established pharmacological approach to obesity is no exception to this rule, including steatorrhea, abdominal pain, flatulence and uncontrolled bowel movement. Interestingly, due to the mechanism of action of Orlistat, adverse effects associated with Orlistat are accentuated when the patients consume an unhealthy diet high in fat. Today, Orlistat is rarely used, except to educate patients and increase awareness of the fat content in their diets, which will become apparent due to their increased adverse effects profile. Similarly, although Sibutramine and Rimonabant showed promise as weight loss therapies, in accordance with the Food and Drug Administration (FDA), their use is now prohibited due to the

associated risk of heart attack and neuropsychiatric disorders, respectively (Krentz, Fujioka and Hompesch, 2016).

Bariatric surgeries are often perceived by many patients as a quick solution to their problems, however, it is important to recognise that any surgery procedure is not only expensive, but also comes with a myriad of complications that necessitates management by the clinical team (Gulliford *et al.*, 2016). Key adverse events post-bariatric surgery encompass gastric leaks, haemorrhage, strictures, gastroesophageal reflux, nutritional deficiencies, and neuropathy, all of which must be clearly communicated to all patients prior to undertaking the surgery (Alsabah *et al.*, 2017). Additionally, a considerable number of patients undergoing bariatric surgery often require plastic surgery procedures such as local area lift surgery, abdominoplasty surgery, or body contouring. These procedures entail significant costs to individuals as they may not be covered by existing health policies or insurance and can come with their own set of post-surgical complications (Gunnarson *et al.*, 2015; Wagenblast, Laessoe and Printzlau, 2014). Furthermore, following weight loss interventions, maintaining weight loss also poses significant challenges for patients, often necessitating further weight management strategies (Althumiri *et al.*, 2024).

Despite global recognition of the mortality and morbidity linked with obesity, addressing this issue presents the WHO and national governance bodies with a formidable global challenge that demands immediate attention. While current therapies for obesity do show promise in tackling this global health crisis, they also have some serious side effects and limitation (Krentz, Fujioka and Hompesch, 2016).

1.5 Possible Alternative Treatment Approaches

Orlistat serves as an effective anti-obesity medication by acting as a pancreatic lipase inhibitor, thereby reducing the digestion and absorption of dietary fats, and consequently reducing calorie intake (Heck, Yanovski and Calis, 2000). However, its undesirable adverse effects limit its suitability for everyday use (Krentz, Fujioka and Hompesch, 2016). The initial success of Orlistat has prompted the exploration of strategies aimed at reducing fat digestion and absorption, thus offering a viable anti-obesity therapy. To achieve this goal without the side effects associated with Orlistat,

research has focused on alternative natural sources (Austin *et al.*, 2018; Zhang *et al.*, 2021; Shannon, Conlon and Hayes, 2023). Over the last decade, seaweeds have received considerable interest as an alternative natural compound capable of suppressing the activity of digestive enzymes and potentially treating obesity (Lu, Gu and Yu, 2024; Shannon, Conlon and Hayes, 2023).

1.6 Seaweed

Seaweed is recognised for its potential health benefits and its association with improved gastrointestinal tract (GIT) health. Moreover, a growing body of evidence demonstrates that specific seaweeds possess the ability to modulate digestive enzyme activity and potentially treat obesity and its associated comorbidities (Chater *et al.*, 2015). Seaweed, a marine macroalgae often found on rocky shores and within the ocean, has been utilised for centuries as a traditional food source and herbal remedy in Southeast Asian countries, including Japan, China, and Korea, with approximately two billion kilograms collected annually (Pérez-Lloréns *et al.*, 2023). Globally, seaweed and its associated businesses have an annual worth of $6 billion, with 85% of this value attributed to human use (Ferdouse *et al.*, 2018, pp. 1-2). Seaweed boasts a wide range of applications, including industrial and medicinal uses such as fertilisers, cosmetics, gelling agents, dyes, and a food source (Janarthanan and Kumar, 2018). Epidemiological studies, conducted in Southeast Asian countries, particularly in Japan, have revealed a low incidence of metabolic disorders and cancer, which has been linked to the daily consumption of seaweed (Fujiki *et al.*, 2015; Hitoe and Shimoda, 2017; Murai *et al.*, 2019). The most edible seaweeds, which are taxonomically classified into three groups based to their pigmentation, chemical composition, and morphological characteristics, are green (Chlorophyceae), brown (Phaeophyceae), and red (Rhodophyceae) (Janarthanan and Kumar, 2018).

Seaweed is considered a nutritious food source boasting high nutritional content with a low caloric value compared to other terrestrial food sources, whether derived from plants or animals (Shannon and Abu-Ghannam, 2019). The health-promoting benefits of seaweed stem from its rich source of dietary fibre, minerals (Zhu *et al.*, 2022), antioxidants (Bhuyar *et al.*, 2021), and vitamins (Koseki *et al.*, 2023). Moreover, seaweed serves as a valuable source of essential amino acids, proteins (Hoffmann *et*

al., 2021), polysaccharides (Bhuyar *et al.*, 2021), polyunsaturated fatty acids (PUFAs) (Afonso *et al.*, 2021), polyphenols (Yuan *et al.*, 2018) and carotenoids (Savira, Amin and Alamsjah, 2021). Although the exact mechanisms underlying the health-promoting benefits of seaweed remain elusive, they are likely attributed to its phytochemicals and unique micro- and macronutrient composition (Cherry *et al.*, 2019).

1.6.1 Seaweed Safety

Seaweed is estimated a nutritious food source owing to its dense concentration of nutrients compared to other land-based food sources. Its nutritional profile is influenced by the aquatic environment in which it grows. A crucial aspect to consider when consuming seaweed is its mineral composition, which surpasses that of terrestrial plants by tenfold, rendering seaweed a rich source of macro-elements. Additionally, seaweeds boast elevated concentrations of minerals such as iodine, calcium, magnesium, sodium, and iron. These minerals, essential for various biochemical functions, are turned over in the human body; hence, consuming foods abundant in seaweeds may confer numerous health benefits to tissues, cells, and other biochemical processes (Wu *et al.*, 2022a).

Despite the array of health benefits associated with seaweed consumption, it is essential to recognise that some seaweeds also contain heavy metals such as mercury, cadmium, arsenic and lead. These heavy metals are absorbed from the surrounding environment and can induce toxicity in human cells and tissues if their concentrations exceed certain thresholds (Kim, Kraemer and Yarish, 2019; Wu *et al.*, 2022a). Consequently, it is imperative to identify the seaweeds and environments where these heavy metals are present to mitigate the risk to human health. Implementing continuous monitoring of edible seaweed and aquatic environments to exclude seaweed with elevated levels of heavy metals and pollutants aims to minimise any potentially deleterious effects of seaweed consumption (Murai *et al.*, 2021; Wu *et al.*, 2022a). In addition, harvesting seaweed from non-polluted seas such as the sea of Norway, minimises any possibility of adverse effects from seaweed consumption (Harman *et al.*, 2019).

1.6.2 Seaweed Bioactive Components

Seaweed possesses a diverse array of nutritional and bioactive compounds linked to health, which have been shown to fluctuate depending on the species, geographic location and season (Table 1. 1) (Jofre *et al.*, 2020; Ptak *et al.*, 2021). Among these bioactive compounds are carotenoids, pigments that are species-specific and have been recognised for their antioxidant, anti-cancer, anti-obesity, anti-diabetic and anti-inflammatory properties (Kurniawan *et al.*, 2023). Additionally, carotenoids have also been used as food dyes (Zahrah, Amin and Alamsjah, 2020), cosmetics (Kang *et al.*, 2017), and fluorescent markers (Liu *et al.*, 2024).

Seaweed also contains various types of lipids, including saturated fatty acids, polyunsaturated lipids and glycoglycerolipids. Polyunsaturated fatty acids present in seaweeds, such as omega-3, omega-6, arachidonic, eicosapentaenoic acid, oleic acid, linolenic, and palmitic acid, have all been cited as anti-inflammatory (da Costa *et al.*, 2021), antioxidant and anti-microbial (Belattmania *et al.*, 2016). Seaweeds are also recognised as a good source of plant protein, containing essential amino acids (Niemi *et al.*, 2023). Although the protein content may vary depending on the seaweed type, with red (*Ahnfeltia plicata*), green (*Cladophora* species) and brown (*Fucus vesiculosus*) containing approximately 201, 139 and 71 g/kg dry weight respectively, offering an alternative to animal protein with a rich source of essential amino acids (Olsson, Toth and Albers, 2020). Peptides contained within seaweed have also been reported to confer anti-hypertensive (Wang *et al.*, 2021), antioxidant and anti-diabetic benefits (Wen *et al.*, 2020).

Polyphenols, another compound present in seaweeds, offer internal and external stress protection and exhibit antioxidant properties, detailed in-depth in Chapter 5. Seaweed contains a variety of polyphenols with diverse health-promoting benefits (Murray *et al.*, 2021). Notably, brown seaweed is distinguished by the presence of a novel polyphenol called phlorotannins, which have gained special attention due to their subtypes and pharmacological activity. Specifically, phlorotannins have been reported to induce anti-tumour (Lee *et al.*, 2020), anti-diabetic, anti-oxidant (Gheda *et al.*, 2021), anti-obesity (Kim and Park, 2023) and neuroprotective effects (Wang *et al.*, 2018).

Polysaccharides are the most extracted bioactive compound derived from seaweeds and have been extensively used for decades in food (emulsifying, stabilising and thickening) (Yang, Liu and Han, 2017), pharmaceutical (Bhuyar *et al.*, 2021), and cosmetic products (Yang *et al.*, 2021). Examples of polysaccharides present in brown seaweeds include alginate, fucoidan, and laminarin, while ulvan is derived from green seaweed, and carrageenan and agar are examples of polysaccharides found in red seaweed (Nagahawatta *et al.*, 2023). The most commonly cited health benefits of most algal polysaccharides are presented in Table 1. 1.

Table 1. 1 Bioactive components contained in seaweeds with the micronutrient type, source, biological activity, and their therapeutic properties.

Biological activity of seaweed bioactive components				
Bioactive components	Types	Seaweed species	biological activity	References
Ulvan	Polysaccharide	Green Seaweed	• Anti-coagulant • Anti-hyperlipidemic • Anti-microbial • Anti-inflammatory	(Kidgell *et al.*, 2019)
Carrageenan	Polysaccharide	Red Seaweed	• Antioxidant. • Anti-tumour. • Anti-microbial. • Anticoagulant. • Respiratory treatment for bronchitis and chronic coughs. • Wound dressing.	(Das and Bal, 2024)
Agar	Polysaccharides	Red Seaweed	• Anti-diabetic. • Anti-tumour. • Anti-microbial.	(Pereira, 2018)
Porphyran	Polysaccharide	Red Seaweed	• Anti-inflammatory. • Anti-diabetic. • Antioxidant. • Anti-hyperlipidemic. • Anti-cancer.	(Qiu *et al.*, 2021)
Alginate	Polysaccharide	Brown Seaweed	• Improves satiation. • Delayed nutrient absorption. • Anti-cancer. • Anti-obesity.	(Pereira, 2018; Wilcox *et al.*, 2014)

			• Anti-bacterial. • Wound healing agent. • Gastric Treatment (for gastritis, gastroduodenal ulcers, and gastroesophageal reflux).	
Fucoidan	Polysaccharides	Brown Seaweed	• Anti-coagulant properties. • Antiviral properties. • Anti-cancer properties. • Anti-inflammatory. • Antioxidant. • Anti-obesity.	(Hwang *et al.*, 2016; Pozharitskaya, Obluchinskaya and Shikov, 2020)
Laminarin	Polysaccharide	Brown Seaweed	• Anti-inflammatory. • Anti-cancer. • Anticoagulant. • Antioxidant. • Anti-microbial. • Anti-hypertensive. • Decrease cholesterol.	(Zargarzadeh *et al.*, 2020)
Phlorotannin	Polyphenol	Brown Seaweeds	• Antioxidant. • Anti-tumour. • Anti-diabetic. • Anti-obesity. • Neuroprotective.	(Gomez and Huovinen, 2020; Lee *et al.*, 2020; Wang *et al.*, 2018)
Fucoxanthin	carotenoid	Brown Seaweed	• Anti-diabetic. • Anti-cancer. • Anti-obesity. • Anti-inflammatory.	(Din *et al.*, 2022)

1.6.3 Benefits of Seaweed and their Components on Human Health

Seaweed has been linked to enhancements in health and well-being, particularly in mitigating diet-related diseases such as type 2 diabetes (Wen *et al.*, 2020), obesity, hyperlipidaemia (Kim and Park, 2023), cardiovascular disease (Wang *et al.*, 2021) and cancer (Lee *et al.*, 2020). Epidemiological studies, predominantly conducted in Japan and Korea, have revealed a reduced incidence of diseases such as heart disease, stroke and various types of cancer when compared to Western countries. While numerous factors contribute to this phenomenon, the daily consumption of seaweeds in Southeast Asian countries has been recognised as playing an important role (Cherry *et al.*, 2019; Lee *et al.*, 2016). Interestingly, the lowest cancer rates were observed in Japan, where the average daily seaweed consumption was 14.3 g per in adults (Shannon and Abu-Ghannam, 2019).

1.6.3.1 Seaweed and Cancer

The relationship between diet and cancer has been a subject of interest for several years. In a study utilising data from the Hokkaido cohort study, which involved 3,158 participants between 1984 and 2002, Khan *et al.* (2004) observed a relationship between dietary habits and the mortality risk associated with specific cancers, including lung, stomach, colorectal and pancreatic cancer. Notably, the consumption of seaweed was found to reduce the risk of pancreatic and stomach cancers (Khan *et al.*, 2004). Subsequently, in a later study, Iso and Kubota (2007) investigated the impact of seaweed consumption and other supplements on cancer-related mortality across various cancer types in Japan. Analysing data from over 110,000 participants between 1988 and 1990 from the Japan Collaborative Cohort (JACC) study. Iso and Kubota (2007) revealed an inverse correlation between seaweed consumption and the death rate associated with lung, pancreatic and kidney cancers. Moreover, Yang *et al.* (2010) examined the association between seaweed consumption and breast cancer. Analysing data collected between 2004 and 2006 from the Samsung Hospital of Sungkyunkwan University in Seoul, the authors included data from 362 Korean women and revealed that the consumption of the red seaweed *Porphyra* was associated with a reduced risk of breast cancer (Yang *et al.*, 2010). Interestingly, the link between

seaweed and breast cancer was documented in an Egyptian medical document dating back to approximately the 16th century BCE, known as the Ebers Papyrus (Yang *et al.*, 2010). Furthermore, more recent data from a study conducted in Shanghai, China, revealed that daily seaweed consumption showed an inverse association with the risk of biliary tract cancer (Nelson *et al.*, 2017).

1.6.3.2 Seaweed and Cardiovascular Diseases

According to the WHO, cardiovascular disease (CVD) accounts for 3.9 million deaths per year in Southeast Asia (Zhao, 2021). However, in Japan, the incidence and mortality associated with CVD are lower than in other countries in the Western world, partly attributed to the daily consumption of seaweed (Shimazu *et al.*, 2007). Hata *et al.* (2001) reported that patients who were given capsules to take three times daily for four weeks containing 420mg of the Japanese brown seaweed *Undaria pinnatifida* had a decrease in systolic and diastolic blood pressure (13 and 9 mmHg, respectively) and cholesterol by 8%. Similarly, Teas *et al.* (2009a) observed a reduction of 10 mmHg in systolic blood pressure in people with high blood pressure (>130 mmHg) following consuming the Japanese brown seaweed *Undaria pinnatifida* for eight weeks at doses of 6g daily. More recently, Murai *et al.* (2019) examined the relationship between seaweed intake and the incidence of CVD using data from the Japan Public Health Centre (JPHC) prospective study between 1990 to 1994. The authors observed that the consumption of seaweed significantly reduced the risk of ischemic heart disease (Murai *et al.*, 2019), leading to an overall decrease in CVD mortality and morbidity (Kishida *et al.*, 2020).

1.6.3.3 Seaweed and Diabetes

The association between type 2 diabetes and obesity is firmly established, with obese individuals being seven times more prone to developing type 2 diabetes compared to those maintaining a healthy weight (Pillon *et al.*, 2021). Lifestyle modification remains the cornerstone of managing obesity and early onset or pre-diabetes. Therefore, dietary modification can have a profound impact on mitigating both conditions. Interestingly, there exists an inverse correlation between daily seaweed intake and the incidence of type 2 diabetes (Montonen *et al.*, 2005; Morimoto *et al.*, 2012). Specifically, AG-dieckol, a naturally occurring phlorotannin derivative extracted from

the Australian brown seaweed *Fucus vesiculosus*, exhibits promise as a potential anti-diabetic agent (Lee and Jeon, 2015). Lee and Jeon (2015) demonstrated that consuming a 500 mg tablet of AG-diekol three times daily for 12 weeks before meals effectively reduced postprandial blood glucose in a cohort of 80 pre-diabetic patients. In a more recent study conducted by Sakai *et al.* (2019), the authors reported that patients consuming a 30ml beverage, containing 810 mg of fucoidan twice a day for over 12 weeks effectively managed their type 2 diabetes just using diet therapy. Similarly, Yoshinaga and Mitamura (2019) evaluated the therapeutic potential of the Japanese brown seaweed *Undaria pinnatifida* in treating type 2 diabetes. The authors identified that pre-diabetes subjects consuming 4 g of dried seaweed with 200 g of rice significantly modulated blood glucose (142.1 ± 12.7 mg/dL) and insulin (25.4 ± 11.7 μU/ml) levels 30 minutes following consumption, compared to the control group consuming rice alone (152.3 ± 16.6 mg/dL and 31.0 ± 18.2 μU/ml, respectively) (Yoshinaga and Mitamura, 2019).

1.6.3.4 Seaweed and Other Disorders

Although seaweed is consumed globally, its clinical significance has been underscored by epidemiological findings suggesting that regular seaweed consumption may confer protective benefits against various diseases, including cancer, type 2 diabetes, and obesity, as previously mentioned (Rajapakse and Kim, 2011). Additionally, there is evidence indicating that the consumption of brown seaweed *Alaria esculenta* may reduce symptoms and enhance the quality of life in healthy postmenopausal women by influencing oestrogen levels (Teas *et al.*, 2009b). Moreover, various species of seaweed, such as the green seaweeds *Halimeda monile* and *Ulva lactuca,* red seaweed *Porphyra yezoensis* and the brown seaweeds *Ecklonia stolonifera,* have been shown to possess regenerative, reparative, and protective properties on renal, hepatic and respiratory tissue (Mohamed, Hashim and Rahman, 2012). These regenerative attributes extend to dermatological applications, where several seaweeds have proven effective moisturisers, antioxidants, enhancers of skin, photoprotective properties, and providers of antiaging benefits (Fluhr *et al.*, 2010; Leelapornpisid *et al.*, 2014; Pimentel *et al.*, 2017).

These immunomodulatory effects of seaweeds are likely to be attributed to the aforementioned properties. Bioactive compounds found in alginate derived from brown seaweed and EEs derived from the red seaweed *Eucheuma cottonii* have demonstrated efficacy in enhancing wound healing (Fard *et al.*, 2011; Tai-fang, Cai-yun and Wen, 2007). Moreover, there is also evidence that consuming 5 g of brown seaweed *Undaria pinnatifida* may enhance immune function in individuals diagnosed with human immunodeficiency virus (HIV) (Teas and Irhimeh, 2012). Interestingly, beyond physical health, improvements in mental health have also been associated with seaweed consumption, as evidenced by an inverse relationship between seaweed intake and depression (Allaert, Demais and Collén, 2018; Nanri *et al.*, 2010). Irrespective of the seaweed type and the underlying mechanisms of action, there appears to be a consistent foundation that seaweeds have the potential to enhance various health outcomes and improve the quality of life in patients. However, it is important to acknowledge that larger longitudinal studies are needed in Western populations to ascertain if these benefits transfer between different populations.

1.6.4 Benefits of Seaweed and their Components on Obesity

The prevalence of obesity is unrelenting despite a variety of attempts to mitigate this. In addition to lifestyle modification, pharmacotherapies such as Orlistat have shown promise in effectively managing weight loss. However, naturally occurring compounds without adverse events have also shown promise as anti-obesity therapies that also enhance GIT function, increase satiety and decrease energy intake (Chater *et al.*, 2015; Wan-Loy and Siew-Moi, 2016). There is also consistent epidemiological evidence demonstrating a strong negative relationship between seaweed intake and weight gain (Park, Lee and Shin, 2022). Commonly cited bioactive components present in seaweeds that have been identified as potential anti-obesity therapies include alginate, fucoidan, phlorotannins, and fucoxanthin (Shannon and Abu-Ghannam, 2019). Chapter 2 includes a detailed overview and critique of in-vitro studies that have demonstrated the ability of different seaweed species and their extracts to inhibit pancreatic lipase activity. Chapter 4 presents the data from previous in-vivo studies showing the modulatory effects of seaweed and their extracts on appetite, GIT function and body weight.

1.6.5 Seaweed and Geographical Locations

Most evidence elucidating the health promoting benefits of seaweeds originates from Southeast Asian nations where seaweed intake surpasses that of Western countries. Furthermore, it is also important to acknowledge the impact of geographical location and seasonal variations on the biodiversity of marine species and other abiotic and biotic variables, including living organisms and non-living physical and chemical components within the ecosystem (Park *et al.*, 2023). Similar to all living organisms, seaweeds must adapt to these geographical and seasonal variations (Jofre *et al.*, 2020; Ptak *et al.*, 2021), which consequently influence the biochemical composition, bioactive compounds, and health-enhancing properties of seaweeds (Park *et al.*, 2023).

The impact of seasonal fluctuations on the distinct chemical composition of various seaweeds, including carbohydrates, proteins, phenols, and lipids, is well-established (Kamal *et al.*, 2023; Vinuganesh *et al.*, 2022). Previous research has shown that the lipid and fatty acid compositions of seaweeds vary depending on the season of harvested, seaweed species and the geographical location , with fat content being higher in Ochrophyta during spring and summer compared to *Chlorophyta* which was higher in winter (Pereira *et al.*, 2021). More recent findings by Vinuganesh *et al.* (2022) revealed variations in seaweed protein and amino acid composition, with higher amino acids levels noted in the post monsoon season to summer. Specifically, concentrations of amino acids such as lysine, glycine and alanine in *Caulerpa prolifera* (green seaweed), *Acanthophora.spicifera* (red seaweed) and *Turbinaria ornata* (brown seaweed) were noted to be at their highest levels when harvested in summer (Kamal *et al.*, 2023). Moreover, a fucoidan, sulfated polysaccharide, of the brown seaweed from Tunisia, *Cystoseira schiffneri* was found to be influenced by the harvesting date (Benslima *et al.*, 2021). Benslima *et al.* (2021) reported that sulfate content of *Cystoseira schiffneri* was the highest in July (summer), at 34.8%. Similarly, Konstantin *et al.* (2023) investigated the biochemical contents of four brown seaweeds *Ascophyllum nodosum, Fucus vesiculosus, Laminaria digitata, Saccharina latissima* from the coastline surrounding Solovetsky Islands over a span of 1 year. The authors noted that the concentrations of carbohydrates, lipids and proteins were highest in samples collected in August (Konstantin *et al.*, 2023). Furthermore, Kumar, Ganesan

and Rao (2015) explored the seasonal effects on the biochemical composition of the red seaweed *Kappaphycus alvarezii* by collecting samples monthly from September 2004 to April 2006. The authors observed variations in protein and fibre content throughout the collection process, with the highest mineral content observed in April and the lowest in January (Kumar, Ganesan and Rao, 2015).

In addition to seasonal variations, a substantial body of evidence illustrates that the geographical location where seaweed grows exerts a significant influence on its biochemical structure and function, as well as its economic value. Hees *et al.* (2017) documented variations in phenolic components of brown seaweed across seven locations along the coastline of Western Australia. The authors revealed that polyphenol concentrations were positively and negatively correlated with salinity and photosynthetically active radiation respectively (Hees *et al.*, 2017). In a recent study, Saraswati *et al.* (2020) investigated the chemical composition of the Indonesian brown seaweed *Sargassum cristaefolium* at four different coastal sites on Java island (Saraswati *et al.*, 2020). The authors revealed that alginate and fucose-containing sulphated polysaccharides yields were the highest in samples from the Sayang Heulang and Ujung Genteng beaches. Conversely, the total sugar and fibre contents were the highest in samples from the Awur Bay and Pari Island samples. Furthermore, the same authors collected brown seaweed S*argassum cristaefolium* samples from the same locations and observed that high levels of polyunsaturated fatty acids and pigment contents were present at Sayang Heulang and Awur Bay beaches (Saraswati *et al.*, 2020). In East Asia, Terasaki *et al.* (2017) conducted a study on the brown seaweed *Sargassum horneri*, which thrives off the coast of Usujiri in Japan. The authors observed that the total lipids, fucoxanthin, and fucosterol contents were notably higher in samples extracted from Usujiri than those from the Matsushima area. Similarly, in a recent study in South Asia, Ajayan *et al.* (2021) examined the variations in carbohydrates, protein and lipids contents from three regions along India's Kerala coast: Kovalam, Thirumullavaram, and Tikotti. Interestingly, the authors noted variance among the three sites, with Kovalam exhibiting the highest concentrations of carbohydrates, and Thirumullavaram and Tikotti, having the highest protein and lipid concentrations, respectively (Ajayan *et al.*, 2021).

These data demonstrate the significant influence that the geographical and seasonal variations, encompassing marine environments and the biotic and abiotic conditions, can exert on the biochemical composition of seaweeds. A more comprehensive understanding is necessary to determine which seasons and geographical locations yield seaweeds processing optimal biochemical qualities for their intended application. For example, identifying a specific location in Japan that yields the highest concentrations of alginic acids during Spring is crucial when considering the seaweed will be used as an anti-obesity agent.

1.7 Kuwait

Despite its relatively small size, Kuwait, situated in the Middle East, borders the shores of the Arabian Gulf and boasts a substantial coastline that hosts a distinctive marine environment that is home to a diverse array of seaweed species (Figure 1. 2A) (Alabdulghani, Elsammak and Sarawi, 2013; AlYamani *et al.*, 2014; Devlin, Quesne and Lyons, 2015). In this section, the Kuwait marine environment in relation to its seaweed species was discussed.

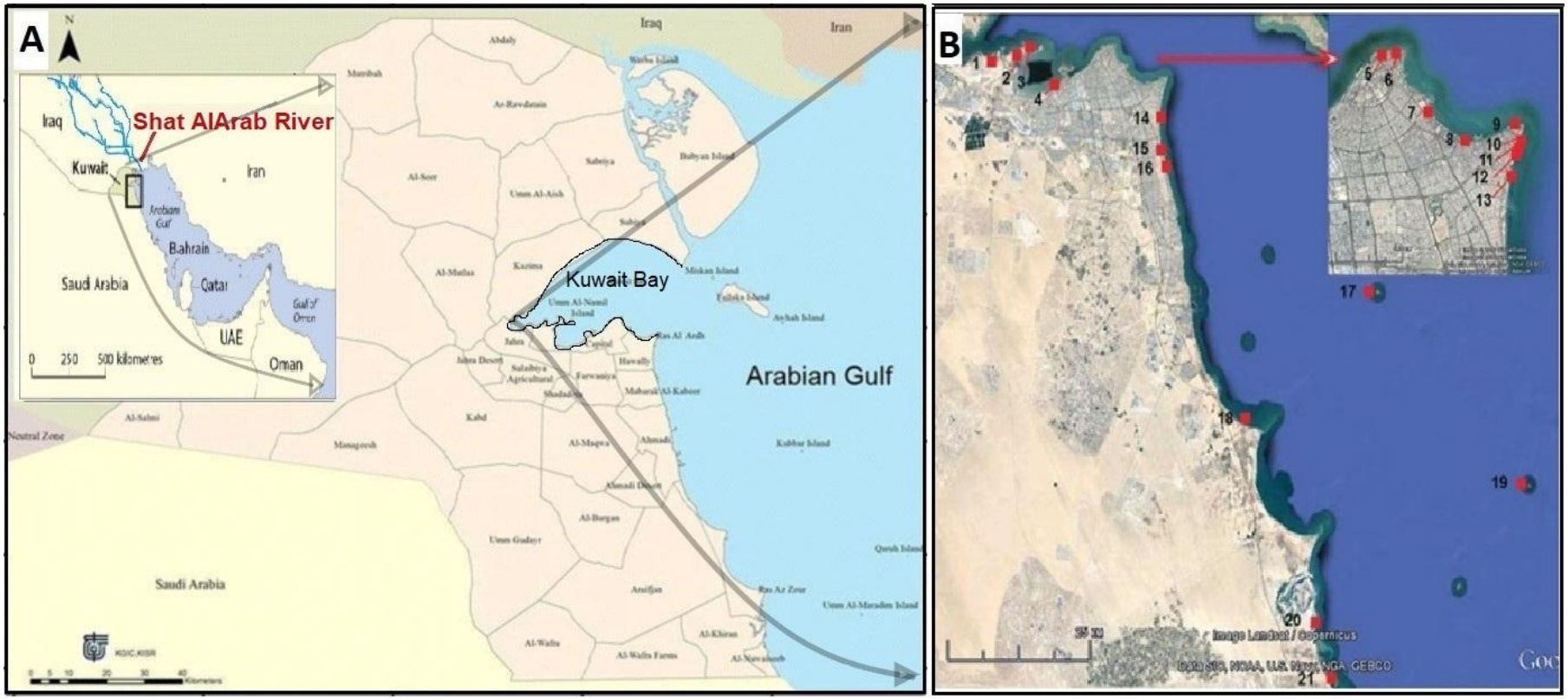

Figure 1. 2 Kuwait map

Figure 1. 2A depicts the primary features of Kuwait's marine environment, including Kuwait Bay and the Shatt Al Arab River. Image adapted from (Alabdulghani, Elsammak and Sarawi, 2013; Devlin, Quesne and Lyons, 2015). Figure 1. 2B displays a magnified view of the Kuwait coastline. Red dots denote specific beach locations where seaweed was collected for this thesis, with dots 3, 6, 10, 14 and 15, associated with Doha, Abraj, Salmiya, Anjifa and Fintas beaches, respectively. Image adapted from (AlYamani *et al.*, 2019, p. 15).

1.7.1 Kuwait Marine Environment (salinity, temperature, and environmental contaminants)

Kuwait is located in the North-Western region of the Arabian Gulf, boasting nearly 500 km of coastline (Devlin *et al.*, 2015) (Figure 1. 2A). The Arabian Gulf serves as the primary source of drinking water for Kuwait, acquired through a desalination process, along with fishing activities carried out in Kuwait and other Gulf countries (Edmonds *et al.*, 2021). The water of the Shatt Al Arab River significantly impacts the physical characteristics of the Gulf's water. Kuwait Bay, also known as Jun Kuwait, receives water from the Shatt Al Arab River, which discharges into the shallow waters of the 750 km^2 Bay (Figure 1. 2A). Kuwait Bay plays a crucial role in Kuwait's economy and that of the Gulf region, offering commercial, industrial, and recreational services (Alabdulghani, Elsammak and Sarawi, 2013; Edmonds *et al.*, 2021). The extensive coastline of Kuwait, coupled with its diverse marine environment, holds great significance in Kuwait's traditions, notably in fishing activities, and serves as a supplementary economic resource alongside oil (Edmonds *et al.*, 2021).

Kuwait Bay boasts a unique marine environment characterised by its elevated summer temperature of 36°C, a salinity level of 45 practical salinity units (PSU), representing the amount of salt in grams per one kilogram of water, and an ultraviolet (UV) index ranging between 8 and 9 (Habeebullah *et al.*, 2019). While anthropogenic activities contribute to pollution, the level of chemical contamination throughout Kuwait's marine environment remains modest compared to global industrial regions (AlSarawi *et al.*, 2015; Devlin, Quesne and Lyons, 2015). The Kuwait Environment Public Authority (KEPA) is tasked with regularly monitoring the physical, chemical, and biological properties of Kuwait's marine environment, ensuring the flourishing of marine life (Almutairi, Abahussain and Elbattay, 2014; Devlin *et al.*, 2015). The diversity of marine life present in Kuwait Bay is an indication of KEPA's effectiveness, evidenced by the presence of various marine species, including coral reefs, coastal habitats, seabirds, turtles and 124 fish species (Edmonds *et al.*, 2021). Furthermore, it has been confirmed that Kuwait's coastline contains 113 different types of seaweed (Habeebullah *et al.*, 2019).

1.7.2 Kuwaiti Seaweed

A diverse array of seaweed species has been collected along the Kuwait coastline in previous years (Figure 1. 3) (AlYamani *et al.*, 2014). Despite the abundance of seaweed available, there is a scarcity of research dedicated to measuring and assessing the potential bioactive components of these seaweeds. In Habeebullah *et al.* (2019) study, the authors investigated the antioxidant properties of water, absolute (100%) and 50% EEs derived from 26 different species of Kuwaiti seaweed, gathered from the Kuwaiti coastline between August 2015 and April 2016. Since polyphenolic compounds have previously been shown to have antioxidant activity and to be beneficial for disease prevention, polyphenolic compounds were isolated from seaweeds for this investigation. Habeebullah *et al.* (2019) revealed that different seaweed species exhibited variable phenolic contents along with different antioxidant activities, suggesting their potential use as natural antioxidants. The water and 50% EEs from the brown seaweeds, including all *Sargassum species*, *Canistrocarpus cervicornis*, *Padina gymnospora, Iyengaria stellata* and *Feldmannia irregularis*, the red seaweeds, including *Polysiphonia platycarpa* and *Chondria species,* and the green seaweed *Cladophora species,* were noted to possess a high polyphenolic content and exhibited antioxidant activity using different antioxidant tests (α,α-Diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging activity and inhibition of liposome oxidation) (Habeebullah *et al.*, 2019).

In addition to antioxidant properties, Habeebullah *et al.* (2020) also investigated chemical variability in Kuwaiti seaweed gathered from the Kuwaiti coastline between September 2016 and March 2017. Chemical variability has been directly linked to different seaweed species and the solvents employed for extraction, which is crucial for optimising polyphenol yield (Habeebullah *et al.*, 2020). To identify an optimal extraction process, the same authors employed an enzyme-assisted extraction approach using various enzymes (carbohydrases and proteases) on seven Kuwait seaweeds, which exhibited the maximum antioxidant capacity in traditional solvent extractions. The highest phenolic content and antioxidant activity in different tests were noted with enzymatic extracts from Kuwaiti seaweeds *Sargassum boveanum*, *Sargassum angustifolium*, and *Feldmannia irregularis* (Habeebullah *et al.*, 2020). Additionally, Habeebullah *et al.* (2020) also revealed that carbohydrase extracts

demonstrated high DPPH radical scavenging activity whereas protease extracts had strong iron chelating activity. These findings underscore the chemical diversity among different seaweeds and the enzymes utilised for their extraction, which can have logistical and clinical implications (Habeebullah *et al.*, 2020). Previous research has demonstrated that the phenolic content of *Sargassum* species from Kuwait exceeds that of the same seaweed species from Korea (Heo *et al.*, 2005) and France (Sánchez-Camargo *et al.*, 2016).

In a more recent study, the fatty acid (FA) and PUFA contents of ten different Kuwaiti seaweed species collected from the Kuwaiti coastline between June 2018 and February 2021 were extracted using the base-catalysed direct transmethylation method and analysed by a gas chromatograph (Aladilah *et al.*, 2021). Aladilah *et al.* (2021) reported that FA contents have been directly linked to different seaweed species. Additionally, Kuwaiti seaweeds showed an increase in saturated FA and a decrease in PUFA contents. Interestingly, the Kuwait coastline is no different to other coastlines worldwide, experiencing typical FA profiles of warm waters. Furthermore, Aladilah *et al.* (2021) revealed that *Ulva* species exhibited high PUFA content, suggesting a substantial potential for human consumption and animal nutrition. The specific PUFAs investigated by Aladilah *et al.* (2021) are exclusively derived from terrestrial dietary sources and are not synthesised by humans, underscoring seaweeds as a unique dietary intake source.

To the best of our knowledge, no studies have evaluated the effect of Kuwaiti seaweeds and their extracts, specifically regarding their potential use as an anti-obesity agent. Considering the diversity of seaweeds along the Kuwait coastline and the unique marine environment, it is important to assess whether Kuwaiti seaweed can be used an anti-obesity agent.

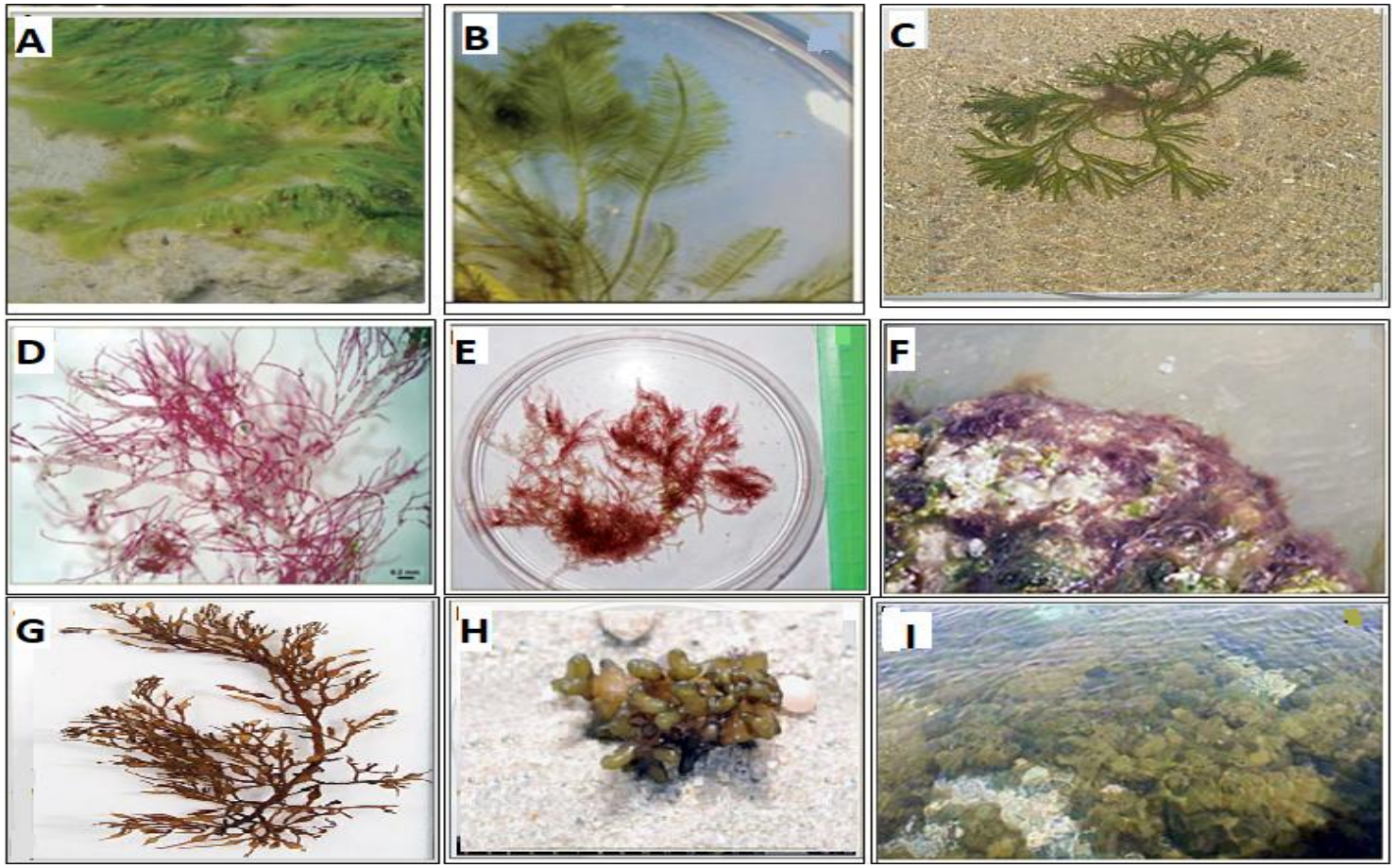

Figure 1. 3 Different Kuwaiti seaweed species.

Figure 1. 3A, B and C are green seaweeds *Cladophora sericioides*, *Bryopsis plumose* and *Codium papillatum*, respectively. Figure 1. 3D, E and F are red seaweeds *Heterosiphonia crispella*, *Hypnea cornuta* and *Gelidium Pusillum*, respectively. Figure 1. 3G, H and I are brown seaweed *Sirophysalis trinodis*, *Iyengaria stellata* and *Colpomenia sinuosa,* respectively. Image adapted from (AlYamani *et al.*, 2014).

1.8 Aims and Objectives

The aim of this thesis was to investigate the biological activities of Kuwaiti seaweeds and their extracts, focusing on their total polyphenol content and activity as a potential anti-obesity agent, including geographical and temporal effects.

To examine the geographical and temporal effects on the seaweed properties through extraction methods:

- The conventional solvent extractions using deionised water (WE), absolute ethanol (100%) and 50% ethanol were applied to seven Kuwaiti seaweeds.
- A comparison between extraction yields was applied according to different extraction solvents, as well as geographical and temporal effects.

To determine if Kuwaiti seaweed and its extracts can inhibit pancreatic lipase activity:

- An in-vitro olive oil turbidity assay was employed to identify if Kuwait seaweed and its extracts can reduce the activity of pancreatic lipase, employing the Michaelis-Menten equation and Lineweaver-Burk plots.
- An in-vitro olive oil turbidity assay was used to compare the effect of different Kuwait seaweeds and their extracts on Pancreatic lipase activity according to different extraction solvents, as well as geographical and temporal effects, employing the Michaelis-Menten equation and Lineweaver-Burk plots.

To evaluate if Kuwaiti seaweed and its extracts can reduce fat digestion under conditions resembling physiological digestion:

- A synthetic model gut system was used. The model gut system replicates sequential digestion steps, including the oral cavity, stomach, and small intestine, while enabling extraction of samples at various intervals.
- Using the synthetic model gut system, it was assessed whether seaweeds showing inhibitory properties in the in-vitro olive oil turbidity assay could translate into synthetic digestion.

- Using the synthetic mode gut system, a comparison of fat digestion with several Kuwait seaweeds and their extracts vs normal digestion was conducted.

To examine and discern the optimal season and geographical location for Kuwait seaweeds and their extracts based on total polyphenol content:

- The Folin-Ciocalteu method was used to quantify the total polyphenol content of seaweeds and their extracts.

The potential beneficial effects of Kuwait seaweeds on pancreatic lipase activity, fat digestion, and antioxidant properties could translate into improved clinical outcomes and potential treatment of obesity in Kuwait. This could pave the way for a collaborative partnership involving The Kuwait Institute for Scientific Research (KISR), KFAS KEPA and Newcastle University.

1.9 Thesis Structure

The thesis is comprised of six chapters with the following structure:

- Chapter 1
  Introduction, a concise literature review, identification of any areas that require further research, aims and objectives and the structure of this thesis.

The following chapters provide the results and are presented using a manuscript-like format: an introduction, methodology, results, discussion, and conclusion.

- Chapter 2
  Title: Anti-lipase Potential of Seven Kuwait Seaweeds and Their Aqueous and Organic Extracts

- Chapter 3
  Title: Kinetics of Pancreatic Lipase Inhibition by Kuwait Seaweed and Their Aqueous and Organic Extracts.

- Chapter 4
  Title: The Effect of Kuwaiti Seaweed and Their Aqueous and Organic Extracts on in-vitro Fat Digestion Using a Synesthetic Model Gut.

- Chapter 5
  Title: Differences in Total Polyphenol Content of Kuwait Seaweed Based on Seaweed Species and Seasonal Variation.

Then, the last chapter

- Chapter 6

  A final discussion with a summary of the data, limitations and suggestions for future research.

1.10 The Sequences of Steps and Scientific Experiments

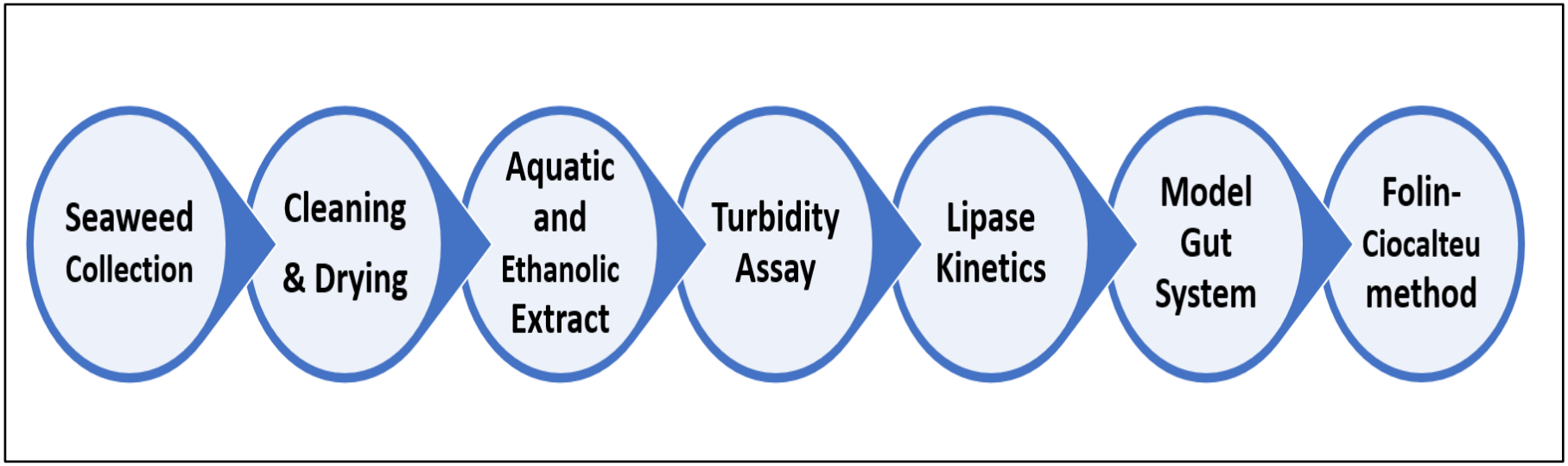

Figure 1. 4 The sequences of steps and scientific experiments in this research.

Chapter 2 Anti-lipase Potential of Nine Kuwait Seaweeds and their Aqueous and Organic Extracts

2.1 Introduction

Obesity, characterised by abnormal or excessive fat accumulation, causes significant health risks to individuals (WHO, 2020). Overweight and obesity are linked to several comorbidities, including diabetes, hypertension, cardiovascular disease, musculoskeletal disorders, asthma, sleep apnea, gastroesophageal reflux, infertility, and cancer (Bray, Kim and Wilding, 2017). Notably, recent research has identified an association between elevated body mass index and obesity with an increased susceptibility to complications and adverse clinical outcomes in coronavirus (Simonnet *et al.*, 2020).

Beyond the health ramifications, obesity imposes a substantial clinical and economic burden, with global estimates reaching as high as $2 trillion dollars (Shephard, 2019). Consequently, there is an urgent need to mitigate obesity levels, enhance clinical outcomes and improve the quality of life in the general population. Currently, three principal approaches are used in order to address obesity: lifestyle modification, pharmacotherapy, and bariatric surgery) (Kushner, 2018).

Although there remains some debate within the literature, a prevailing consensus indicates that the most effective strategy for managing obesity involves long-term lifestyle modification, including dietary adjustments and physical activity (Fenton *et al.*, 2021; Kushner, 2018). However, it is noteworthy that even when diligently adhered to lifestyle interventions, focusing on physical activity and/or diet typically yields a modest reduction in body weight of around 7 to 10% within the context of a comprehensive lifestyle change program (Fenton *et al.*, 2021). Moreover, a key limitation inherent in any lifestyle intervention is ensuring sustained engagement. Failure to achieve consistent adherence often leads to suboptimal outcomes, including inadequate reduction in body weight and eventual relapse. Consequently, pharmaceutical interventions such as Orlistat, along with more recent developments like the use of the diabetic drug glucagon-like peptide 1 (GLP-1) Ozempic, have been employed in the

treatment of obesity (Heck, Yanovski and Calis, 2000; May, Schindler and Engeli, 2020; Wilding *et al.*, 2021).

2.1.1 Anti-obesity Medications

Currently, in the UK, three medications have received licencing approval for weight loss as a viable treatment option for obesity. These medications function as either inhibitor of fat digestion, exemplified by Orlistat, or act as appetite suppressants, as demonstrated by the GLP-1 medications Wegovy and Saxenda (Guglielmi *et al.*, 2023). Despite patients demanding these medications and some clinicians advocating the use of them for weight loss, their prescription rates remain low, with less than 3% of obese patients being prescribed them. Several potential explanations exist for the low prescription of the aforementioned obesity medications, including concerns regarding cost, limited efficacy, safety considerations, adverse side effects, inadequate participant enrolment and outcomes that fail to meet patients' expectations ultimately resulting in poor adherence (Kosmalski *et al.*, 2023; Kushner, 2018).

2.1.2 Pancreatic Lipase Inhibitor

Orlistat, a medication recognised for over two decades for its efficacy in inducing weight loss, continues to be a prescribed drug for weight management (Kwon *et al.*, 2022). Orlistat’s mechanism of action involves binding to and inhibiting the active site of pancreatic lipase, thereby impeding the digestion and absorption of the calorie-rich fats in the GIT. Despite its effectiveness, Orlistat is associated with adverse side effects, including increased defecation, oily patches, flatulence with discharge, faecal urgency, and oily stool, ultimately leading to poor adherence (Kushner, 2018). Consequently, despite its success in promoting weight loss, the adverse effects of Orlistat have spurred interest in alternative compounds that may inhibit pancreatic lipase without causing such adverse side effects. Compounds derived from natural sources have emerged as a potential option in this regard (Lange *et al.*, 2015).

2.1.3 Seaweed as Natural Pancreatic Lipase Inhibitor

Seaweeds are commonly consumed as part of the daily diet in various regions worldwide. Seaweeds offer a low-calorie food rich in dietary fibre content, which can enhance gastrointestinal health, increase satiety, and reduce energy intake (Shannon and Abu-Ghannam, 2019). Beyond its recognised health benefits within a balanced

diet, emerging evidence suggests that compounds found in seaweed may present a promising avenue for obesity treatment. Numerous pre-clinical research (in-vitro studies), as summarised in Table 2. 1, along with clinical trials, have indicated the potential inhibitory properties of various seaweed species and their extracts on pancreatic lipase, satiety regulation (El Khoury *et al.*, 2014; Jensen *et al.*, 2012), and body weight management (Abidov *et al.*, 2010; Hall *et al.*, 2012; Hitoe and Shimoda, 2017; Jensen, Kristensen and Astrup, 2012).

2.1.3.1 Seaweed Compounds as Anti-pancreatic Lipase Agents

The inclusion of seaweed in the traditional diets of Southeast Asian nations has been linked to a decreased prevalence of ischemic heart disease, obesity, and cancer (Murai *et al.*, 2021). However, the specific bioactive compound responsible for these observed benefits remains to be conclusively identified. Seaweed exhibits a diverse array of bioactive compounds, contributing to its high nutritional richness and associated health benefits. These compounds include antioxidants, phenolic compounds, and alkaloids. Additionally, seaweeds are an abundant source of fibre, vitamins, and minerals, all of which can contribute to their health-promoting properties (Pradhan *et al.*, 2022; Shannon and Abu-Ghannam, 2019).

Concerning the potential use of seaweed in combatting obesity, a spectrum of bioactive compounds derived from seaweed has been examined for their ability to inhibit pancreatic lipase (Wan-Loy and Siew-Moi, 2016). Polysaccharides, polyphenols and carotenoids have emerged as particularly promising candidates for their role in inhibiting pancreatic lipase activity, thereby indicating their potential utility as natural inhibitors for managing obesity and associated metabolic diseases (Chater *et al.*, 2015). Notably, alginates, particularly those rich in guluronic acid (G-residues) and fucoidans, both derived from the cell walls of brown seaweed, have demonstrated inhibitory effects on pancreatic lipase (Wilcox *et al.*, 2014; Zhang *et al.*, 2021). Moreover, polyphenolic compounds, regarded as secondary metabolites alongside phlorotannins derivatives and the carotenoid fucoxanthin, all found abundantly in brown seaweed have exhibited multiple health benefits and displayed an inhibitory effect on pancreatic lipase activity (Austin *et al.*, 2018; Catarino *et al.*, 2019; Eom *et al.*, 2013; Matsumoto *et al.*, 2010; Montero *et al.*, 2018).

Table 2. 1 In-vitro studies of seaweed and its extract as a lipase inhibitor.

In-vitro studies of seaweed and its extract as a lipase inhibitor				
Author and year	**Seaweed origin**	**Samples tested, solvent used with ratios and times for extraction.**	**Aims**	**Summary of key findings**
Bitou *et al.* (1999)	Fifty-four Japanese seaweed	• **ME** ○ 100 mg/ml (~20°C for 24 hrs) • **EthAE** ○ 100 mg/ml (~20°C for 24 hrs)	• Examine ⊥ extracts on PL.	• A Significant ↓ in PL activity was observed from 27 seaweeds. • The ME of *C. toxifolia* and *A. taxiformis* showed complete ⊥ on PL activity. • Both extracts from *C. toxifolia* exhibited significant ⊥ on PL activity. • Caulerpenyne was isolated from an EthAE and proposed as competitive ⊥ of PL.
Ben Rebah *et al.* (2008)	Seven Tunisian seaweed species	• **EE** ○ 1:20 (w:v) at ~37°C for 24 hrs	• Study ⊥ these extracts on digestive lipase.	• *C. prolifera* showed ⊥ on PL activity. • The degree of ⊥ varied depending on the type of lipase (gastric or pancreatic) and the type of substrates (short or long-chain TAG). • Several variables such as the seaweed species, geographic location, and season influence the degree of ⊥.
Kim *et al.* (2012)	Korean *Ecklonia cava*	• **EE** (94%) ○ 1:10 (~20°C for 24 hrs) • Re-suspended with WE (1:10 for 1 hr) • Then resuspended samples were mixed with different solvents (at a 1:1 ratio)	• study ⊥ the EE and its other extract on PL.	• The hexane, chloroform, ethyl acetate and n-butanol fractions exhibited higher PL ⊥ ability effects than WE fractions and EE.
Balasubramaniam *et al.* (2013)	Three Malaysian red seaweeds	• **Fresh and dried Seaweed** • **EE** ○ 1:20 (w:v) at ~20°C for 6 hrs	• Study ⊥ these extracts on pancreatic lipase.	• Both the EE and dried seaweed significantly reduced pancreatic lipase activity by 80% and 60% respectively. • No significant ⊥ effect on lipase activity was seen in other examined samples.

Eom *et al.* (2013)	Korean *Eisenia bicyclis*	• **ME** ○ 1:10 (w:v) (~20°C) • Second extract of the supernatant ○ with dichloromethane, EthAE and n-butyl alcohol (at 1:6)	• Examine ⊥ the ME on PL.	• The EthAE showed significant ⊥ PL activity (IC_{50} 0.03 mg/ml).
Jung *et al.* (2013)	Korean *Eisenia bicyclis*	• **EE** ○ 1:10 (w:v) (~20°C for 24 hrs • dried extract ○ was suspended in WE and n-hexane (1:1(v:v) for 2 hrs) ○ was suspended in WE and chloroform (1:1 (v:v) for 2 hrs) ○ was suspended in water and ethyl acetate (1:1 (v:v) for 2 hrs) • was suspended in WE and butanol (1:1 (v:v) for 2 hrs)	• Study the effect of EE and its extracts on PL activity.	• EthAE significantly ↓ PL activity by 66, 59 and 38% at 5 2.5 and 1 mg/ml.
Wilcox *et al.* (2014)	Two brown seaweeds	• **Alginate** with different proportions of sugar residues ○ Guluronate residues • Mannuronate residues	• Study the effect of alginate structure on PL activity.	• PL activity was Significant ↓ when using alginate from *L. hyperborea* with high guluronic acid content.
Chater *et al.* (2016)	Three Hebridean brown seaweeds	• **Seaweed homogenate** • **EE and EP** ○ 1:100 (w:v), at ~20°C for 30 min • **SCE** ○ 0.2 HCL overnight ○ 1: 30 WE at ~20°C overnight. ○ After centrifugation, 1:20 1M sodium carbonate was added to the pellet at 70°C for 3 hrs.	• Study ⊥ these extracts on pancreatic lipase. • Evaluate the possible ⊥ *F. Vesiculosus* (homogenate and EE) on fat digestion using an artificial MG.	• There was significantly ↓ PL activity in all samples and extracts. • This ↓ followed a dose-response pattern. • IC_{50} value indicates that the seaweed homogenate and EP are the most potent lipase ⊥ (with a range of 0.12 to 0.75 mg/ml). • *F. vesiculosus* had the highest ⊥effect on lipase activity (IC_{50} 0.12 mg/ml). • In MG, all *F. vesiculosus* samples exhibited reduced fat digestion. • The EE showed the highest ↓ in fat digestion.

Austin *et al.* (2018)	Hebridean *Ascophyllum nodosum*	• **UWE** (1:10, ~20°C for 1 hr) • **AAE** (50%) 1:10 (~20°C for 1 hr)	• Study the effect of the extracts on PL activity.	• Both the UWE and AAE showed ⊥ on PL activity (IC_{50} 0.15 and 0.2 mg/ml, respectively).
Yuan *et al.* (2018)	Four Chinese brown seaweeds	• **MAE** ○ **EE** (70% at 1:10)	• Extract phenolic compounds with MAE. • Examine ⊥ phenolic compounds on PL activity.	• The extracts contained derivatives of phenolic acid, phlorotannin and gallocatechin. • *L. trabeculate* significantly ↓ on PL activity by 70%.
Catarino *et al.* (2019)	Portugal *Fucus vesiculosus*	• **AE** (70%) ○ 0.01 g/ml (~20°C for 3 hrs) • Concentrated supernatant (until half of the solvent evaporated) then: ○ HE (1:1), then EthAE (1:1)	• Study the effect of the AE and the EthAE on PL activity.	• The EthAE exhibited higher PL ⊥ effects than the AE.
Zhang *et al.* (2021)	Chinese *Laminaria japonica*	• **PSE** ○ **EE** (70°C for 2 hrs) • **EAE**	• Study the effect of PSE on lipase activity.	• ↑ the concentration of PSE resulted in ↓ in PL activity, demonstrating an inverse correlation.
Shannon, Conlon and Hayes (2023)	Three green and brown Australian seaweeds	• **PSE** ○ **AE** (200 mg/ml) at ~20°C for 3 hrs) ○ Then, the pellet was suspended in NaCl (2.5 mg/ml) at 60 °C for 30 min. ○ 10mg SuAP was added (60°C for 24 hrs) and then deactivated (95°C for 10 min) • **PE** ○ **EE** (96%) (100 mg/ml) (~20°C for 10 min)	• Examine ⊥ the extracts on PL activity.	• The PE and PSE significantly ↓ PL activity. • The ⊥ effect of PE on PL activity is higher than PSE. • The PE from *P. comosa* showed the highest ⊥ followed by *E. radiata* and *U. ohnoi* (with IC_{50} =52, 61 and 62 µg/ml, respectively), which showed very low compared to other papers.
Lu, Gu and Yu (2024)	Chinese *Laminaria japonica*	• **Fucoidan**	• Examine ⊥ fucoidan on PL activity.	• There was significant ↓ PL activity in all concentrations (0.5 -3 mg/ml).

ME: mEE; ml: millilitre; mg: milligrams; °C: degrees Celsius; hrs: hours; EthAE: ethyl acetate extract; ⊥: inhibition effect of; PL: pancreatic lipase; ↓: decrease; *C. toxifolia*: *Caulerpa toxifolia*; *A. taxiformis*: *Asparagopsis taxiformis*; EE: EE; (w:v): weight in grams of seaweed: mililiters of solvent; *C. prolifera*: *Caulerpa prolifera*; TAG: Triacylglycerides*;* WE: distilled water; (v:v): volume of WE: volume of solvent; *L. hyperborea*: *Laminaria hyperborea;* EP: Ethanol pellet; min:

minutes; SCE: Sodium carbonate extract; *F. Vesiculosus*: *Fucus Vesiculosus*; MG: model gut; UWE: Ultrapure water extract; AAE: aqueous acetonitrile extracts; MAE: Microwave assisted extraction; *L. trabeculate*: *Lessonia trabeculate;* AE: acetone extracts; HE: hexane extract; PSE: Polysaccharide extraction; EAE: Enzyme-assisted extracted; ↑: increase; NaCl: sodium chloride; SuAP: Subtilisin A protease; PE: polyphenol extracts; *P. comosa*: *Phyllospora comosa*; *E. radiata*: *Ecklonia radiata*; *U. ohnoi*: *Ulva ohnoi*; IC_{50}: Half-maximal Inhibitory Concentration; µg: micrograms.

2.1.4 Pancreatic Lipase

Effective digestion of dietary lipids commences in the stomach, facilitated by preduodenal lipase and peristalsis, which generates a partially digested emulsion. This emulsion is then propelled into the duodenum through the pyloric sphincter (Xu *et al.*, 2021). Pancreatic lipase, also known as triacylglyceride acyl hydrolase, is a water-soluble enzyme produced by pancreatic acinar cells secreted into the second part of the duodenum. Pancreatic lipase is mixed with pancreatic juice, which is secreted by the exocrine cells of the pancreas. As the pancreatic secretions move toward the duodenum, the pancreatic duct converges with the common bile duct, where they mix with bile salts. These bile salts are essential for emulsion formation and aid in the digestion of dietary lipids. Subsequently, this mixture of pancreatic lipase, pancreatic juices, and bile acids enter the second part of the duodenum via the ampulla of Vater (Lim, Steiner and Cridge, 2022).

The bile salts released by the liver and gallbladder play a pivotal role in fat digestion, serving two crucial functions: Firstly, bile salts act as detergents, facilitating the emulsification of large fat droplets into smaller, more manageable droplets. This process is essential for increasing the surface area available for pancreatic lipase activity. Secondly, bile salts form small micelles with emulsified lipids, ensuring that the fat-containing micelles remain water soluble, thus aiding in their digestion and absorption (Ahmed, 2022).

Moreover, pancreatic lipase requires a co-factor, which is also secreted by the pancreas into the duodenum in an inactive form known as procolipase. Upon reaching the duodenum, procolipase is activated by trypsin, resulting in the formation of colipase. Colipase is an amphipathic protein comprising ninety-four amino acids, characterised by three prominent protrusions (fingers) containing hydrophobic amino acids, interconnected by five disulphide bridges (Lim, Steiner and Cridge, 2022). To ensure optimal activity colipase and pancreatic lipase are homogenised at a ratio of 1:1 to prevent any inhibitory action of the bile salts that could impede fat digestion (Tilbeurgh *et al.*, 1999).

Upon reaching the second part of the duodenum and mixing with bile and pancreatic secretions, the pH of the emulsified digesta is between 6-8 (Omer and Chiodi, 2024). This pH range optimally supports pancreatic lipase activity, enabling triacylglycerides

hydrolysis into one monoacylglyceride, two free fatty acids and often cholesterol, which are subsequently absorbed by enterocytes (Zhu *et al.*, 2021). Pancreatic lipase is composed of 449 amino acids and comprises two structural domains, namely the N and C-terminal domains (Figure 2. 1). The N-terminal domain spans amino acids 1-335, forming β-sheets at the core and encircled by α-helices. The N-terminal domain houses the catalytically active site and lid domain, situated at residues 237-261, which are pivotal for pancreatic lipase activity. The active site comprises a catalytic triad composed of serine 152, histidine 263 and aspartic acid 176, or glutamic acid. Conversely, the shorter C-terminal domain encompasses amino acids 336-499, which fold into β-sheets and harbour the colipase binding site (Lim, Steiner and Cridge, 2022; Tilbeurgh *et al.*, 1999).

Pancreatic lipase exists in two distinct forms: the active opened form, as well as the inactive closed form (Figure 2. 1). The active site of pancreatic lipase exhibits a hydrophobic nature and is enveloped by an amphipathic lid. This amphipathic lid comprises a hydrophobic side facing the active site and a hydrophilic side orientated towards the external environment. Structurally, the lid covering the active site consists of a short α-helix cap spanning residues 248 to 255, connected to two polypeptide chains (residues 237-247 and 256-261) through a disulphide bridge (cysteine 237-cysteine 261). The integrity of the lid domain is maintained by van der Waals interactions between β5 (residues75-84) and β9 (residues 203-223) in the closed form (Halimi *et al.*, 2005; Lim, Steiner and Cridge, 2022; Tilbeurgh *et al.*, 1999).

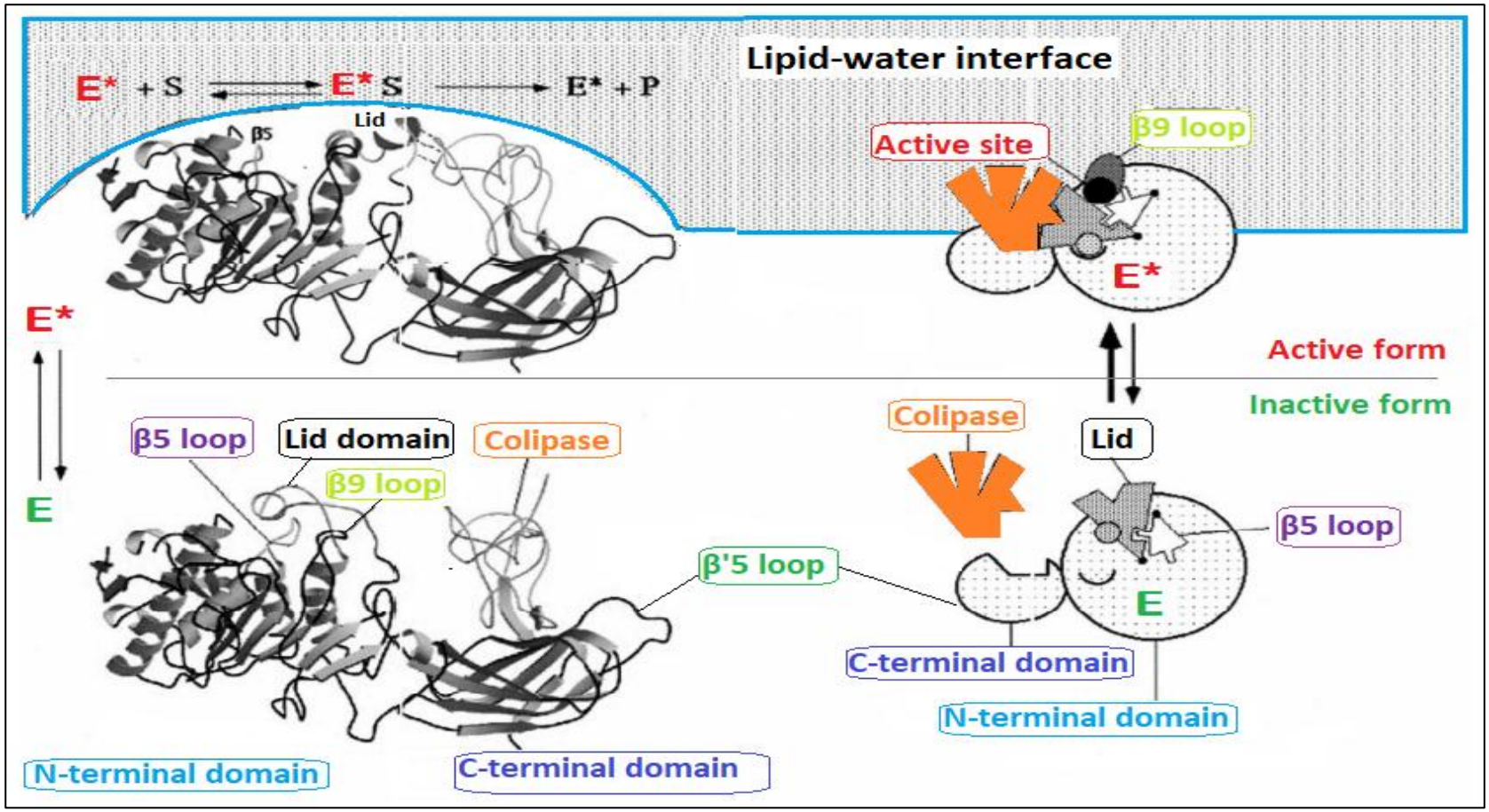

Figure 2. 1 Active and inactive forms of pancreatic lipase.
The three-dimensional structure and schematic diagram show both active and inactive forms of pancreatic lipase, describing the top and bottom views, respectively. The β5 subunit of the pancreatic lipase enzyme assumes the crucial role of maintaining the lid that envelopes the active site during the enzyme's inactive phase (E). Upon interacting with a lipid-water interface, the β5 subunit undergoes displacement from the lid, facilitating the transition of pancreatic lipase into its active state (E*). This movement of the β5 subunit initiates the opening of the active site, denoted as β9, thereby priming it for substrate (S) binding and subsequent hydrolysis to yield products (P). Image adapted from (Tilbeurgh *et al.*, 1999).

2.1.4.1 Pancreatic Lipase Catalytic Activity

Upon encountering emulsified lipids, pancreatic lipase undergoes a conformational change wherein the β5 loop opens, exposing the active site and facilitating the binding of dietary lipids. The activity of pancreatic lipase is further augmented by its co-factor, colipase, which anchors pancreatic lipase to the surface of lipid micelles (Lim, Steiner and Cridge, 2022; Xiao and Lowe, 2015). Upon assuming its open conformation, a second binding site is exposed for colipase. The interaction between the fingers of colipase and the β9 loop generates an unstable reactive hydrophobic corona, also known as an oxyanion hole. This unstable reactive hydrophobic corona around the binding site is produced by increasing negative charge, creating an alkoxide ion in serine. This process, facilitated by conformation change of the active site lid and colipase binding, enhances lipase interaction with lipid-water interfaces, thereby promoting the hydrolysis of triacylglycerides, while simultaneously preventing any inhibitory effects of bile salts (Tilbeurgh *et al.*, 1999; Lim, Steiner and Cridge, 2022).

Initially, the electronic environment of the catalytic triad at the active site of pancreatic lipase is altered by deprotonating serine, resulting in the formation of an alkoxide ion (O^-) (Figure 2. 2A). This alteration is facilitated by the hydrogen bond between aspartic acid and histidine. The oxyanion (O^-) ion acts as a nucleophile, cleaving the carbonyl carbon of the ester bond (sn1) in the triacylglyceride, leading to the formation of an unstable tetrahedral intermediate 1 (Figure 2. 2B) (Reis *et al.*, 2009). To stabilise intermediate 1, a proton is transferred from histidine, resulting in the release of diacylglyceride. Subsequently, histidine attracts a hydrogen atom from the surrounding molecules at its interface, such as water (H_2O) or alcohol (R-OH) (Figure 2. 2C). This mechanism, which is a proton shuttle, culminates in the liberation of one fatty acid and a return to the original configuration of the catalytic site in pancreatic lipase (Figure 2. 2C, respectively). Pancreatic lipase is then able to repeat the process of hydrolysis for the next bond (sn-3) and substrate (Figure 2. 2D) (Reis *et al.*, 2009).

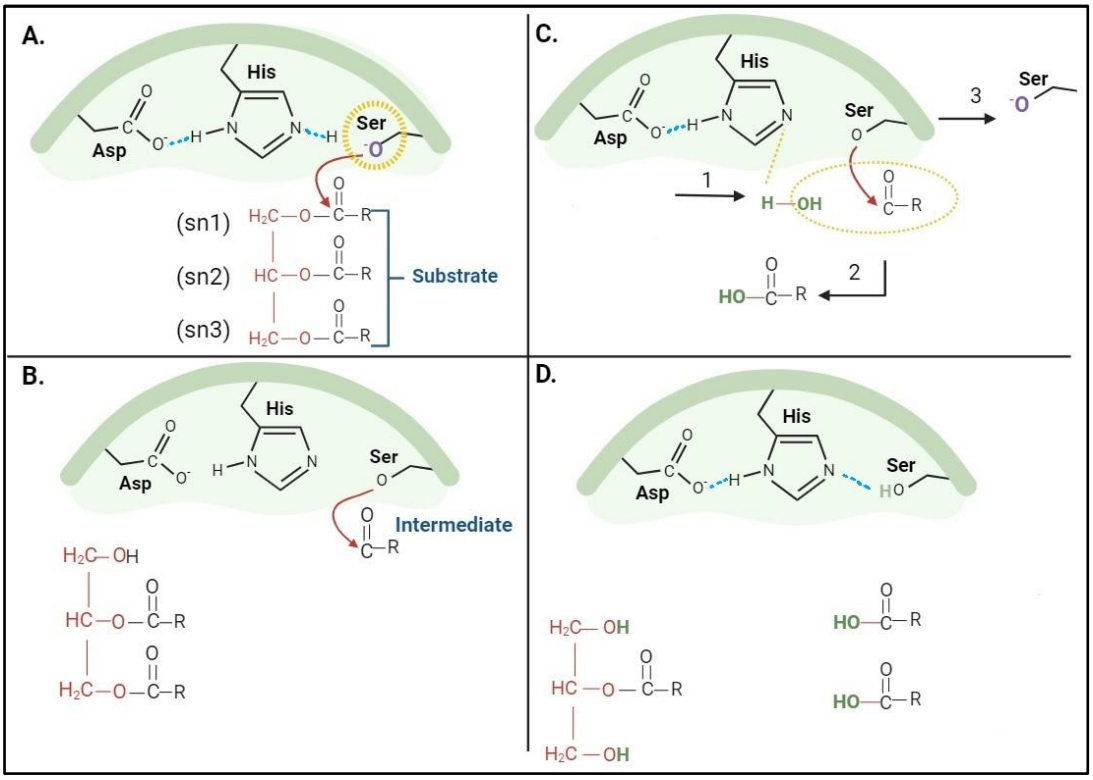

Figure 2. 2 Pancreatic lipase catalysis mechanism.

The schematic diagram illustrates the process of triacylglyceride hydrolysis by pancreatic lipase. Figure 2. 2A depicts the formation of an alkoxide ion in serine, initiated by the deprotonation of serine by histidine, which is, in turn, deprotonated by aspartate. Subsequently, serine engages in a nucleophilic attack on the carbonyl group of the ester bond (sn1) in the triacylglyceride. Figure 2. 2B shows the tetrahedral intermediate and the transfer of the histidine proton, resulting in the release of diacylglyceride. Figure 2. 2C depicts histidine attracting a hydrogen atom from the surrounding molecules, such as water (H2O) or alcohol (R-OH). The hydroxide ion attacking the carbonyl carbon facilitates the liberation of one fatty acid. Pancreatic lipase repeats the sequences of reactions from A to C, subsequently liberating the fatty acid at sn-3. Figure 2. 2D demonstrates pancreatic lipase's ability to return to its original conformation following two sequential reactions, resulting in the production of two fatty acids and glycerol. Image adapted from (Reis *et al.*, 2009) and created from biorender.com.

2.1.4.2 Pancreatic Lipase Inhibition Mechanism

Orlistat is a saturated derivative produced through hydrogenation, which emerged in 1983 as a notable development. It was recognised as a more stable and straightforward compound compared to Lipstatin, which is synthesised by the bacterium *Streptomyces toxytricini* (Heck, Yanovski and Calis, 2000; Katimbwa *et al.*, 2022). Lipstatin has been investigated as a potential treatment for obesity, owing to its irreversible inhibition of pancreatic lipase (Lucas and Kaplan-Machlis, 2001). However, its limited productivity rendered the cost of manufacturing Lipstatin prohibitively expensive. In contrast, Orlistat's stability and simplicity made it more cost-effective to produce than Lipstatin. Consequently, Orlistat has garnered significant attention for its ability to inhibit pancreatic lipase and its potential as an anti-obesity medication (Białecka-Florjańczyk *et al.*, 2018).

Orlistat obtained licencing approval from the Food and Drug Administration (FDA) in 1999, designating it as a treatment for obesity. Derived from Lipstatin, Orlistat acts as a pancreatic lipase inhibitor, forming a covalent bond with the enzyme's active site, specifically the serine residue. The O- of serine acts as a nucleophile, initiating an attack on the carbon of the-C=O on the β-lactone ring, resulting in the opening of the β-lactone ring (Figure 2. 3B) (Białecka-Florjańczyk *et al.*, 2018). Inhibition of the active site of pancreatic lipase subsequently prevents the digestion of triacylglycerides, causing them to pass through the GIT without being absorbed and ultimately excreted in the faces (Heck, Yanovski and Calis, 2000; Kwon *et al.*, 2022). While the exact figures may vary among studies, typically 30% of dietary fat remains undigested due to the inhibitory effects of orlistat on lipases (Kwon *et al.*, 2022; Van Gaal *et al.*, 2004).

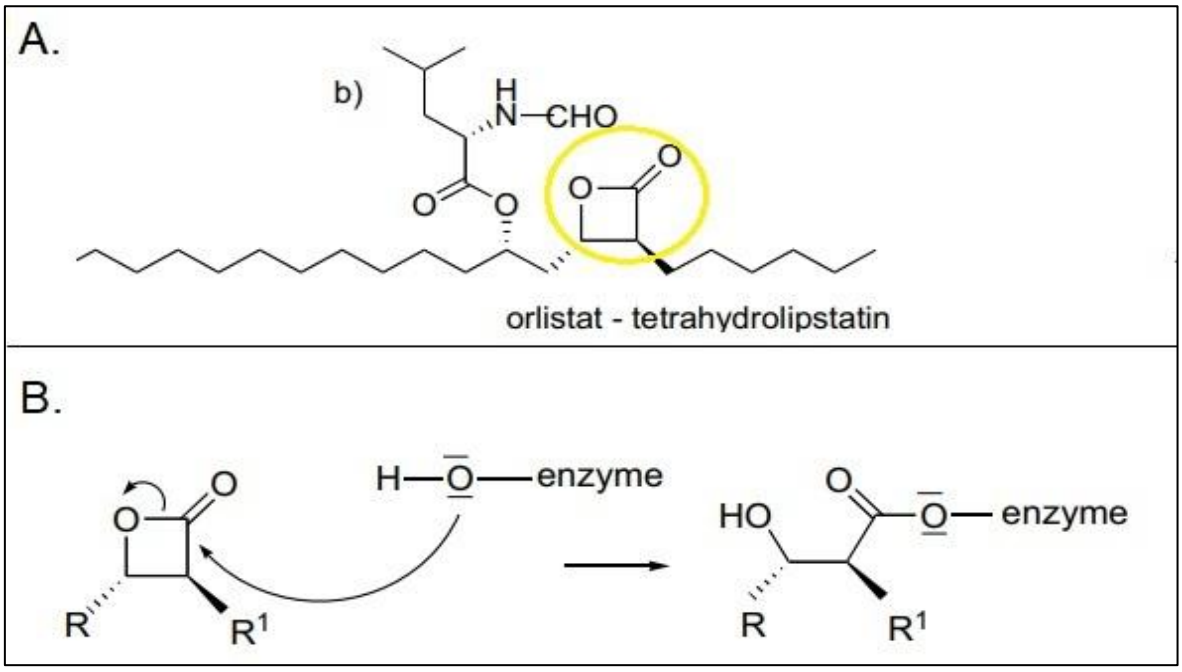

Figure 2. 3 Structure of Orlistat and its mechanism of action as a pancreatic lipase inhibitor.

Figure 2. 3A depicts the structure of Orlistat and its reactive β-lactone ring, highlighted by the yellow circle. Figure 2. 3B demonstrates the interaction of Orlistat with the serine residue located at the active site of pancreatic lipase, depicting the opening of the β-lactone ring. Image adapted from (Białecka-Florjańczyk *et al.*, 2018).

Although Orlistat has demonstrated significant potential as an anti-obesity treatment, its use is not without concern. GI adverse events are common and include abdominal pain, flatulence, urgent bowel movements, oily stools, and oily rectal spotting. However, the severity of these adverse events is reported to diminish with prolonged therapy, which can also lead to potential complications. In addition to GI adverse events, there are concerns regarding vitamin deficiency following prolonged use of Orlistat, particularly fat-soluble vitamins A, D, E and K (Heck, Yanovski and Calis, 2000). Furthermore, prolonged use of Orlistat may increase the risk of developing cholelithiasis and nephrolithiasis (Handing *et al.*, 2022; Katimbwa *et al.*, 2022). In severe cases, Orlistat has been associated with the pathophysiology of colon cancer. While there is clear clinical potential for the use of Orlistat, longitudinal studies are necessary, and patients using it must be appropriately monitored (Jin *et al.*, 2021).

Due to the adverse events associated with Orlistat and the lack of pharmaceutical alternatives, a significant body of research is being conducted to identify natural compounds capable of inhibiting digestive lipases (Catarino *et al.*, 2019; Kurniawan *et al.*, 2023; Lu, Gu and Yu, 2024). Seaweeds are an integral component of diets in various parts of the world and have been shown to offer numerous health benefits, including high fibre content, ω-3 fatty acids, essential amino acids, and vitamins A, B, C, and E. Furthermore, seaweeds contain a diverse range of bioactive compounds. Interestingly, previous research has suggested that specific seaweeds may have promise as treatments for obesity due to their ability to inhibit pancreatic lipase activity (Shannon and Abu-Ghannam, 2019). Various seaweeds from different regions have demonstrated similar potential in previous studies, including those from Japan (Bitou *et al.*, 1999), Tunisia (Ben Rebah *et al.*, 2008), Korea (Kim *et al.*, 2012; Eom *et al.*, 2013; Jung *et al.*, 2013), Malaysia (Balasubramaniam *et al.*, 2013), Scotland (Chater *et al.*, 2016; Austin *et al.*, 2018), China (Lu, Gu and Yu, 2024; Zhang *et al.*, 2021), Portugal (Catarino *et al.*, 2019) and Australie (Shannon, Conlon and Hayes, 2023). However, to the best of our knowledge, no studies have been conducted to date investigating the potential of Kuwaiti seaweed and its extracts as pancreatic lipase inhibitors.

2.2 Aims

The aims of this chapter are:

- To assess the impact of different Kuwaiti seaweed species and their extracts on pancreatic lipase activity using turbidity assays.
- To explore any dose-response relationships of Kuwait seaweed and their extracts on pancreatic lipase activity.
- To determine the influence of seasonal variations on pancreatic lipase activity in Kuwait seaweeds.

2.3 Methods

Pancreatic lipase activity can be measured using several assays, including titration, spectrophotometry, fluorescence. These assays measure the hydrolysis products of fat substrate using pH salt titration (Chatzidaki *et al.*, 2016), p-Nitrophenyl substrate (Vo *et al.*, 2022) and Fluorescent substrate (rhodamine B-labelled triglycerides) (Jerbi *et al.*, 2017), respectively.

In this study, pancreatic lipase activity was assessed using the turbidity assay, representing the hydrolysis of triacylglyceride to free fatty acids, as evidenced by a reduction in turbidity (Wilcox *et al.*, 2014). Since the turbidity test has been used in my supervisor's lab and in previous PhD studies, it was selected for this study. Additionally, the chemicals and equipment required, such as a spectrophotometer and standard reagents, were available in the lab, making this assay a viable option. Furthermore, it is quick, easy, and economical.

Various concentrations of Kuwaiti seaweeds and their extracts, ensuring consistency with previous studies in the research group thus enabling data comparison, were suspended in an oil substrate solution (seaweed solution) to evaluate their impact on pancreatic lipase activity. Commercially purchased olive oil was the substrate to stimulate lipase activity, while Orlistat was employed as a positive lipase inhibitor.

2.3.1 Materials

Pancreatic lipase (≥200 units/mg), colipase, orlistat and tris (hydroxymethyl)-methylamine were purchased from Sigma-Aldrich (Poole, UK) and sodium deoxycholate was purchased from Fluka (Buchs, Switzerland). Absolute ethanol, acetone and aluminium oxide were obtained from Fisher Scientific (Loughborough, UK). Olive oil was obtained from the Cooperative food company.

2.3.2 Equipment

The 96-well plate reader (EL808 Bio Teck) was used at 405 nm (Bedfordshire, UK). A pH meter with a 213 microprocessor was used to measure pH (Hana Instruments, Leighton Buzzard, UK).

2.3.3 Kuwaiti Seaweed

A total of seven different seaweed species collected from the Kuwaiti coastline between 2019 and 2022 were generously provided by the Marine Science Department of KISR and Kuwait University (Appendix 3 and Appendix 4). Among these, two green seaweeds were collected: *Cladophora sericioides (C. sericioides)* and *Codium papillatum (C. papillatum)*, each from distinct geographical locations, Salmiya and Anjifa, respectively (Table 2. 2). Additionally, four brown seaweeds were harvested: *Sirophysalis trinodis (S. trinodis*) from Salmiya and *Padina boergesenii (P. boergesenii), Colpomenia sinuosa (C. sinuosa)*, and *Iyengaria stellata* (*I. stellata*) from Fintas (Table 2. 2). *S. trinodis* collected from the Salmiya coastline of Kuwait in November 2020 (N) and April 2021 (A) was performed to assess any seasonal variation in the seaweed's properties. One red seaweed, *Gelidium Pusillum (G. Pusillum)*, was obtained from the Abraj coastline of Kuwait in May 2022. Following collection from the Kuwait coastline, all seaweeds were washed in the marine science department of KISR using deionised water, followed by air-drying before being crushed into a powder and then stored in polythene bags. The powdered form of the seaweed was used for all experiments conducted in this study.

- Kuwaiti Seaweed samples

This study used four different types of seaweed samples, including seaweed powder (homogenate), deionised water extract (WE), ethanol extract (EE) and ethanol pellet remaining after ethanol extraction (EP).

Table 2. 2 Kuwaiti seaweed species.

Seaweed	Species	Collected time	Collected place	Supplier
Cladophora sericioides	Green	Oct. 2019	Salmiya	KISR
Sirophysalis trinodis	Brown	Nov. 2020 Apr. 2021	Salmiya	KISR
Codium papillatum	Green	May 2022	Anjifa	Kuwait University
Gelidium Pusillum	Red	May 2022	Abraj	Kuwait University
Colpomenia sinuosa	Brown	June 2022	Fintas	Kuwait University
Iyengaria stellata	Brown	June 2022	Fintas	Kuwait University
Padina boergesenii	Brown	June 2022	Fintas	Kuwait University

KISR: the Kuwait Institute for Scientific Research.

2.3.4 Kuwait Seaweed Extraction

Three different extraction solvents, WE, 100% EE and 50% EE were used. The ratio of solid (in gram) to solvent (in millilitres) was 1:10. All seven seaweed species underwent extraction using WE at a ratio of 1:10. Seaweeds *C. sericioides and S. trinodis* were extracted using 100% EE at a 1:10 ratio. Seaweeds *C. papillatum*, *G. Pusillum, P. boergesenii, C. sinuosa*, and *I. stellata* were subjected to EE diluted by 50% using WE to evaluate EE efficiency. The latter extraction method (50% EE) was modified due to the ineffective inhibition of pancreatic lipase activity observed using absolute (100%) EE.

Figure 2. 4 illustrates the sequential process of collecting, preparing and extracting various extracts from different seaweed species collected along Kuwait's coastline. Upon collection from the coastline, all seaweeds were individually dried and ground into a powder using a grinder. The powder was then mixed with a solvent (WE, 100% EE or 50% EE) at a solid-to-solvent ratio of 1:10, homogenised and placed on a rolling platform for 24 hours at 4°C in a dark room. Subsequently, each sample underwent centrifugation at 1,157x g for 10 minutes at 20°C. Following centrifugation, the supernatant and pellet were collected and analysed separately. The water supernatants were transferred to a tube and frozen at -20°C before undergoing lyophilisation. Ethanol supernatants were poured into a weighing boat and dried in a fume hood. The resulting dried extracts were re-suspended using 10 ml of WE and transferred to a 25 ml tube for freeze-drying. All pellets were air-dried and stored in a freezer until further analysis (Chater *et al.*, 2016). Each seaweed was extracted in triplicate and the extraction yield was determined using Equation 2. 1.

$$\textbf{Extraction yield } \% = \frac{\boldsymbol{the\ dry\ wieght\ of\ extract}}{\boldsymbol{the\ dry\ wieght\ of\ seaweed}} \times \mathbf{100}$$

Equation 2. 1 Extraction Yield.

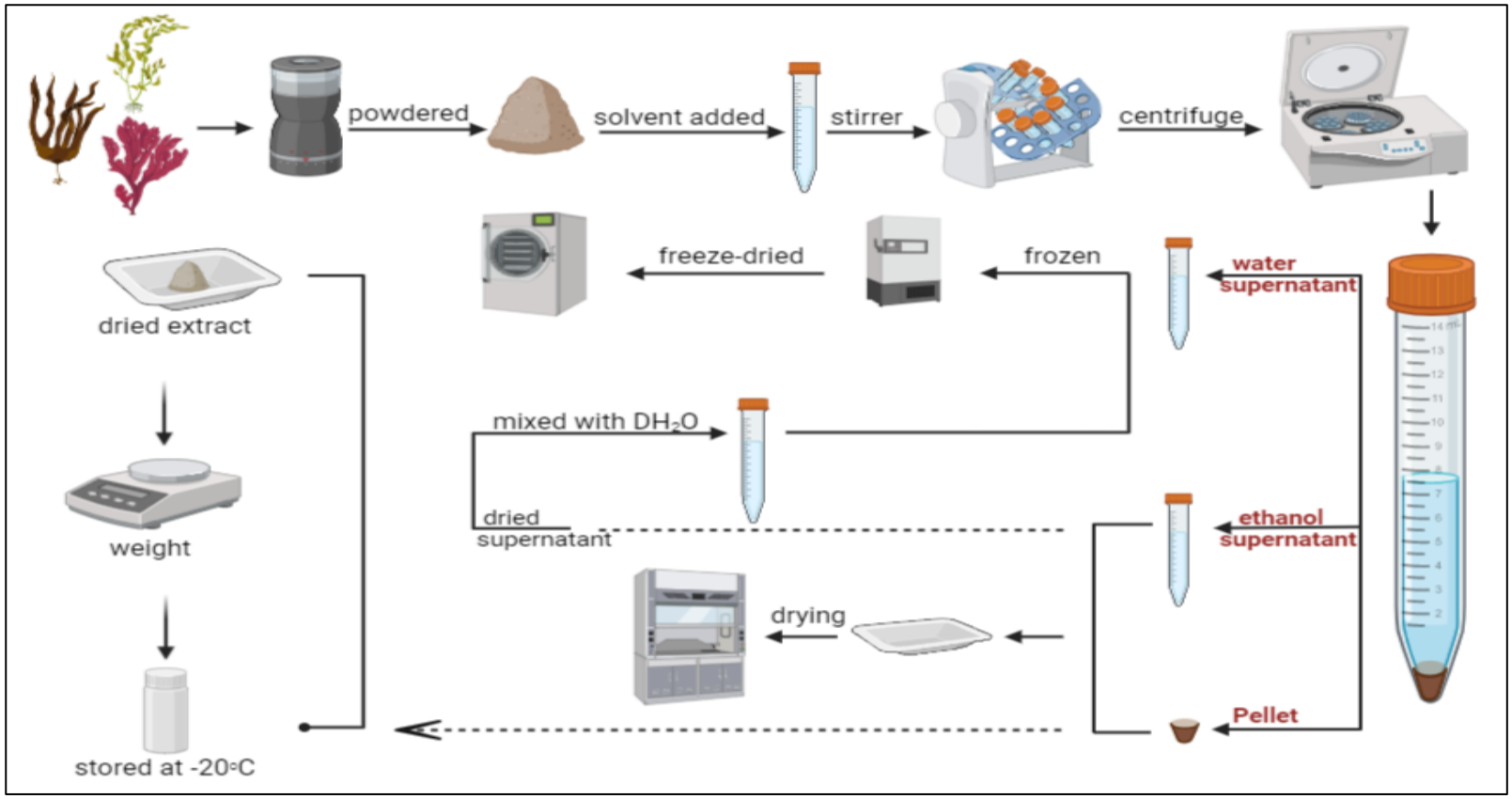

Figure 2. 4 Preparation procedures for seaweed extracts.
Following their collection from the coastline, all seaweeds were individually dried before ground using a grinder to create a powder. The powder was then added to a solvent (deionised water extract (WE), 100% ethanol extract (EE) or 50% EE) using a solid-to-solvent ratio of 1:10, before being homogenised and placed on a rolling platform for 24 hours at 4°C in a dark room. Each sample was then centrifuged at 1,157x g for 10 minutes at 20°C. Following centrifugation, the supernatant and pellet were collected and analysed separately. The water supernatants were frozen at -20°C before being lyophilised. Ethanol supernatants were poured into and dried using a weighing boat in a fume hood. The dried extracts were then re-suspended using 10 ml of WE before being transferred to a 25 ml tube for freeze-drying. All pellets were air-dried and stored until further analysis in a freezer until further analysis. The image was created with permission from bioRender.com.

2.3.5 Reagent Setup

- Buffer solution

The buffer solution consisted of 0.05 M Tris (hydroxyl methyl) aminomethane, which was diluted using WE and then titrated to a pH of 8.3 using 6M hydrochloric acid (HCL). After that, 0.35% sodium deoxycholate was added to the buffer solution and stirred using a magnetic stirrer. This solution should be retained as a stock solution for additional experiments.

- Substrate solution

To ensure triacylglycerides were the substrate being hydrolysed, olive oil was passed through an 8cm deep layer of aluminium oxide in a glass chromatography column to remove free fatty acids. Subsequently, 10 g of the processed olive oil was mixed with 100 ml of acetone to create a 10% olive oil solution which was then refrigerated at 4°C for future experiments. Before each experiment, a 1% olive oil solution was prepared by taking 1 ml of a 10% olive oil solution and diluting it with 10 ml acetone.

Subsequently, 100 ml of Tris buffer was heated to 70°C. Following this, 4 ml of 1% olive oil solution was mixed with the heated Tris buffer for 10 minutes. All experiments using the pre-heated oil substrate solution were conducted within 6 hours of heating after the oil substrate solution returned to room temperature.

- Orlistat solution

The positive control, Orlistat, a well-known pancreatic lipase inhibitor, was first diluted and then mixed in an oil substrate solution to achieve a concentration of 0.025 mg/ml.

- Pancreatic lipase

Pancreatic lipase was added and homogenised in WE to attain a concentration of 1.29 mg/ml (5 units/ml). Following this, 18 µg/ml of colipase was added to the pancreatic lipase solution and then vortexed for thorough mixing.

- Seaweed solution

Four different types of seaweed solutions were used in separate experiments. The seaweed homogenate, WE, EE and EP were then diluted in the oil substrate solution

to achieve concentrations of 5, 2.5, 1.25, 0.5 and 0.25 mg/ml, thus providing the four different seaweed solution solutions.

2.3.6 Procedures

The enzymes and substrate solutions were preincubated separately using two 96-well microplates (Figure 2. 5). On a 96-well plate (Plate A), 10µl of pancreatic lipase combined with colipase was then added to the wells (Row A-C) on the 96-well plate labelled 'enzyme', while 10µl of WE was added to the wells (Row E-G) on the 96-well plate labelled 'blank', which served as a negative control (Figure 2. 5A). On a 96-well plate (Plate B), 240 µl of seaweed solutions at concentrations 5, 2.5, 1.25, 0.5 and 0.25 mg/ml, diluted in oil substrate solution, was added to the appropriate wells (in separate Columns from 1 to 5, respectively) (Figure 2. 5B). Additionally, 240 µl of the oil substrate solution alone and Orlistat diluted to 0.025 mg/ml in oil substrate solution were dispensed into their wells (Columns 6 and 7, respectively) (Figure 2. 5B). Plates A and B were subsequently incubated for 10 minutes at 37°C. Following incubation, 200 µl (volume 1(V_1)) of the solutions contained on Plate B, comprising of the seaweed samples at different concentrations (C_1), substrate, and Orlistat, were transferred to Plate A (volume 2 (V_2) 210 µl) (Figure 2. 5). Then, plate A was immediately inserted into a spectrophotometer and read every 5 minutes at 405 nm for 45 minutes. After the transfer of seaweed solutions containing seaweeds at concentrations (C_1) of 5, 2.5, 1.25, 0.5 and 0.25 mg/ml, the final seaweed concentrations (C_2) were calculated using Equation 2. 2 as 4.8, 2.4, 1.2, 0.48 and 0.24 mg/ml, respectively.

$$\boldsymbol{C1 \times V1 = C2 \times V2}$$

Equation 2. 2 Concentration of Dilute Solution
C1: concentration of the seaweed solution diluted in oil substrate, V1: volume of seaweed solution (200µl) added to 10µl pancreatic lipase, V2: final volume in the assay (210 µl), C2: final concentration of seaweed solution in the assay.

The activity of pancreatic lipase was then calculated using Equation 2. 3, from which any pancreatic lipase inhibition could be calculated as depicted Equation 2. 3, following the subtraction of each blank from its corresponding sample.

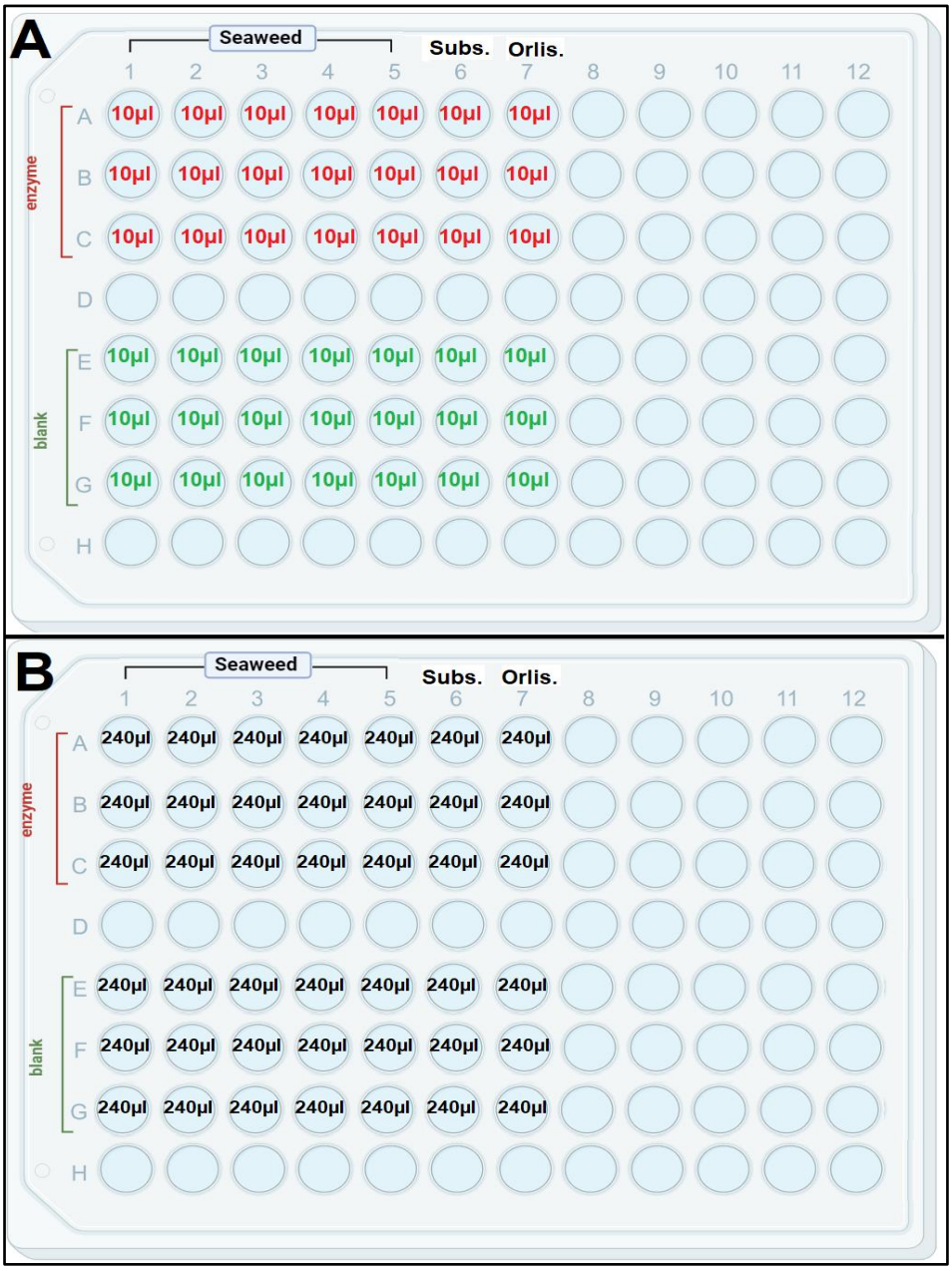

Figure 2. 5 96-well plate layout for pancreatic lipase activity assay.

Figure 2. 5A shows plate A, which contained 10µl of pancreatic lipase at 1.29 mg/ml combined with colipase (Row A-C) labelled 'enzyme', and 10µl of WE (Row E-G) labelled 'blank', which served as a negative control. Figure 2. 5B shows Plate B, which contained 240 µl of seaweed solutions at concentrations 5, 2.5, 1.25, 0.5 and 0.25 mg/ml (in separate Columns from 1 to 5, respectively). In addition, 240 µl of the oil substrate solution alone and Orlistat diluted to 0.025 mg/ml in oil substrate solution were dispensed into Columns 6 and 7, respectively (Figure 2. 5B). After 10 minutes of incubation at 37°C, a 96-well plate A was inserted into the microplate reader. Next, 200 µl of the seaweed at different concentrations (in Columns 1 to 5), substrate control (in Column 6) and orlistat solutions (in Column 7) from Figure 2. 5B were added to plate A and immediately read every 5 minutes at 405 nm for 45 minutes.

2.3.7 Calculations

- The percentage of lipase inhibition was calculated as depicted in Equation 2. 3.

$$\textbf{\textit{Lipase inhibition}}\ \% = \mathbf{1} - \left(\frac{\textbf{\textit{inhibition control} - \textit{seaweed sample}}}{\textbf{\textit{inhibition control} - \textit{lipase control}}}\right) \times \mathbf{100}$$

Equation 2. 3 Pancreatic Lipase Inhibition %.
Inhibition positive control is Orlistat, seaweed sample is the seaweed and lipase negative control is substrate only.

2.3.8 Statistical Analysis

All raw data were extracted from the spectrophotometer and uploaded onto a Microsoft Excel spreadsheet. Subsequently the data were then extracted and analysed using GraphPad Prism 9 software. To evaluate the differences in WE and EE yields from seaweed, an unpaired t-test was used. In addition, the correlation between absorbance values measured at 405 nm and time was determined using the Spearman correlation coefficient. To evaluate pancreatic lipase activity at different seaweed concentrations, a one-way ANOVA with post-hoc Dunnett's test was conducted after assessing normality using the Shapiro-Wilk test. The significance was determined at $P<0.05$. In addition, a two-way ANOVA using post-hoc Bonferroni tests was used to examine any differences in pancreatic lipase activity inhibition among seaweed extracts and different seaweeds. Furthermore, the relationship between seaweed concentrations and pancreatic lipase inhibition was determined using Pearson correlation coefficients. Finally, IC_{50} was calculated by generating a dose-response curve (non-linear regression) between the percentage of pancreatic lipase inhibition and the logarithm of inhibitor (seaweed) concentrations. Each experiment was performed in three independent experiments, each with three biological replicates. All data are presented as mean ± standard deviation (SD), Unless otherwise stated.

2.4 Results

2.4.1 Extraction Yields

The extraction yields of green Seaweed *C. sericioides* demonstrated a higher yield when extracted with 100% EE compared to WE, yielding 21.5 ± 0.2% vs. 16.5 ± 5.1% (p=0.3), respectively (Table 2. 3). In addition, the extraction yields of brown Seaweed *S. trinodis* collected in November 2020 demonstrated a higher yield when extracted with 100% EE than WE, yielding 7.0% (± 3.0) vs. 5.5% (± 1.9) (p=0.5), respectively. Conversely, in the case of *S. trinodis* collected in April 2021, a reverse trend was observed, with the WE extraction yield significantly higher than the yield from 100% EE yielding, 15.7% (± 3.7) vs. 1.1% (± 0.1) (p=0.002), respectively (Table 2. 3).

Regarding seasonal variability, the WE extraction yield for *S. trinodis* was significantly higher in April than in November, yielding 15.7% (± 3.7) and 5.5% (± 1.9) (p=0.01), respectively. However, when extracted with 100% EE, *S. trinodis* showed a significantly larger extraction in November compared to April, 7.0% (± 3.0) and 1.1% (± 0.1) (p=0.03), respectively (Table 2. 3).

Furthermore, a comparison of WE vs. 50% EE was conducted across three different seaweed species (Table 2. 3). *C. papillatum* and *C. Sinuosa* exhibited significantly higher extraction yields when extracted with 50% EE compared to WE, yielding 76.7% (± 1.5) vs. 62.7% (± 4.9) (p=0.0004) and 31.4% (± 1.3) vs. 16.3% (± 1.3) (p=0.0001), respectively (Table 2. 3). Conversely, in the case of *I. stellata*, a reverse trend was observed, with the WE extraction yield significantly higher than the yield from 50% EE yielding, 4.2% (± 0.4) vs. 2.9% (± 0.2) (p=0.002), respectively However, no significant differences in extraction yields were noted between WE and 50% EE for *P. boergesenii* (Table 2. 3).

Table 2. 3 The extraction yields using deionised water vs. 100% or 50% ethanol from Kuwaiti seaweed

Seaweed Species	Seaweed	Extraction Yield %			
		Extraction solvent			p-value
		WE	**100% EE**	**50% EE**	
Green seaweed	*Cladophora sericioides*	16.5 ± 5.1	21.5 ± 0.2	-	0.3
	Codium papillatum	62.7 ± 4.9	-	76.7 ± 1.5	0.0004
Brown seaweed	*Sirophysalis trinodis* N	5.5 ± 1.9 [b]	7.0 ± 3.0 [a]	-	0.5
	Sirophysalis trinodis A	15.7 ± 3.7 [a]	1.1 ± 0.1 [b]	-	0.002
	Colpomenia Sinuosa	16.3 ± 1.3	-	31.4 ± 1.3	0.0001
	Iyengaria stellata	4.2 ± 0.4	-	2.9 ± 0.2	0.002
	Padina boergesenii	36.6 ± 4.3	-	36.7 ± 1.	0.9
Red seaweed	*Gelidium Pusillum*	22.3 ± 2.2	-	23.6 ± 0.9	0.3

Seaweeds were subject to extraction using a solid-to-solvent ratio of 1:10 using deionised water (WE) and ethanol (EE) (100 or 50%). The yield variations were analysed using an unpaired t-test, significant at $p<0.05$. Value with different letters in each column shows significant difference Data are presented as mean ± standard deviation (n=3). N and A identify if the extraction was performed in November or April, respectively.

2.4.1.1 Comparing the Extraction Yields from Kuwaiti Seaweed Collected between 2019 to 2022

The comparison between WE extraction yields of eight seaweeds was determined, as shown in Appendix 1A. The WE yield extraction of the green seaweed *C. papillatum* was significantly the highest among all seaweeds 62.7% (± 4.9) ($p \leq 0.03$) (Appendix 1A). The WE yield extraction of *P. boergesenii* was significantly higher than that from red seaweed (*G. pusillum*), green seaweed (*C. sericioides*) and other brown seaweed (*C. sinuosa*, *S. trinodis* collected in April and *I. stellata*) ($p \leq 0.03$) (Appendix 1A). However, there was no significant difference between the WE extraction yield from brown seaweed *P. boergesenii* and brown seaweed (*S. trinodis* collected in November) ($p = 0.06$) (Appendix 1A).

The red seaweed *G. pusillum* had significantly higher WE extraction yield than brown seaweeds, including *C. sinuosa*, *S. trinodis* collected in November and April and *I. stellata*, ($p \leq 0.03$) (Appendix 1A). However, there was no significant difference between the WE yield of red seaweed *G. pusillum* and green seaweed (*C. sericioides*) ($p = 0.7$). There were no significant differences between the WE extraction yield of green seaweed (*C. sericioides*) compared to that of brown seaweeds, including *C. sinuosa, S. trinodis* N, *S. trinodis* A and *I. stellata* ($p > 0.9$) (Appendix 1A). In addition, there was no significant difference between the WE extraction yield of brown seaweeds *C. sinuosa* and that of *S. trinodis* collected in April. However, the brown seaweed *C. sinuosa* had significantly higher WE extraction yield than brown seaweed *S. trinodis* collected in November and *I. stellata* ($p < 0.03$). There was no significant difference between the WE extraction yield of brown seaweeds *I. stellata* and *S. trinodis* N and *S. trinodis* A ($p > 0.05$) (Appendix 1A).

Regarding the 100% EE yields, the green seaweed *C. sericioides* had significantly the highest yields ($p = 0.02$). However, there was no significant difference between the extraction yield of brown seaweed *S. trinodis* collected in November and April ($p = 0.1$) (Appendix 1B).

Regarding the 50% EE yields, the green seaweed *C. papillatum* had the highest extraction yield, followed by *P. boergesenii*, *C. sinuosa*, *G. pusillum*, and *I. stellata* ($p < 0.05$) (Appendix 1C).

2.4.2 Pancreatic Lipase Activity with a Positive Control Inhibitor (Orlistat)

Triacylglycerides hydrolysis into monoglyceride and free fatty acids by the enzyme pancreatic lipase can be quantified by measuring the change in the absorbance in the turbidity assay (Figure 2. 6). Reduced pancreatic lipase activity results in a decrease or absence of transparency in the olive oil substrate solution. In this study, inhibition was assessed using Orlistat as a positive control, which showed a consistent absorbance pattern over time, indicating a reduced or negligible pancreatic lipase activity. A significant reduction in absorbance was noted when comparing Orlistat with the olive oil substrate control over a 35-minutes (Appendix 2). The reduction over time was supported by a significantly strong negative correlation between absorbance and time (r_s= -1) (p =0.0004). To determine any reduction in pancreatic lipase activity, data obtained at the 20-minute mark were used to estimate any reductions in pancreatic lipase activity in all subsequent seaweed samples, indicating that the enzyme had reached its peak activity.

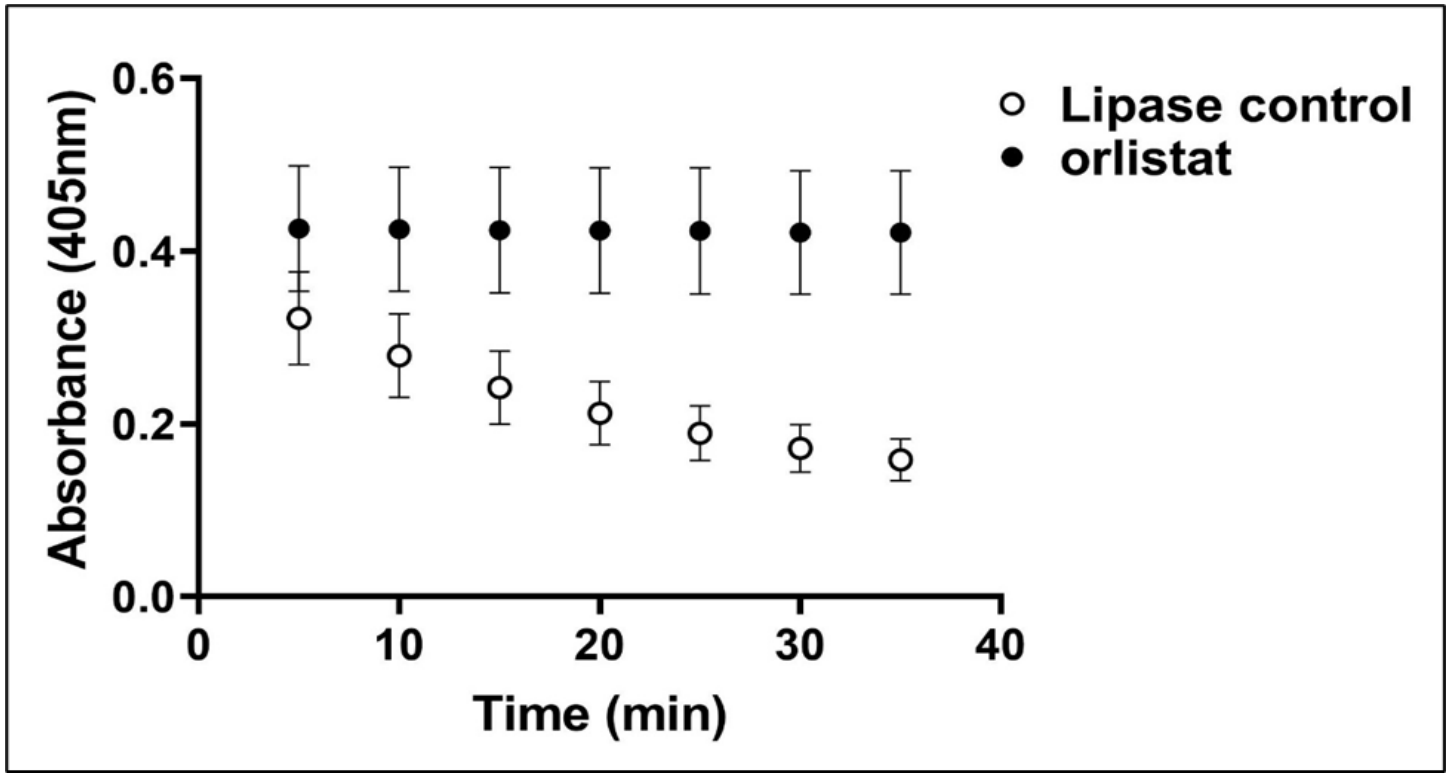

Figure 2. 6 Changes in absorbance at 405nm using the turbidity assay to assess pancreatic lipase activity and positive inhibitory control, Orlistat.

Changes in absorbance at 405 nm reflect changes in turbidity over a 35-minute duration. Diminished turbidly signifies the hydrolysis of triacylglycerides and was employed as an indicator of pancreatic lipase activity. ○ denotes pancreatic lipase activity using the olive oil substrate control and ● denotes pancreatic lipase activity with olive oil substrate and Orlistat, a well-established pancreatic lipase inhibitor. Data are presented as mean ± standard deviation (n=6).

2.4.3 Effect of Kuwait Seaweed Extracts on Pancreatic Lipase Activity Collected between 2019 to 2022

An assessment was conducted to evaluate the effects of different extraction methods from eight Kuwait seaweeds on pancreatic lipase activity. The extracted products included the homogenate, WE extract, 100% or 50% EE, and their EP at 4.8 and 2.4 mg/ml. The extraction products were obtained from green seaweed (*C. sericioides* and *C. papillatum*), brown seaweed (*S. trinodis* N, *S. trinodis* A, *C. Sinuosa, I. stellata*, *P. boergesenii*) and red seaweed (*G. Pusillum*). The findings revealed that most seaweed extracts collected from the Kuwait coastline between 2019 and 2021 showed significant inhibition of pancreatic lipase activity against the oil olive substrate alone (Table 2. 4).

The homogenate and EP from *C. sericioides* exhibited significant inhibition of pancreatic lipase activity at 4.8mg/ml, with inhibition rates of 99% (± 1.9, p = 0.0003) and 90% (± 7.2, p = 0.006), respectively (Table 2. 4). At a concentration of 2.4 mg/ml, inhibition rates of 87% (± 3.7, p = 0.002) and 67% (± 14.5, p = 0.04) were observed for *C. sericioides* homogenate and EP, respectively (Table 2. 4). Additionally, there was a significant albeit lower inhibition rate of pancreatic lipase, at 11.0% (± 1.1, p = 0.008) in the 100% EE from *C. sericioides* at 4.8mg/ml. No other significant reductions in pancreatic lipase activity were observed in any other extracts or concentrations for *C. sericioides* (Table 2. 4). Another green seaweed *C. papillatum* showed a significant reduction in pancreatic lipase at 4.8 mg/ml in the WE and EP of 73% (± 16.4, p=0.04) and 74% (± 11.8, p=0.02), respectively (Table 2. 4). No other significant differences in pancreatic lipase activity were noted in any other seaweed extracts at either 4.8 or 2.4mg/ml in *C. papillatum* (Table 2. 4).

Significant inhibition of pancreatic lipase activity was observed in the turbidity assay when utilising the homogenate and WE from *S. trinodis* N and *S. trinodis* A (Table 2. 4). At a concentration of 4.8 mg/ml, inhibition rates of 88% (± 6.4, p = 0.005)) and 99% (± 3.2, p = 0.0009) inhibition were recorded, while at 2.4 mg/ml, inhibition rates of 84% (± 14, p = 0.02) and 97% (± 5.3, p = 0.003) were noted for *S. trinodis* N homogenate and WE, respectively (Table 2. 4). Similarly, at 4.8 mg/ml, inhibition rates of 79% (±12.4, p = 0.02) and 92% (± 4.5, p = 0.002) were observed, and at 2.4 mg/ml, inhibition rates of 64% (± 3.3, p = 0.002) and 81% (± 3.9, p = 0.002) were recorded for *S. trinodis*

A homogenate and WE, respectively (Table 2. 4). No other significant levels of inhibition were observed for the 100% EE and EP from either *S. trinodis* N or *S. trinodis* A (Table 2. 4). In the other brown seaweeds *C. Sinuosa, I. stellata*, and *P. boergesenii*, the seaweed homogenate at 4.8 mg/ml significantly inhibited pancreatic lipase activity, with inhibition rates of 67% (± 9.2, p=0.02), 71% (± 5.8, p=0.006) and 59% (± 12.6, p=0.04), respectively (Table 2. 4). Similarly, at 2.4 mg/ml from the homogenates of *C. Sinuosa, I. stellata*, and *P. boergesenii*, inhibition rates of 71% (± 12.6, p=0.03), 66% (± 3.9, p=0.003) and 62% (± 7.4, p=0.01) were observed, respectively (Table 2. 4). Furthermore, a reduction in pancreatic lipase activity was noted in *P. boergesenii* WE and EP at 4.8 mg/ml of 82% (± 1.6, p=0.0003) and 52% (± 2.1, p=0.001), respectively (Table 2. 4). At a concentration of 2.4 mg/ml, an inhibition rate of 61% (± 3.0, p=0.002) was noted in *P. boergesenii* EP. No other significant inhibition of pancreatic lipase activity was observed in any other seaweed extracts at either 4.8 and 2.4mg/ml in *C. Sinuosa, I stellata* or *C. papillatum* (Table 2. 4).

In the red seaweed *G. Pusillum*, both the homogenate and EP exhibited an inhibitory effect on pancreatic lipase activity. The homogenate at 4.8 and 2.4mg/ml resulted in inhibition rates of 66.0% (± 7.0, p=0.009) and 62% (± 7.0, p=0.01), respectively ((Table 2. 4). Similarly, the EP induced a significant reduction in pancreatic lipase activity by 67% (± 8.3, p=0.01) and 61% (± 2.1, p=0.001) at concentrations of 4.8 and 2.4mg/ml, respectively (Table 2. 4). No other significant inhibition of pancreatic lipase was observed in the WE and 50% EE for *G. Pusillum* at 4.8 or 2.4mg/ml (Table 2. 4)

Table 2. 4 Pancreatic lipase inhibition collected between 2019 to 2022.

Seaweed Type	Seaweed		Kuwaiti seaweed extracts Extracts					
	Seaweed	Concentration	homogenate	WE	100% EE	100% EP	50% EE	50% EP
Green	*C. sericioides*	4.8 mg/ml	99.0 ± 1.9 (P= 0.0003)	36.0 ± 13.7 (P= 0.1)	11.0 ± 1.1 (P= 0.008)	90.0 ± 7.2 (P= 0.006)	-	-
		2.4 mg/ml	87.0 ± 3.7 (P= 0.002)	38.0 ± 17.2 (P= 0.2)	15.0 ± 5.5 (P= 0.1)	67.0 ± 14.5 (P= 0.04)	-	-
	C. papillatum	4.8 mg/ml	37.0 ± 17.0 (P= 0.2)	73.0 ± 16.4 (P= 0.04)	-	-	44.0 ± 14.5 (P= 0.1)	74.0 ±11.8 (P= 0.02)
		2.4 mg/ml	46.0 ± 18.0 (P= 0.1)	65.0 ± 17.6 (P= 0.1)	-	-	62.0 ± 17.8 (P= 0.2)	49.0 ± 22.2 (P= 0.2)
Brown	*S. trinodis* N	4.8 mg/ml	88.0 ± 6.4 (P= 0.005)	99.0 ± 3.2 (P= .0009)	37.7 ± 11.2 (P= 0.1)	37.0 ± 12.9 (P= 0.1)	-	-
		2.4 mg/ml	84.0±14.0 (P= 0.02)	97.0 ± 5.3 (P= 0.003)	36.0 ±15.2 (P= 0.1)	34.0 ± 9.8 (P= 0.1)	-	-
	S. trinodis A	4.8 mg/ml	79.0±12.4 (P= 0.02)	92.0 ± 4.5 (P= 0.002)	11.3 ± 3.2 (P= 0.1)	24.0 ± 13.3 (P= 0.2)	-	-
		2.4 mg/ml	64.0 ± 3.3 (P= 0.002)	81.0 ± 3.9 (P= 0.002)	6.0 ± 1.7 (P= 0.1)	40.0 ± 15.4 (P= 0.1)	-	-
	C. Sinuosa	4.8 mg/ml	67.0 ± 9.2 (P= 0.02)	45.0 ± 14.3 (P= 0.1)	-	-	8.0 ± 6.7 (P= 0.4)	54.0 ±21.2 (P= 0.1)
		2.4 mg/ml	71.0 ± 12.6 (P= 0.03)	36.0 ± 12.7 (P= 0.1)	-	-	3.0 ± 3.2 (P= 0.6)	45.0 ± 24.3 (P= 0.2)

	I. stellata	4.8 mg/ml	71.0 ± 5.8 (P= 0.006)	74.0 ± 23.0 (P= 0.1)	-	-	18.0 ±15.7 (P= 0.4)	30.0 ± 10.5 (P= 0.1)
		2.4 mg/ml	66.0 ± 3.9 (P= 0.003)	46.0 ± 14.6 (P= 0.1)	-	-	5.0 ± 3.6 (P= 0.3)	7.0 ± 4.2 (P= 0.3)
	P. boergesenii	4.8 mg/ml	59.0 ± 12.6 (P= 0.04)	82.0 ± 1.6 (P= .0003)	-	-	15.0 ± 12.0 (P= 0.4)	52.0 ± 2.1 (P= 0.001)
		2.4 mg/ml	62.0 ± 7.4 (P= 0.01)	35.0 ± 17.2 (P= 0.2)	-	-	3.0 ± 1.7 (P= 0.2)	61.0 ± 3.0 (P= 0.002)
Red	***G. Pusillum***	4.8 mg/ml	66.0 ± 7.0 (P= 0.009)	46.0 ± 17.5 (P= 0.1)	-	-	4.0 ± 3.6 (P= 0.4)	67.0 ± 8.3 (P= 0.01)
		2.4 mg/ml	62.0 ± 7.0 (P= 0.01)	25.0 ± 11.9 (P= 0.2)	-	-	5.0 ± 3.4 (P= 0.3)	61.0 ± 2.1 (P= 0.001)

Seaweed homogenate and extracts in deionised water (WE), 100% or 50% ethanol (EE), and an ethanol pellet remaining after ethanol extraction (EP) were evaluated for their ability to inhibit pancreatic lipase activity at concentrations of 4.8 and 2.4 mg/ml. A one-way ANOVA with a Dunnett's post-hoc analysis was employed to assess the significant impact of seaweed concentrations on pancreatic lipase activity ($p<0.05$). Data are presented as mean ± standard deviation (n=3).

2.4.4 Seaweeds and Seaweed Extracts Selected for Further Analysis Relating to Pancreatic Lipase Inhibition

After evaluating various seaweeds and their extracts, the extracts capable of significantly inhibiting pancreatic lipase activity were identified. Table 2. 5 illustrates the seaweeds selected to further investigate their ability to inhibit pancreatic lipase. Subsequently, all the seaweeds and their extracts presented in Table 2. 5 were then tested using concentrations of 4.8, 2.4, 1.2, 0.48, and 0.24 mg/ml.

Table 2. 5 Selected seaweed and their extracts for subsequent experiments based on their ability to inhibit pancreatic lipase.

Seaweeds	Seaweed Extracts		
	Homogenate	**WE**	**Ethanol pellet**
Green	*Cladophora sericioides*		*Cladophora sericioides*
		Codium papillatum	*Codium papillatum*
Brown	*Sirophysalis trinodis N*	*Sirophysalis trinodis N*	
	Sirophysalis trinodis A	*Sirophysalis trinodis A*	
	Colpomenia Sinuosa		
	Iyengaria stellata		
	Padina boergesenii	*Padina boergesenii*	*Padina boergesenii*
Red	*Gelidium Pusillum*		*Gelidium Pusillum*

Seaweed species and extracts that significantly inhibited pancreatic lipase were used for subsequent experiments. N and A denote extraction of *Sirophysalis trinodis* in November and April, respectively.

2.4.5 Effect of Seaweed Homogenate on Pancreatic Lipase Activity

The effect of seven Kuwaiti seaweed homogenates from green, red and brown seaweeds was evaluated for their ability to inhibit pancreatic lipase against a lipase-only substrate control (Figure 2. 7 and Figure 2. 8).

2.4.6.1 Effect of Green and Red Homogenate on Pancreatic Lipase

significant inhibition rates were observed of 99% (± 1.9, P = 0.0003), 87% (± 3.7, p = 0.002) and 82% (± 4.3, p = 0.002) on pancreatic lipase activity at concentrations of 4.8, 2.4, and 1.2 mg/ml, respectively, from the green seaweed *C. sericioides* homogenate when compared to the substrate only control (Figure 2. 7A). However, no significant inhibition was observed at concentrations 0.48 and 0.24 mg/ml for the homogenate extracted from *C. sericioides* (Figure 2. 7A).

Similarly, inhibition rates of 66% (± 7.0, p = 0.009), 62% (± 7.0, p = 0.01) and 69% (± 12.2, p = 0.03) were observed at concentrations of 4.8, 2.4 and 1.2 mg/ml in the presence of red seaweed *G. Pusillum* homogenate (Figure 2. 7B). No other significant inhibition of pancreatic lipase was identified at any concentrations below 1.2 mg/ml in homogenates extracted from *G. Pusillum* (Figure 2. 7B).

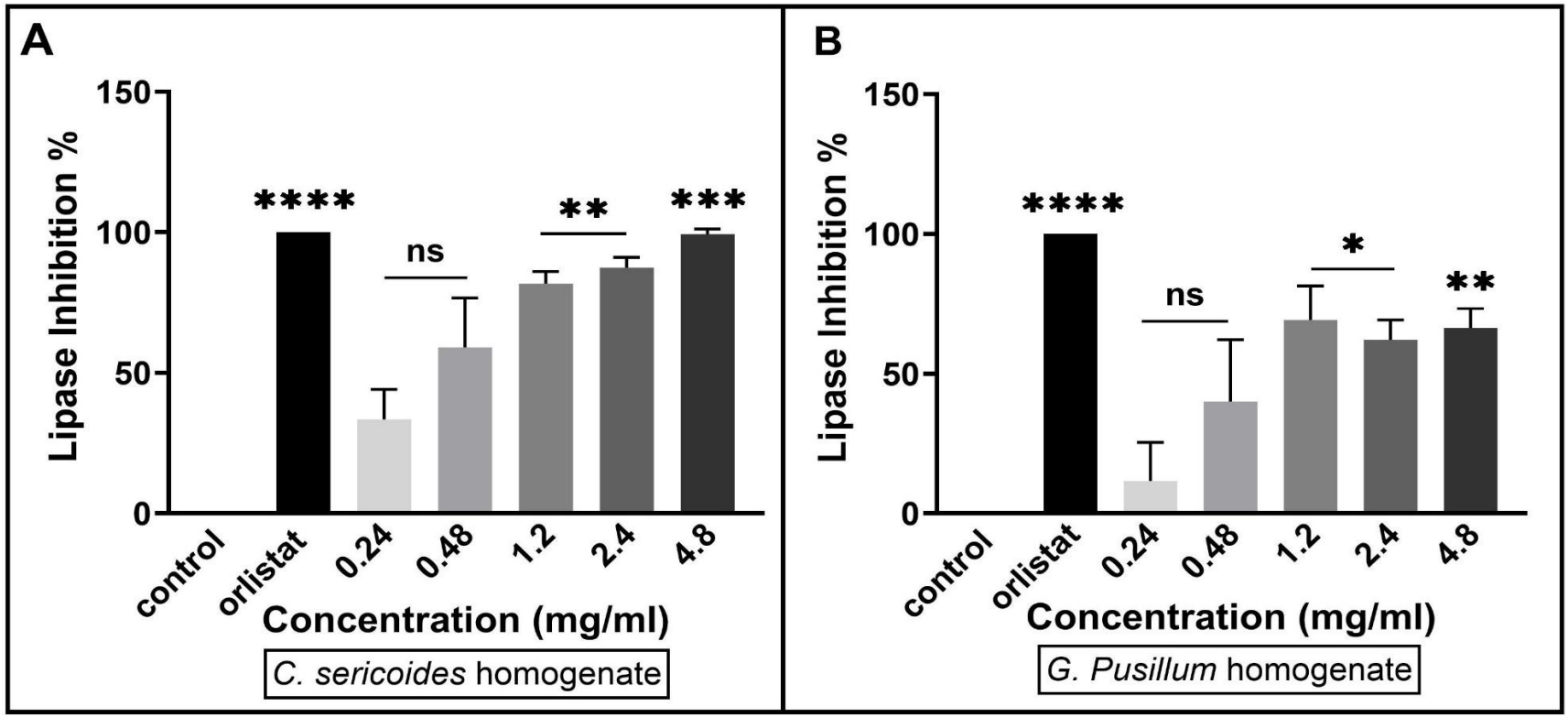

Figure 2. 7 Pancreatic lipase inhibition in the presence of homogenates extracted from green and red seaweeds and Orlistat.

Pancreatic lipase activity was evaluated in the presence of a substrate-only control, Orlistat, and green and red homogenates at various concentrations following extraction from various seaweeds. Figure 2. 7A depicts the inhibition upon adding the homogenate extracted from green seaweed *Cladophora sericioides* on pancreatic lipase. Figure 2. 7B depicts the inhibition of pancreatic lipase following adding the homogenate from red seaweed *Gelidium Pusillum*. To assess the effect of seaweed concentrations on pancreatic lipase activity against a lipase-only substrate control, a one-way ANOVA was conducted with a Dunnett's post-hoc analysis. Data are presented as mean ± standard deviation (n=3). *, **, ***, and **** denote significance at $p \leq 0.05$, ≤ 0.009, ≤ 0.0003, and < 0.0001, respectively.

2.4.6.2 Effect of Brown Homogenate on Pancreatic Lipase

Figure 2. 8 illustrate the activity of pancreatic lipase in the presence of homogenate extracted from brown seaweeds and Orlistat compared against a substrate-only control. The homogenate extracted from *S. trinodis* N inhibited pancreatic lipase at 4.8 and 2.4 mg/ml with inhibition rates of 88% (± 12.4, p = 0.005) and 84% (± 14.0, p = 0.02), respectively (Figure 2. 8A). However, there was no significant inhibition of pancreatic lipase activity in any other concentration for the homogenate extracted from *S. trinodis* N (Figure 2. 8A).

Similarly, the homogenate extracted from *S. trinodis* A inhibited pancreatic lipase at 4.8 and 2.4 mg/ml, with inhibition rates of 79% (± 6.4, p = 0.02), 64% (± 3.3, p = 0.002), respectively (Figure 2. 8B). Furthermore, the homogenate extracted from *S. trinodis* A also inhibited pancreatic lipase at 1.2 mg/ml by 57% (± 11.8, p = 0.04) (Figure 2. 8B). However, there was no significant inhibition of pancreatic lipase activity at concentrations 0.48 and 0.24 mg/ml for the homogenate extracted from *S. trinodis* A (Figure 2. 8B).

The homogenate extracted from *C. Sinuosa* at concentrations 4.8 and 2.4 mg/ml Significantly inhibited Pancreatic lipase with inhibition rates of 67% (± 9.2, p = 0.02) and 71% (± 12.6, p = 0.03), respectively (Figure 2. 8C). However, no other significant inhibition of pancreatic lipase was observed at any other concentrations for *C. Sinuosa* (Figure 2. 8C).

Similarly, the homogenate extracted from *I. stellata* significantly inhibited pancreatic lipase at concentrations of 4.8 and 2.4 mg/ml with inhibition rates of 71% (± 5.8, p = 0.006) and 66% (± 3.9, p = 0.003), respectively (Figure 2. 8D). However, no other significant inhibition of pancreatic lipase was observed at any other concentrations for *I. stellata* (Figure 2. 8D).

Figure 2. 8E depicts the inhibition rates of pancreatic lipase, with significant inhibition rates of 59% (± 12.6, p = 0.04), 62% (± 7.4, p = 0.01) and 55% (± 9.6, p = 0.03) in the presence of *P. boergesenii* homogenate at 4.8, 2.4 and 1.2 mg/ml, respectively. No other significant inhibition of pancreatic lipase was identified at any concentrations below 1.2 mg/ml in homogenates extracted from *P. boergesenii* (Figure 2. 8E).

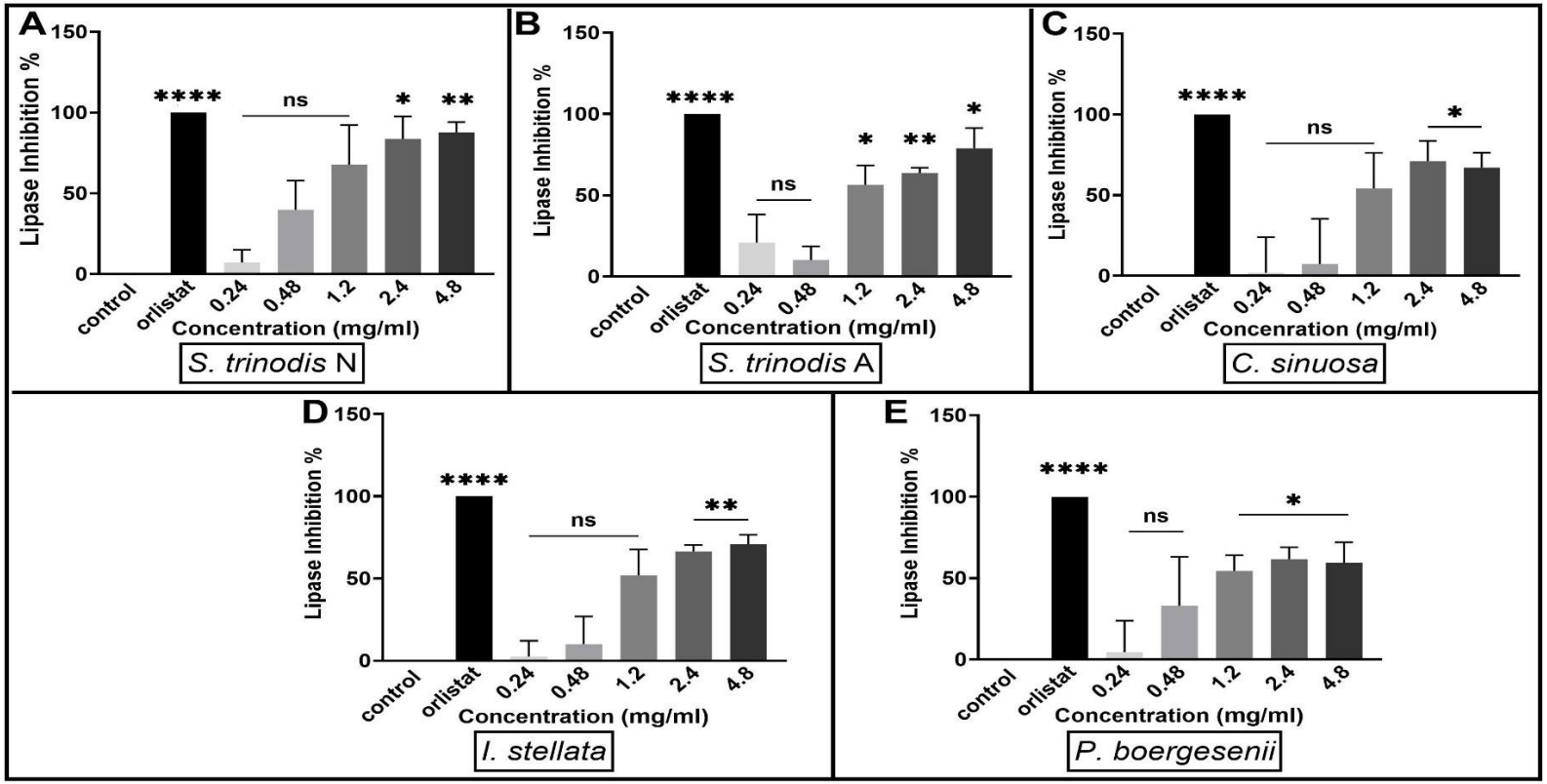

Figure 2. 8 Pancreatic lipase inhibition in the presence of homogenates extracted from brown seaweeds and Orlistat.

Pancreatic lipase activity was evaluated in the presence of a substrate-only control, Orlistat and seaweed homogenate at various concentrations following extraction from various brown seaweeds. Figure 2. 8A to E illustrate the inhibition of pancreatic lipase following the addition of homogenates from brown seaweeds *Sirophysalis trinodis* N, *Sirophysalis trinodis* A, *Colpomenia Sinuosa*, *Iyengaria stellata* and *Padina boergesenii*, respectively. To assess the effect of seaweed concentrations on pancreatic lipase activity against a lipase-only substrate control, a one-way ANOVA was conducted with a Dunnett's post-hoc analysis. Data are presented as mean ± standard deviation (n=3). *, **, ***, and **** denote significance at p ≤0.05, ≤0.009, ≤ 0.0003, and < 0.0001, respectively.

2.4.6 Effect of Seaweed WE on Pancreatic Lipase Activity

The effects of a WE from four Kuwaiti seaweeds and Orlistat were evaluated for their ability to inhibit pancreatic lipase (Figure 2. 9).

The WE from the green seaweed *C. papillatum* significantly inhibited pancreatic lipase, with inhibition rates of 73% (± 16.4, p = 0.04) and 42% (± 4.2, p = 0.009) at concentrations of 4.8 and 0.48 mg/ml, respectively. However, no significant inhibition of pancreatic lipase was observed at concentrations 2.4, 1.2 or 0.24 mg/ml (Figure 2. 9A).

The data presented in Figure 2. 9B to D present the inhibition properties of the WE extracted from brown seaweeds *S. trinodis* N, S. trinodis A and *P. boergesenii*. There was a significant reduction in pancreatic lipase activity, with inhibition rates of almost 99% (± 3.2, p = 0.0009) and 97% (±5.3, p = 0.003) for 4.8 and 2.4 mg/ml, respectively. In addition, a significant reduction in pancreatic lipase activity, with inhibition rates of 75% (± 16.0, p = 0.04) and 70% (± 6.4, p = 0.007) were observed for the WE from *S. trinodis* N at 1.2 and 0.48 mg/ml, respectively (Figure 2. 9B). However, no significant inhibition of pancreatic lipase activity was observed at a concentration of 0.24 mg/ml (Figure 2. 9B).

The WE extracted from *S. trinodis* A exhibited significant inhibitory effects on pancreatic lipase activity at all concentrations, with inhibition rates of 92% (± 4.5, p = 0.002), 81% (± 3.9, p = 0.002), 81% (± 4.7, p = 0.003), 92% (± 4.1, p = 0.002) and 37% (± 8.7, p = 0.05) at concentrations 4.8, 2.4, 1.2, 0.48, and 0.24 mg/m, respectively (Figure 2. 9C).

Finally, a significant reduction in pancreatic lipase activity of 82% (± 1.6, p = 0.0003) was observed when adding the WE from *P. boergesenii* at 4.8mg/ml (Figure 2. 9D). No other significant inhibition of pancreatic lipase was noted at any other concentrations for the WE from *P. boergesenii* (Figure 2. 9D).

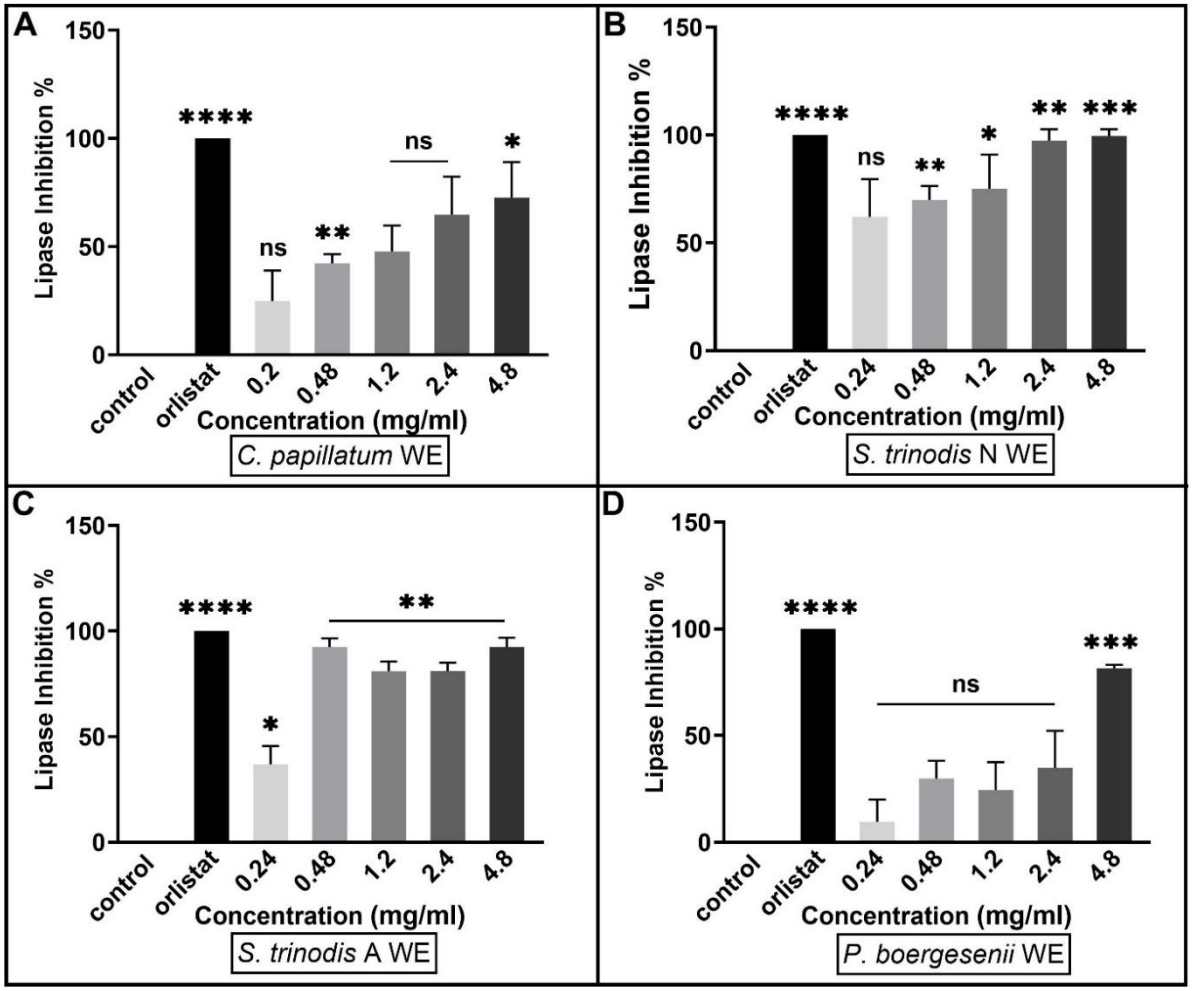

Figure 2. 9 The effect of seaweed WE on pancreatic lipase activity.
Pancreatic lipase activity was evaluated in the presence of the substrate-only control, Orlistat, and WE (deionised water extract) at various concentrations and from different seaweeds. Figure 2. 9A shows the effect of WE from the green seaweed *C. papillatum* on pancreatic lipase activity. Figure 2. 9B and C show the effect of WE from the brown seaweed *S. trinodis* N and *S. trinodis* A, respectively, on pancreatic lipase activity. Figure 2. 9D shows the effect of WE from the brown seaweed *P. boergesenii* on pancreatic lipase activity. To assess the effect of seaweed concentrations on pancreatic lipase activity against a lipase-only substrate control, a one-way ANOVA was performed with a Dunnett's post-hoc analysis. Data presented as mean ± standard deviation (n=3). *, **, *** and **** denote significance at p≤0.05, ≤0.008), ≤0.0009, and <0.0001, respectively.

2.4.7 EP Effect on Pancreatic Lipase Activity

The effects of EP extracted from four different seaweeds and Orlistat were evaluated for their ability to inhibit pancreatic lipase (Figure 2. 10).

A significant reduction in pancreatic lipase activity was observed from the 100% EP from the green seaweed *C. sericioides*, with inhibition rates of 90% (± 7.2, p = 0.006) and 67% (± 14.5, p = 0.04) at concentrations of 4.8 and 2.4 mg/ml, respectively (Figure 2. 10A). No other significant inhibition of pancreatic lipase activity was observed at any other concentration in *C. sericioides* 100% EP.

Additionally, significant inhibition of pancreatic lipase activity was noted from the 50% EP from the green seaweed *C. papillatum*, with inhibition rates of 74% (± 11.8, p = 0.02) and 70% (± 13.6, p = 0.03) at concentrations of 4.8 and 0.48 mg/m, respectively (Figure 2. 10B). No other significant inhibition of pancreatic lipase activity was observed at any other concentration in *C. papillatum* EP (Figure 2. 10B).

The 50% EP from the brown seaweed *P. boergesenii* demonstrated inhibition rates of 52% (± 2.1, p = 0.001), 61% (± 3.0, p = 0.002) and 35% (± 3.0, p = 0.007) at concentrations of 4.8, 2.4, and 1.2 mg/ml, respectively (Figure 2. 10C). No other significant inhibition of pancreatic lipase activity was observed at any other concentration in *P. boergesenii* 50% EP (Figure 2. 10C).

In the red seaweed, *G. Pusillum*, the 50% EP resulted in inhibition rates of 67% (± 8.3, p = 0.01) and 61% (± 2.1, p = 0.001) at concentrations of 4.8 and 2.4 mg/ml, respectively (Figure 2. 10D). No other significant inhibition in pancreatic lipase activity was observed at any other concentrations for the EP from *G. Pusillum* (Figure 2. 10D).

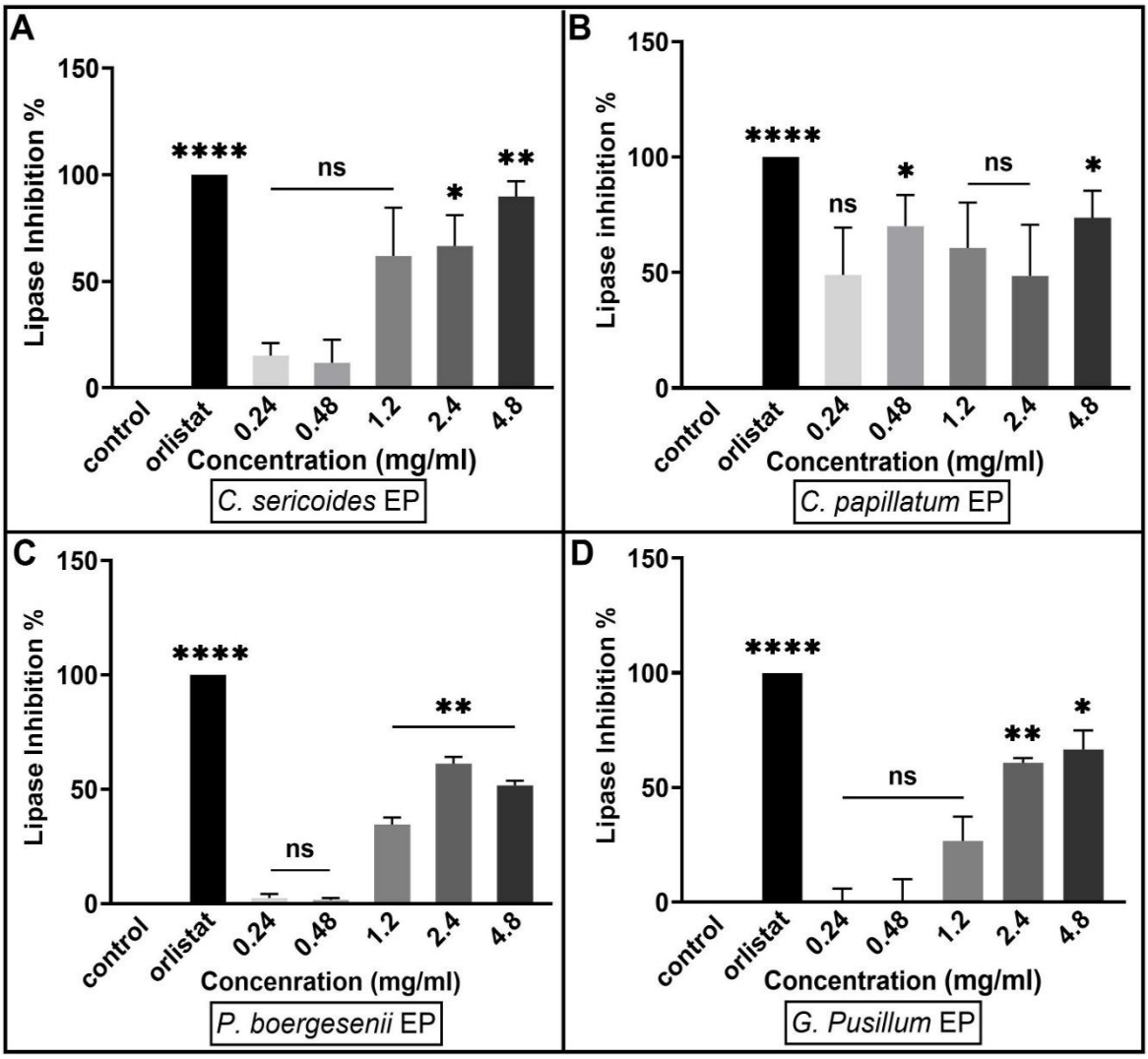

Figure 2. 10 The effect of seaweed EP on pancreatic lipase activity.
Pancreatic lipase activity was assessed in the presence of the substrate-only control, Orlistat, and ethanol pellets (EP) at various concentrations extracted from different seaweeds at 50 or 100% ethanol. Figure 2. 10A shows the effect of the EP extracted from *Cladophora sericioides* using 100% ethanol on pancreatic lipase activity. Figure 2. 10B shows the effect of the EP extracted from *Codium papillatum* using 50% ethanol on pancreatic lipase activity. Figure 2. 10C shows the effect of the EP extracted from *Padina boergesenii* using 50% ethanol on pancreatic lipase activity. Figure 2. 10D shows the effect of the EP extracted from *Gelidium Pusillum* using 50% ethanol on pancreatic lipase activity. A one-way ANOVA with Dunnett's post-hoc analysis was conducted to evaluate the effect of seaweed concentrations on pancreatic lipase activity. Data was presented as mean ± standard deviation (n=3). *, **, ***, and **** denote significance at p≤0.05, ≤0.008, ≤0.0009, and <0.0001, respectively.

2.4.8 Effects of Temporal Variation on Inhibitory Potential in Brown Seaweed

The temporal effects of homogenate and WE from the brown seaweed *S. trinodis* were assessed by comparing their ability to inhibit pancreatic lipase activity in seaweed collected in November and April. No significant temporal effects were observed when comparing the homogenate extract from *S. trinodis* N and S. trinodis A at any concentration (Figure 2. 11A). This finding persisted despite reduced pancreatic lipase activity at all concentrations. In contrast, a significant temporal effect on pancreatic lipase activity was noted in the WE from *S. trinodis*. Specifically, significantly greater inhibition of pancreatic lipase was observed in the November extract compared to the April extract at 2.4 and 0.24 mg/ml, ($p = 0.04$) and ($p = 0.006$), respectively. However, at concentrations of 0.48 mg/ml, there was a significantly higher pancreatic lipase inhibition in the April extract ($p = 0.003$) (Figure 2. 11B).

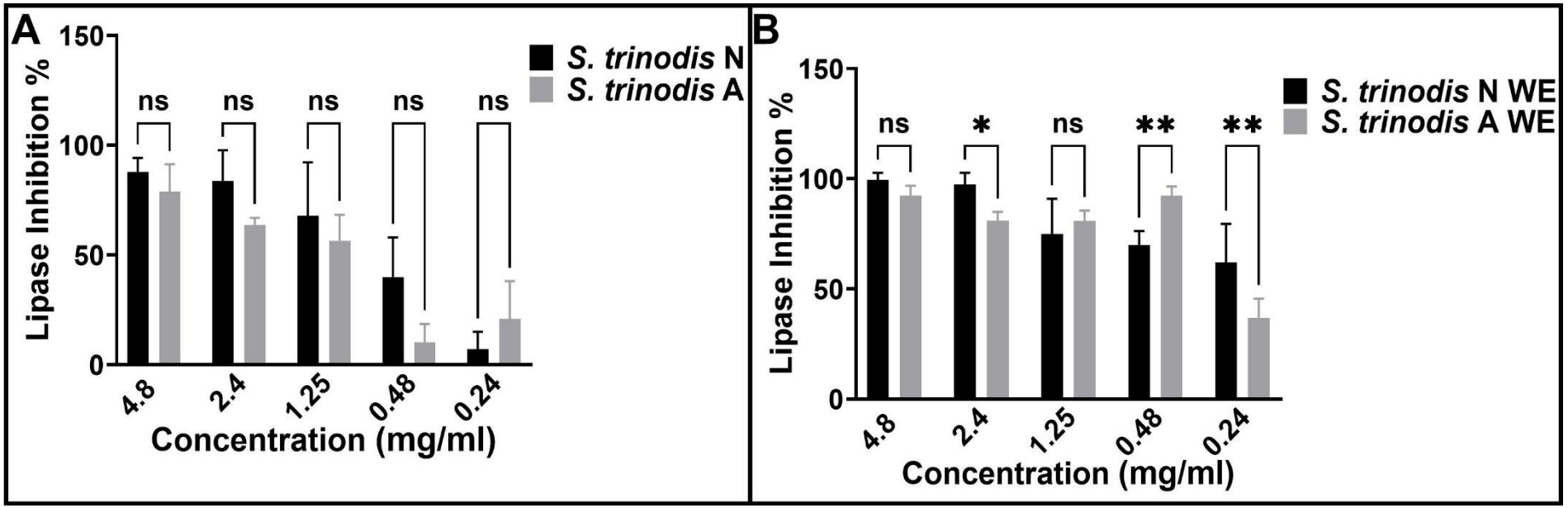

Figure 2. 11 Impact of temporal variation on pancreatic lipase activity in the brown seaweed *Sirophysalis trinodis*.
The brown seaweed *Sirophysalis trinodis* was collected from the Kuwait coastline in November and April. Figure 2. 11A shows the effect of the homogenate extracted from *Sirophysalis trinodis* collected in November and April on pancreatic lipase activity. Figure 2. 11B shows the effect of the WE (deionised water extract) from *Sirophysalis trinodis* N and *Sirophysalis trinodis* A on pancreatic lipase activity. To assess any differences in pancreatic lipase inhibition between samples collected in November and April, a two-way ANOVA with Bonferroni's post-hoc analysis was performed. Data presented as mean ± standard deviation (n=3). * and ** denote a significant difference at $p<0.05$ and <0.006, respectively. N and A denote *Sirophysalis trinodis* collected in November and April, respectively.

2.4.9 Evaluation of any Associations between Pancreatic Lipase Inhibition, Different Seaweed Species, Extracts, and Concentrations

To explore potential associations between pancreatic lipase inhibition and different factors such as seaweed species, seaweed extracts and concentrations, Pearson correlation coefficient tests were conducted. Notably, no significant association were found between pancreatic lipase inhibition and green, brown and red seaweed homogenates ($p > 0.05$), as shown in Figure 2. 12A, Figure 2. 13A and Figure 2. 14A, respectively. Conversely, a strong positive correlation was detected between the level of pancreatic lipase inhibition and the concentration of the WE from the green seaweed *C. papillatum* ($r = 0.91$, $p = 0.03$) (Figure2. 12B).

Moreover, a noteworthy strong positive correlation was identified between the level of pancreatic lipase inhibition and the concentrations of the ethanol pellet extracted from *C. sericioides* using 100% ethanol ($r = 0.89$, $p = 0.04$) (Figure 2. 12C). However, there was no such association between pancreatic lipase inhibition and the concentration of the EP using 50% ethanol from *C. papillatum* ($p= 0.47$) (Figure 2. 12C).

The data presented in Figure2. 13 illustrates the associations between the brown seaweeds and the level of pancreatic lipase inhibition. While no significant associations were detected between the seaweed homogenate concentrations from *P. boergesenii* and *S. trinodis* and pancreatic lipase inhibition (Figure 2. 13A), a significantly strong positive correlation emerged between the concentrations of WE extracted from *S. trinodis* N and *P. boergesenii* and the level of pancreatic lipase inhibition ($r = 0.90$ ($p= 0.03$) and 0.95 ($p= 0.01$), respectively) (Figure 2. 13B). However, no other significant associations were found between pancreatic lipase inhibition and the concentrations of WE *S. trinodis* A ($p= 0.37$). In addition, the data presented in Figure 2. 13C illustrates no associations between the different concentrations of the EP using 50% ethanol from *P. boergesenii* and the level of pancreatic lipase inhibition ($p= 0.11$).

Figure 2. 14 demonstrates no significant associations between the different concentrations of the homogenate from red seaweed *G. Pusillum* and the level of pancreatic lipase inhibition (p= 0.23) (Figure 2. 14A). Conversely, a significant strong correlation between pancreatic lipase inhibition and the different concentrations of the EP using 50% ethanol was obtained from *G. Pusillum* using (r = 0.91, p= 0.03) (**Figure 2. 14**B).

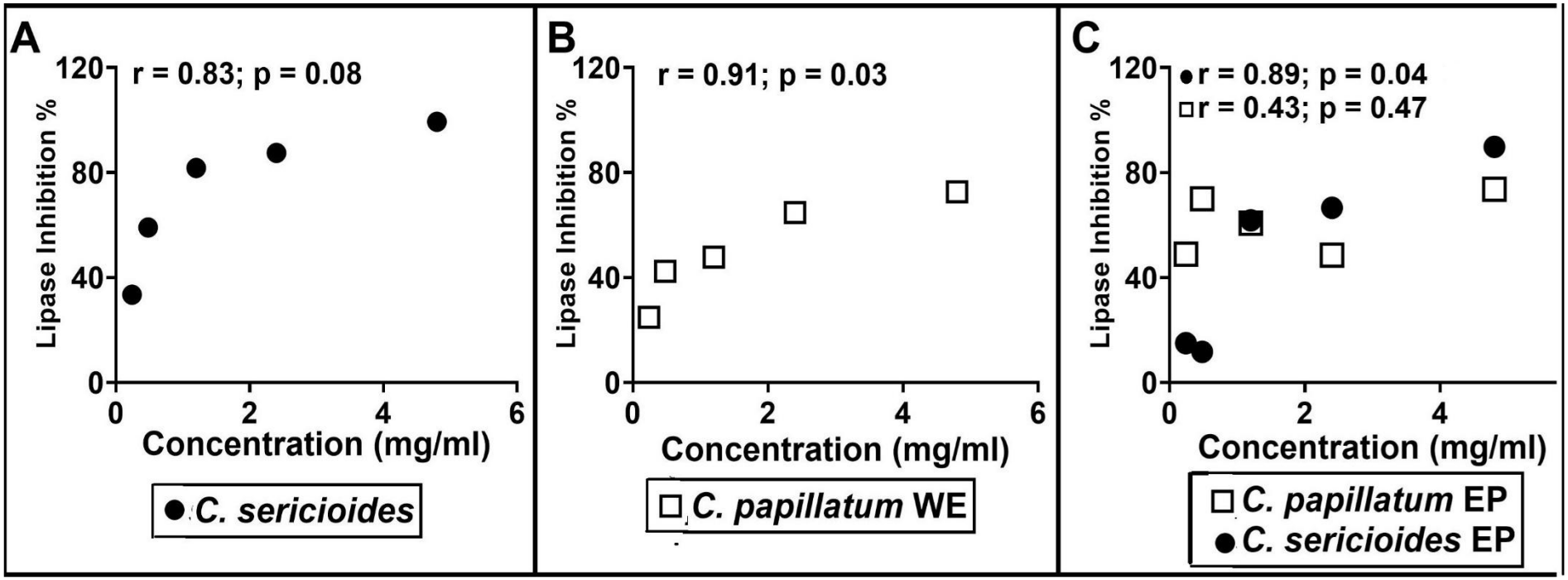

Figure 2. 12 Pearson correlation coefficients between pancreatic lipase inhibition and green seaweed concentrations.
Figure2.12A shows no significant correlation between pancreatic lipase inhibition and homogenate concentration extracted from *Cladophora sericioides* (p=0.08). Figure 2. 12BB shows a strong positive correlation between pancreatic lipase inhibition and WE (deionised water) concentrations extracted from *Codium papillatum* (r = 0.91, p=0.03). Figure 2. 12C shows associations between the EP (ethanol pellet) using 100% ethanol concentrations extracted from *Cladophora sericioides* and pancreatic lipase inhibition showing a strong positive correlation with (r = 0.89, p=0.04). There is no significant correlation between concentrations of the 50% ethanol pellet extracted from *Codium papillatum* (p=0.47).

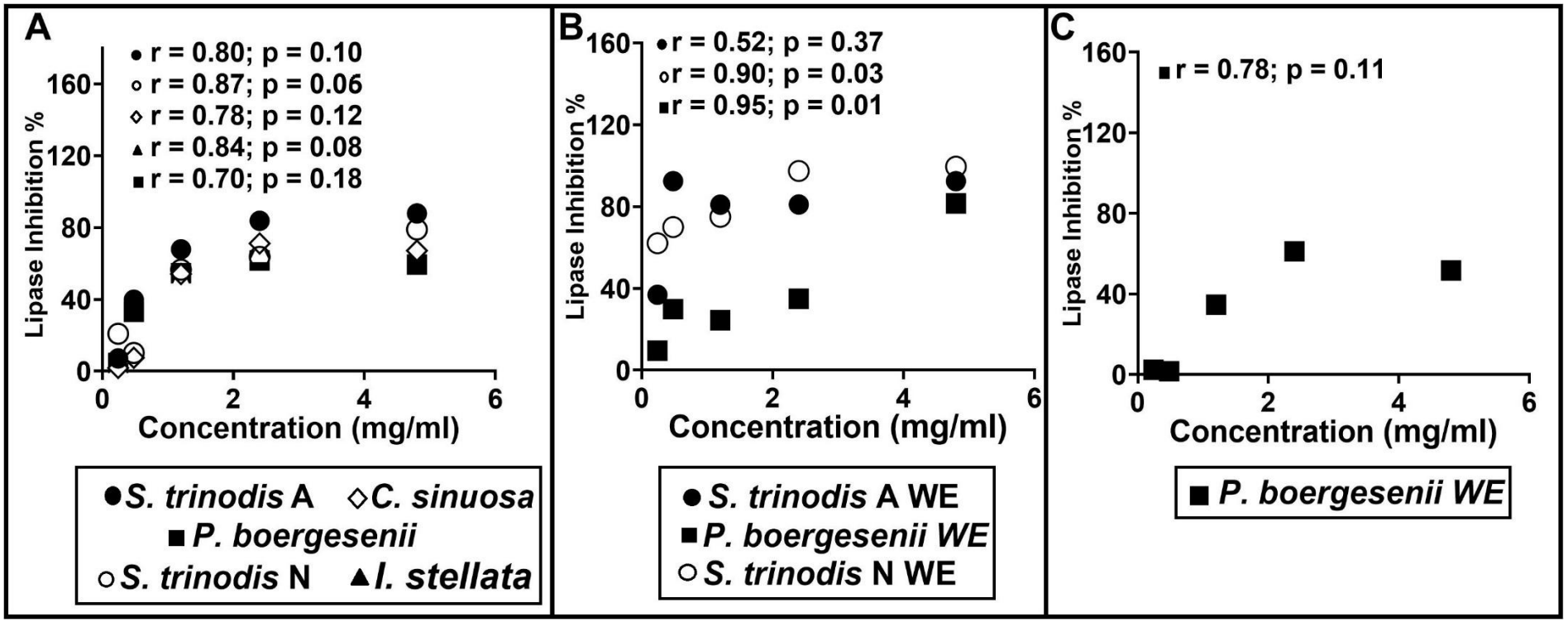

Figure 2. 13 Pearson correlation coefficients between pancreatic lipase inhibition and brown seaweed concentrations.
All experiments were performed using brown seaweeds *Sirophysalis trinodis* collected in April and November, *Colpomenia sinuosa*, *Iyengaria stellata* and *Padina boergesenii*. Figure 2. 13A depicts no significant associations between pancreatic lipase inhibition and concentration of the seaweed homogenate in any of the brown seaweeds ($p>0.05$). Figure 2. 13B shows a significant and strong positive correlation between pancreatic lipase inhibition and deionised water extract (WE) concentrations extracted from *Sirophysalis trinodis* collected in November ($r = 0.90$, $p=0.3$) and *Padina boergesenii* ($r = 0.95$, $p=0.01$). No significant association between pancreatic lipase inhibition and the concentration of WE from *Sirophysalis trinodis* collected in April ($p=0.37$). Figure 2. 13C shows no significant association between concentrations from the ethanol pellet following 50% ethanol extraction (EP) from *Padina boergesenii* and pancreatic lipase inhibition ($p=0.11$). N and A denote *Sirophysalis trinodis* collected in November and April, respectively.

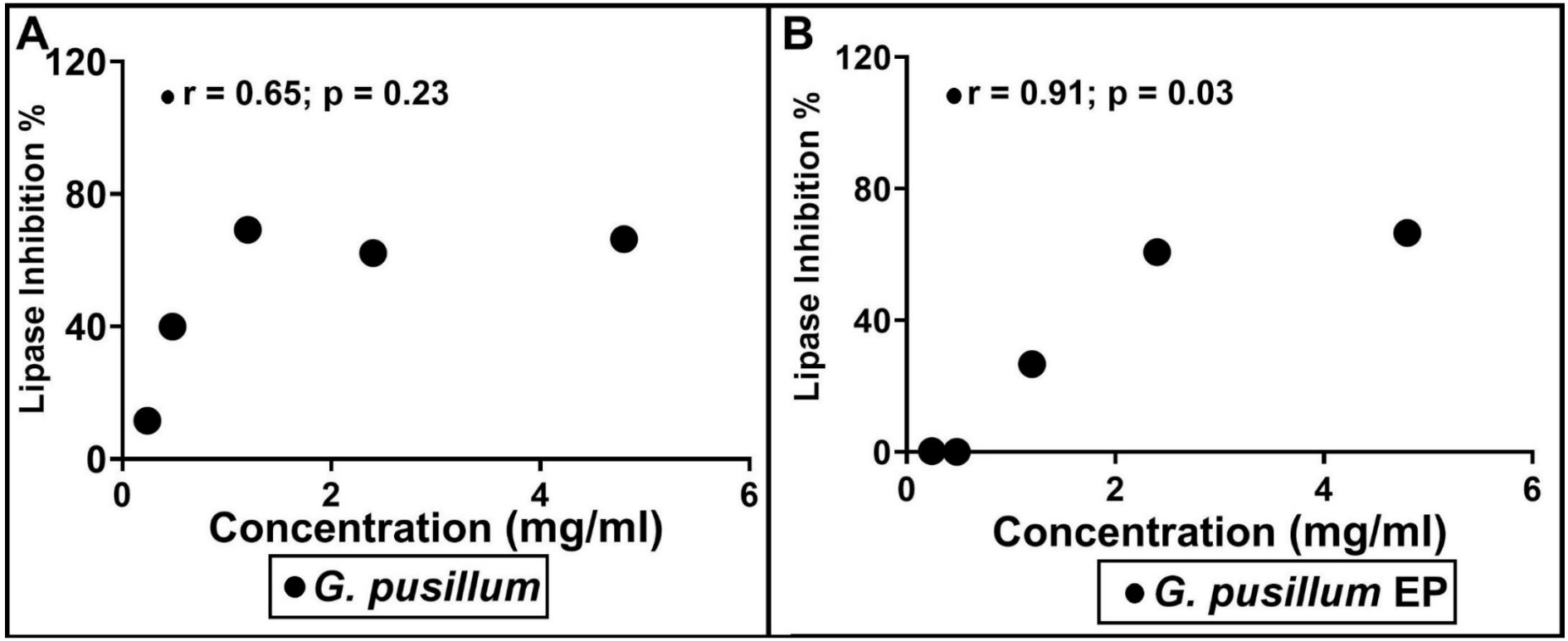

Figure 2. 14 Pearson correlation between pancreatic lipase inhibition and red seaweed concentrations.
Figure 2. 14A shows no significant association between pancreatic lipase inhibition and the concentration of the homogenate from *Gelidium Pusillum* (p=0.23). Figure 2. 14B shows a significant and strong positive correlation (r = 0.91, p=0.03)) between pancreatic lipase inhibition and the concentration of the 50% ethanol pellet (EP) extracted from *Gelidium Pusillum*. Data presented as mean (n=3).

2.4.10 The Half-maximal Inhibitory Concentration (IC_{50}) of Seaweed and its Extracts

The IC_{50} calculated from green, brown and red seaweed species and its extracts is presented in Table 2. 6. In the green seaweeds, a range of IC_{50} values were noted between 0.10 and 1.16 mg/ml. The IC_{50} values of *C. sericioides* homogenate and EP for inhibition of pancreatic lipase were 0.38 and 1.16 mg/ml, respectively (Table 2. 6). In addition, the IC_{50} values of *C. papillatum* WE and EP for inhibition of pancreatic lipase were 1.07 and 0.10 mg/ml, respectively (Table 2. 6).

In the brown seaweeds, the IC_{50} values were between 0.15 and 2.71 mg/ml. The IC_{50} values of the homogenate and WE extracted from *S. trinodis* N for inhibition of pancreatic lipase were 0.75 and 0.15 mg/ml, respectively (Table 2. 6). In addition, the IC_{50} values of *S. trinodis* A homogenate and WE for pancreatic lipase inhibition were 1.34 and 0.27 mg/ml, respectively (Table 2. 6). Moreover, the IC_{50} values of homogenate extracted from *C. sinuosa* and *I. stellata* for pancreatic lipase inhibition were1.51 and 0.87 mg/ml, respectively (Table 2. 6). The IC_{50} values of *P. boergesenii* homogenate, WE and EP for pancreatic lipase inhibition were 1.60, 2.46 and 2.71 mg/ml, respectively (Table 2. 6).

In the red seaweed *G. pusillum*, the IC_{50} values were 1.03 and 2.30 mg/ml for the homogenate and EP, respectively (Table 2. 6).

Table 2. 6 Half-maximal inhibitory concentration (IC50) in different seaweeds and their extracts.

Seaweed species	Half-maximal inhibitory concentration (IC50)		
	Seaweed	Extract	IC50 (mg/ml)
Green	*Cladophora sericioides*	Homogenate	0.38
		EP	1.16
	Codium papillatum	WE	1.07
		EP	0.10
Brown	*Sirophysalis trinodis N*	Homogenate	0.75
		WE	0.15
	Sirophysalis trinodis A	Homogenate	1.34
		WE	0.27
	Colpomenia sinuosa	Homogenate	1.51
	Iyengaria stellata	Homogenate	0.87
	Padina boergesenii	Homogenate	1.60
		WE	2.46
		EP	2.71
Red	*Gelidium pusillum*	Homogenate	1.03
		EP	2.30

The half-maximal inhibitory concentration (IC50) was the concentration of seaweed required to inhibit 50% of pancreatic lipase activity based on hydrolysis from the olive oil substrate alone. To calculate the IC50, non-linear regression analysis was performed by plotting the percentage of lipase inhibition against the logarithm of seaweed concentrations. WE: de-ionised water; EP: ethanol pellets remaining after ethanol extracts; N and A denote *Sirophysalis trinodis* collected in November and April, respectively.

2.5 Discussion

The incidence of obesity has increased exponentially in recent times and has been associated with a myriad of metabolic, cardiovascular, systemic and musculoskeletal comorbidities. While lifestyle modifications remain the cornerstone of anti-obesity treatment, pharmaceutical interventions are increasingly sought after. However, these approaches are often expensive and are commonly associated with a variety of adverse side effects. As an alternative, exploring natural products with anti-obesity properties, such as seaweeds, has garnered interest. The purpose of this study was to evaluate the impact of seven different Kuwait seaweed species, considering seasonal variation and various extracts, including homogenate, WE, EE and EP on pancreatic lipase activity. The key findings from this study are that Kuwait seaweeds can significantly inhibit pancreatic lipase. The extent of pancreatic lipase inhibition varied depending on the seaweed species, extraction method, and collection time.

2.5.1 Extraction

In the current study, various seaweeds were collected from the Kuwait coastline from 2019 to 2021 and subsequently ground into a powder before being exposed to different extractions using WE and 100% EE (Table 2. 3). Consequently, seaweed collected in 2022 was extracted using either WE or 50% EE, based on the principles aimed at raising the polarity of the solvent, which may improve extraction yields (Table 2. 3). The primary objectives of this chapter were threefold: firstly, to conduct a direct comparison of different seaweed species based on geographical origin; secondly, to determine any potential temporal effects; and finally, to ascertain an optimal extraction method. Among all the seaweeds examined, the green seaweed *C. papillatum* exhibited the highest yields post-extraction with WE and 50% EE (Table 2. 3). Notably, a temporal influence on extraction yields was observed, specifically, *S. trinodis* N (Autumn), demonstrated greater extraction yields than those collected in April (spring) when using 100% EE. However, when employing a WE method for *S. trinodis*, the extraction yields were higher in April (spring) compared to November (Autumn) (Table 2. 3).

2.5.2 Extraction Methods and Pancreatic Lipase Inhibition

The observations revealed that in the green seaweed *C. sericioides*, there was significant pancreatic lipase inhibition in the homogenate, 100% EE and EP. However, there was no significant inhibition of pancreatic lipase with *C. sericioides* WE (Table 2. 4). Conversely, the effect of *S. trinodis* N and *S. trinodis* A WE on pancreatic lipase activity showed a significant reduction in pancreatic lipase activity (Table 2. 4). Nonetheless, *S. trinodis* 100% EE and EP showed a non-significant reduction in pancreatic lipase activity.

The data presented here illustrate the significant impact that seaweed species, and even seaweeds originating from the same species, can have on extraction yields. Furthermore, the extraction process, a topic that remains an area of contention, also had a significant impact on the extraction yield, most likely due to the water-soluble and ethanol-soluble compounds present in WE and EE, respectively (Cotas *et al.*, 2020; Shannon and Abu-Ghannam, 2019). These findings are consistent with the work of Leandro *et al.* (2020), who reported that each seaweed has its own unique phytochemical compounds distinguished by their polar properties. Austin *et al.* (2018) previously suggested that seaweeds contain a mixture of water-soluble compounds such as soluble polysaccharides, proteins and soluble polyphenols, as well as ethanol-soluble compounds such as polyphenols, carotenoids and steroids. The data in this study suggest that some of the seaweeds examined here are likely to have a mixture of water and ethanol-soluble compounds, whereas others may have larger concentrations of water-soluble or ethanol-soluble compounds. These findings are supported by the different extraction yields following various extraction approaches. Moving forward, it is unlikely that one extraction method is suitable for all different seaweeds. It is important to identify which extraction approach is most suitable for the seaweed being studied to ensure extraction yield is optimised based on the seaweed's structure and phytochemical compounds. Some insight into the phytochemical compounds of each seaweed, which may help identify optimal extraction methods, is likely to be provided by their geographical location and timing of collection, but these are topics which require further investigation.

2.5.3 Pancreatic Lipase Inhibition by Seaweed 2019 to 2022

Previous pre-clinical studies have demonstrated the ability of different seaweeds to inhibit pancreatic lipase activity (Table 2. 1), with up to 75% inhibition, and human trials have shown their potential to improve body weight and fat deposition (Hall *et al.*, 2012; Hitoe and Shimoda, 2017). In the current study, it was observed that various levels of pancreatic lipase inhibition ranged from 6-99% (Table 2. 4). All seaweed homogenates showed significant pancreatic lipase inhibition levels. It is also found that WE from *C. papillatum*, *S. trinodis* (collected in November and April) and *P. boergesenii* significantly inhibited pancreatic lipase activity by more than 73%. Furthermore, it was demonstrated that the EP also possessed inhibitory properties on pancreatic lipase, including *C. papillatum* and *C. sericioides* (both green seaweeds), *P. boergesenii* (brown seaweed) and *G. pusillum* (red seaweed). Despite a significant inhibition of 11% of pancreatic lipase activity at 4.8mg/ml in the 100% EE from *C. sericioides,* no other significant inhibition of pancreatic lipase activity was observed in the 50% or 100% EE in any of the examined seaweeds.

A potential explanation for why the seaweed homogenate resulted in the highest levels of pancreatic lipase inhibition may be attributed to the comprehensive presence of phytochemical compounds inherent in the seaweed homogenate. This is compared to the WE, which may contain only water-soluble compounds, including soluble polysaccharides and polyphenols. Similarly, the EP may exclusively comprise ethanol-insoluble components, such as insoluble carbohydrates including alginate and fucoidan (Austin *et al.*, 2018).

This study also showed significant pancreatic lipase inhibition with WE from brown seaweeds, including *P. boergesenii* and *S. trinodis* (collected in November and April), and green seaweed *C. papillatum*. This finding is consistent with those documented by Austin *et al.* (2018), wherein they observed that the WE from Hebridean *Ascophyllum nodosum* exhibited greater efficiency in reducing pancreatic lipase activity than its polysaccharides extract. The findings of the significant effect of seaweed homogenates and some of the seaweed EP are consistent with the work of (Chater *et al.*, 2016), who noted that both the homogenate and the EP from three Hebridean brown seaweeds exhibited the highest level of pancreatic lipase inhibition when compared with a sodium carbonate extract predominantly containing alginate. These findings are likely

attributable to the fact that the remaining pellet and homogenate encompass all the bioactive compounds of the seaweeds implicated in inhibiting pancreatic lipase activity. Furthermore, these findings suggest that active components persist following the various extraction methods and thus retain their ability to inhibit pancreatic lipase. However, it is evident that when all active components are concurrently present, the inhibitory levels of pancreatic lipase are highest.

While the findings of the present study hold promise, they diverge from those of Balasubramaniam *et al.* (2013). Balasubramaniam *et al.* (2013) observed a significant reduction in pancreatic lipase activity, ranging from approximately 80 to 90%, upon treatment with an EE derived from three dried Malaysian red seaweeds. These contrasting results imply that the seaweeds under examination in the current study, as well as the red seaweeds studied by Balasubramaniam *et al.* (2013), likely harbour distinct active components with a unique affinity for inhibiting pancreatic lipase, depending upon specific structural attributes of the bioactive compounds. An earlier study by Wilcox *et al.* (2014), previously outlined that alginate containing a high content of guluronic acid, derived from *Laminaria hyperborean* had the highest inhibitory effects on pancreatic lipase activity. However, alginate extracted from *Lessonia nigrescens,* containing a high content of mannuronic acid, showed poor affinity for inhibiting pancreatic lipase (Wilcox *et al.*, 2014).

Although the precise mechanism of how Kuwait seaweeds and their extracts inhibit pancreatic lipase remains elusive, potential mechanisms include the modification of the water/oil interface, thereby reducing the effectiveness of pancreatic lipase (Corstens *et al.*, 2017), and the ability to protonate the active site of pancreatic lipase, consequently impairing pancreatic lipase from hydrolysing fats in the duodenum (Picot-Allain *et al.*, 2022; Wilcox *et al.*, 2014). Notably, this study represents the initial endeavour to evaluate the inhibitory effects of Kuwait seaweed and various extraction methodologies on pancreatic lipase activity. Nevertheless, further research is required to characterise the physical and phytochemical properties of Kuwait seaweeds comprehensively and gain deeper insight into their potential mechanism of action as prospective anti-obesity therapies.

2.5.4 The Temporal Effect of *S. trinodis* in Relation to Pancreatic Lipase Inhibition

Seaweeds are exposed to a variety of seasonal and geographical changes, including factors such as sunlight, temperature, and salinity have been shown to significantly influence the physical and phytochemical properties of seaweeds (Konstantin *et al.*, 2023; Yesilova, Balkis and Taskin, 2017). The Kuwait coastline, similar to coastlines across the globe, undergoes diverse seasonal variation, evidenced in a previous study by Devlin *et al.* (2019), who documented increased temperatures and decreased salinity in April compared to November, both of which are likely to affect seaweed's structure and function. In the present study, it was compared the homogenate and WE from *S. trinodis* N and *S. trinodis* A, both of which significantly inhibited pancreatic lipase activity (Figure 2. 11). The homogenate extract from *S. trinodis* did not show significant temporal effects between November and April. However, significant differences in the WE were noted. Surprisingly, the inhibition of pancreatic lipase activity was greater in the WE from November compared to April at 2.4mg/ml, but at concentrations of 0.48 and 0.24 mg/ml, the inhibition of pancreatic lipase was greater in the WE from April.

The data presented herein substantiates prior literature, which has consistently highlighted significant seasonal fluctuations capable of influencing the levels of bioactive chemicals present in seaweed, encompassing phenols, carotenoids, polysaccharides and other crude components (GÜR and Polat, 2023; Kumar, Sahoo and Levine, 2015). Previous evidence establishing a link between bioactive compounds with seasonal variation was articulated by Connan and Stengel (2011), who determined a positive correlation between polyphenol content and water salinity, a parameter that varies across seasons. Subsequent investigations by Kumar, Sahoo and Levine (2015) and, more recently, Pandey *et al.* (2022), have further confirmed the effects of seasonal variation on bioactive compounds. Both research groups reported elevated polyphenol contents in seaweeds harvested during autumn compared to those obtained in spring (Kumar, Sahoo and Levine, 2015; Pandey *et al.*, 2022). Similarly, recent studies have demonstrated higher carotenoid concentrations in seaweeds collected during autumn (GÜR and Polat, 2023). Conversely, Rioux, Turgeon and Beaulieu (2009) and Kumar, Sahoo and Levine (2015) both noted a decrease in polysaccharide contents extracted from brown seaweed collected during

autumn, implying that seasonal variation may also impact the abundance of specific compounds of interest.

In the present study, it was observed that the influence of seasonal variations on the capacity of seaweed extracts, namely homogenate and WE to inhibit pancreatic lipase activity was negligible. One plausible explanation for this could be that the particular brown seaweed, *S. trinodis*, obtained from Kuwait between November 2020 and April 2021, maintains consistently high concentrations of bioactive compounds throughout the year, which effectively inhibit pancreatic lipase. Consequently, any seasonal fluctuations across seasons may be mitigated by other biological compounds that remain efficacious.

Another rationale for the observed lack of significant temporal effects may stem from a cumulative effect of active components acting in concert. Generally, brown seaweeds, including *S. trinodis* are known to harbour elevated concentrations of active components compared to other seaweed species (Leandro *et al.*, 2020). Noteworthy among these active compounds likely to be present in *S. trinodis* include phlorotannins (Eom *et al.*, 2013), fucoidans (Zhang *et al.*, 2021) alginates (Wilcox *et al.*, 2014) and fucoxanthins (Matsumoto *et al.*, 2010), all of which have demonstrated notable inhibitory effects on pancreatic lipase. Although clear seasonal variations were not evident, these findings underscore the consistent capability of *S. trinodis*, regardless of the time of collection from the Kuwait coastline, to significantly inhibit pancreatic lipase activity. While further investigations are warranted to delineate the specific active compounds responsible for inhibiting pancreatic lipase activity, these data suggest that *S. trinodis* may be a promising candidate for potential use as an anti-obesity therapy.

2.5.5 Correlation

The level of pancreatic lipase inhibition was assessed in relation to the concentrations of seaweed extract. A significant strong positive correlation was observed, indicating a dose-dependent inhibition association. Specifically, significant positive correlations were identified between the different concentrations of WE from *C. papillatum*, *S. trinodis* N and *P. boergesenii* with the degree of pancreatic lipase inhibition. Furthermore, notable positive correlations were identified between the concentration of the EP from *G. pusillum* and *C. sericioides* and the level of pancreatic lipase

inhibition. These findings elucidate that the extent of pancreatic lipase inhibition in Kuwait seaweed and their extracts is influenced by the specific seaweed species and the concentration of seaweed used. Additionally, it is important to acknowledge that not all Kuwaiti seaweed and their extracts exhibit uniform levels of pancreatic lipase inhibition, which may be attributed to the varying active components present in different seaweed species. These results indicate that seaweed is a potential candidate used as a nutraceutical for inhibiting pancreatic lipase activity, hence treating obesity.

2.5.6 IC50

Calculating the IC50 holds paramount importance in pharmaceutical therapy, as it delineates the concentration of a drug required to inhibit a biological process by half (Caldwell *et al.*, 2012). The associations identified in the current study between seaweed extract concentration and pancreatic lipase inhibition indicate a dose-dependent relationship. It was observed that the 50% EP from green seaweed *C. papillatum* and WE from the brown seaweed *S. trinodis* N, exhibited the most potent inhibitory effect, as evidenced by the lowest IC50 values (Table 2. 6).

2.6 Conclusion

The objective of this chapter was to investigate the effects of seven Kuwaiti seaweed species and their extracts on pancreatic lipase activity and to evaluate for any temporal variations in the activity of *S. trinodis* on pancreatic lipase activity. Kuwaiti seaweed homogenates, WE and ethanol pellets exhibited significant inhibition of pancreatic lipase activity to varying extents. However, this study revealed that EE from Kuwaiti seaweed failed to induce any significant decrease in pancreatic lipase activity. Fifteen seaweed samples significantly inhibited pancreatic lipase were all seaweed homogenates, including *C. sericioides S. trinodis* (collected in November 2020 and April 2021), *P.* boergesenii, *C, sinuosa*, *I. stellata* and *G. Pusillum*, the WE, including *C. papillatum*, *S. trinodis* N, *S. trinodis* A and *P. boergesenii*, and EP, including *C. sericioides*, *C. papillatum*, *P*. *boergesenii* and *G. Pusillum*. The diversity in pancreatic lipase inhibition across different seaweeds and their extracts is likely attributable to the presence of species-specific bioactive compounds.

Furthermore, it was noted that the temporal fluctuation of brown seaweed's inhibitory effect on pancreatic lipase activity was absent in the homogenate and very small in the

WE. These findings suggest that the brown seaweed *S. trinodis* may either be unaffected by seasonal variation or contain various bioactive compounds capable of inhibiting pancreatic lipase activity throughout the year. These findings provide valuable insights into the potential presence of highly polar bioactive chemicals in brown seaweed (*S. trinodis*), especially considering the notable efficacy of the WE as a pancreatic lipase inhibitor. Further research is necessary to elucidate which bioactive components may be responsible for pancreatic lipase inhibition in *S. trinodis*.

In summary, the findings presented here underscore the significant ability of Kuwaiti seaweed to inhibit pancreatic lipase activity, thus potentially providing natural bioactive compounds suitable for use in anti-obesity therapies. While further research is required, these findings promise to enhance the commercial value of seaweeds as either a food supplement or a natural bioactive.

Chapter 3 Determination of Kinetics Parameters of Kuwait Seaweeds and their Aqueous and Organic Extracts for the Inhibition of Pancreatic Lipase

3.1 Introduction

Enzymes are large proteins synthesised by living cells to catalyse biochemical reactions. Their creation is facilitated by the natural process in which linear polypeptide chains fold spontaneously (Copeland, 2023, pp. 39-41). The structure of enzymes is complex and directly related to their functional properties. All enzymes exhibit primary, secondary, and tertiary structures, with some also having quaternary structures. Enzymes with a three-dimensional structure have an active site that exhibits a high degree of specificity, binding only with specific substrates (Copeland, 2023, pp. 48-61). Despite their unique structures, the rates of enzyme-catalysed reactions are influenced by factors such as substrate and enzyme concentrations, cofactors, temperature, and pH (Robinson, 2015).

Enzyme reactions are complex and involve a multi-step sequence beginning with substrate binding, followed by the formation of an enzyme-substrate (ES) complex, and concluding with the release of the enzyme and products in a time-dependent manner (Berg *et al.*, 2019, p. 245). In 1913, Leonor Michaelis and Maud Menten identified that enzyme reaction sequences could be mathematically described using basic assumptions about the reaction mechanism between a substrate and an enzyme, (Equation 3. 1) (Berg *et al.*, 2019, p. 245).

$$E + S \underset{K-1}{\overset{K1}{\rightleftharpoons}} ES \xrightarrow{K2} E + p$$

Equation 3. 1 Enzyme reaction mechanism.
E: enzyme, S: substrate, ES: enzyme-substrate complex, P: product, K_1: rate constant for ES formation, K_{-1}: rate constant for the dissociation of ES complex adversely to E+S, K_2: rate constant for the dissociation of ES complex to E+P.

3.2 Michaelis-Menten Model

The Michaelis-Menten model posits that an ES complex forms as a precursor to the reaction, which can either dissociate advantageously to release a product or dissociate adversely with no product formation (Equation 3. 1) (Berg *et al.*, 2019, p. 245). The first phase of the Michaelis-Menten reaction involves two kinetic rate constants: the rate constant for ES formation (K_1) and the rate constant for the dissociation of ES complex back to enzyme + substrate (E+S) (K_{-1}). These constants determine the equilibrium between the formation and dissociation of the ES complex. The second phase of the Michaelis-Menten reaction involves the ES complex dissociating into enzyme (E) and product (P), characterised by a kinetic rate constant (K_2). These constants can be used to determine the Michaelis-Menten constant (K_m), as depicted in Equation 3. 2 (Berg *et al.*, 2019, p. 246).

$$\boldsymbol{Km = \frac{K-1+K2}{K1}}$$

Equation 3. 2 The Michaelis-Menten constant.
Km: Michaelis-Menten constant, K-1: rate constant for the dissociation of enzyme (E) and substrate (S) complex adversely to E+S, K2: rate constant for the dissociation of the ES complex to E+P, K1: rate constant for ES formation.

K_m is defined as the substrate concentration required to reach half of the maximum reaction rate (V_{max}) and serves as an indicator of an enzyme's affinity for the substrate (Berg *et al.*, 2019, p. 247). The reaction rate and the amount of product released over time can also be influenced by the substrate concentration. Consequently, the initial rate of P formation (V_0) in an enzyme-catalysed reaction can be represented by the Michaelis-Menten equation (Equation 3. 3) (Berg *et al.*, 2019, p. 247). The Michaelis-Menten equation, represented in Equation 3. 4, indicates that K_m is equivalent to the substrate concentration at which the reaction rate is half of V_{max} (Berg *et al.*, 2019, p. 247).

$$\boldsymbol{V0 = Vmax\frac{[S]}{Km+[S]}}$$

Equation 3. 3 The Michaelis-Menten equation.
V_0: initial reaction rate, V_{max}: maximum reaction rate, [S]: substrate concentration, K_m: Michaelis-Menten constant.

$$V0 = \frac{Vmax}{2}$$

Equation 3. 4 The Michaelis-Menten equation at half of the maximum reaction rate.
V0: initial reaction rate, Vmax: maximum reaction rate.

3.2.1 Michaelis-Menten Plot

Kinetics refers to the study of chemical reaction rates (Berg *et al.*, 2019, p. 246). Enzyme kinetics are examined by measuring the progressive formation of P over time at various substrate concentrations. As substrate concentrations increase, the enzyme efficiently converts substrate into product. However, a threshold exists where further increases in substrate concentrations do not affect the reaction rate, due to enzyme saturation with substrate. This threshold is reached when the reaction attains its V_{max} (Figure 3. 1A). The Michaelis-Menten plot is then constructed by plotting V_0 against S concentration (Figure 3. 1B) (Berg *et al.*, 2019, p. 246).

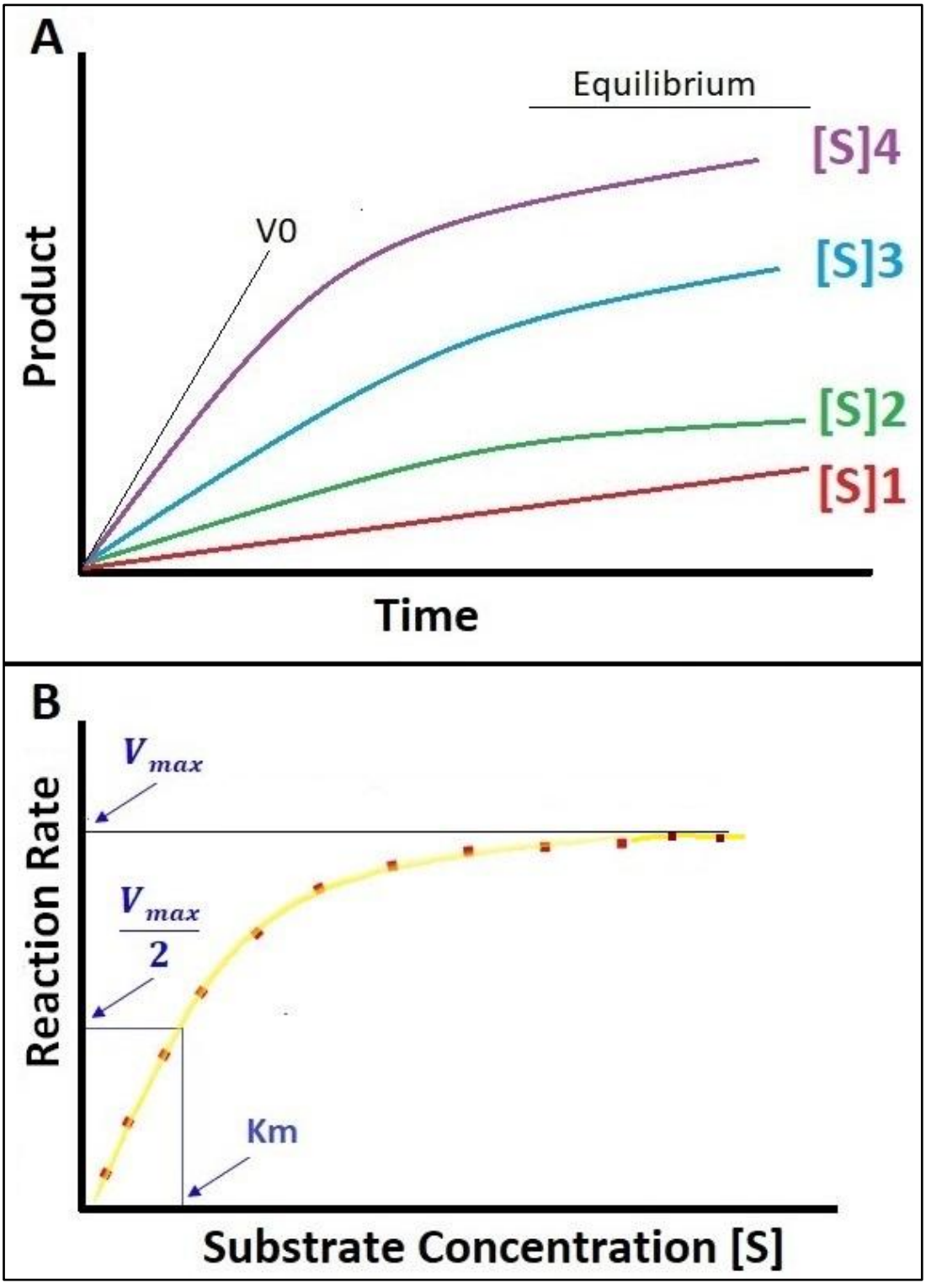

Figure 3. 1 The Michaelis-Menten plots.

Figure 3. 1A shows the formation of product (P) over time at various substrate (S) concentrations, denoted by different coloured lines increasing from S1 to S4. Figure 3. 1B shows the reaction rate versus S concentrations, denoting the maximum rate (V_{max}), half of V_{max} and Michaelis-Menten constant (K_m). Image adapted from (Berg *et al.*, 2019, pp. 245-246).

3.2.2 Lineweaver-Burk Plot

An efficient approach for measuring enzyme kinetics, such as V_{max} and K_m, is to manipulate the Michaelis-Menten equation to obtain a linear plot, as depicted in Equation 3. 5. This linear form is obtained by taking the reciprocal of Equation 3. 3. The Lineweaver-Burk plot of 1/V versus 1/[S], can then be constructed (Figure 3. 2) (Berg *et al.*, 2019, p. 248).

$$\frac{\boldsymbol{1}}{\boldsymbol{V}} = \frac{\boldsymbol{Km}}{\boldsymbol{Vmax}} \times \frac{\boldsymbol{1}}{\boldsymbol{S}} + \frac{\boldsymbol{1}}{\boldsymbol{Vmax}}$$

Equation 3. 5 Reciprocal Michaelis-Menten equation.
V_0: initial reaction rate, Vmax: maximum reaction rate, S: substrate concentration, K_m: Michaelis-Menten constant.

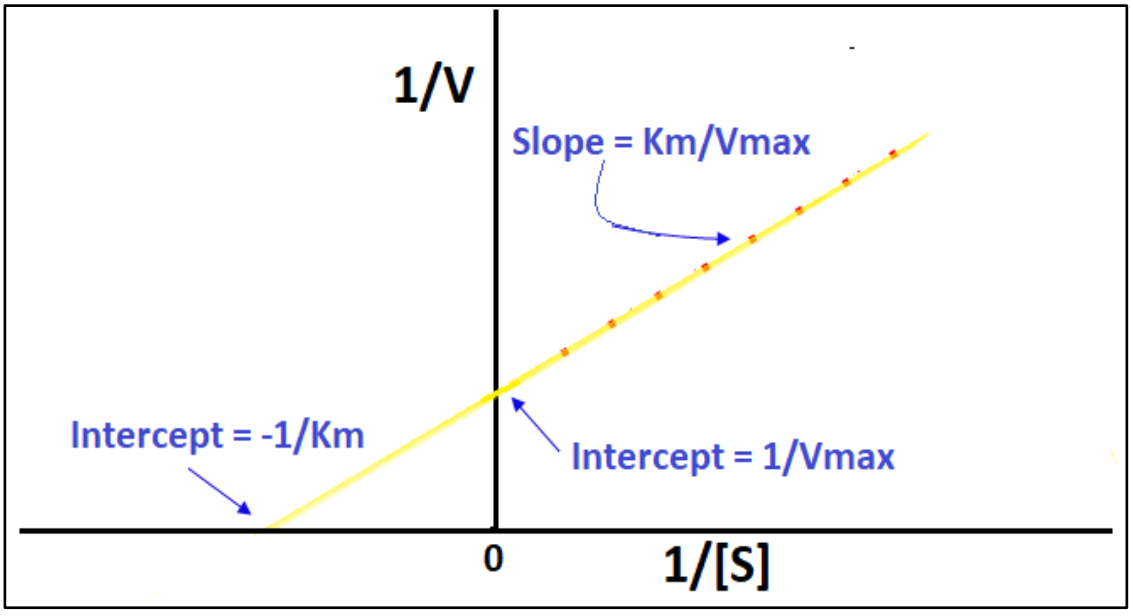

Figure 3. 2 Lineweaver-Burk plot.
Reciprocal of the initial reaction rate (V_0) versus the reciprocal of various substrate (S) concentrations. The intercept on the y-axis is the reciprocal of the maximum reaction rate (V_{max}). The intercept on the x-axis is the reciprocal of the Michaelis-Menten constant (K_m). Image adapted from (Berg *et al.*, 2019, p. 248).

3.2.3 Enzyme Inhibitors

Enzyme reaction rates can be influenced by various factors, including molecules that inhibit enzyme activity (Robinson, 2015). The study of molecules capable of modulating and inhibiting enzymatic activity has been a significant focus of recent research, particularly in the pharmaceutical industry (Copeland, 2013, p. 2). Inhibitors are substances or molecules that can alter the affinity of E to S, thereby reducing the activity of enzymatic reactions. The affinity of an inhibitor to free enzyme and ES complex is defined by the inhibition constants (K_{ia} and K_{ib}, respectively). The enzyme reaction mechanisms, including the effect of inhibitors, are shown in Figure 3. 3 (Copeland, 2013, p. 58).

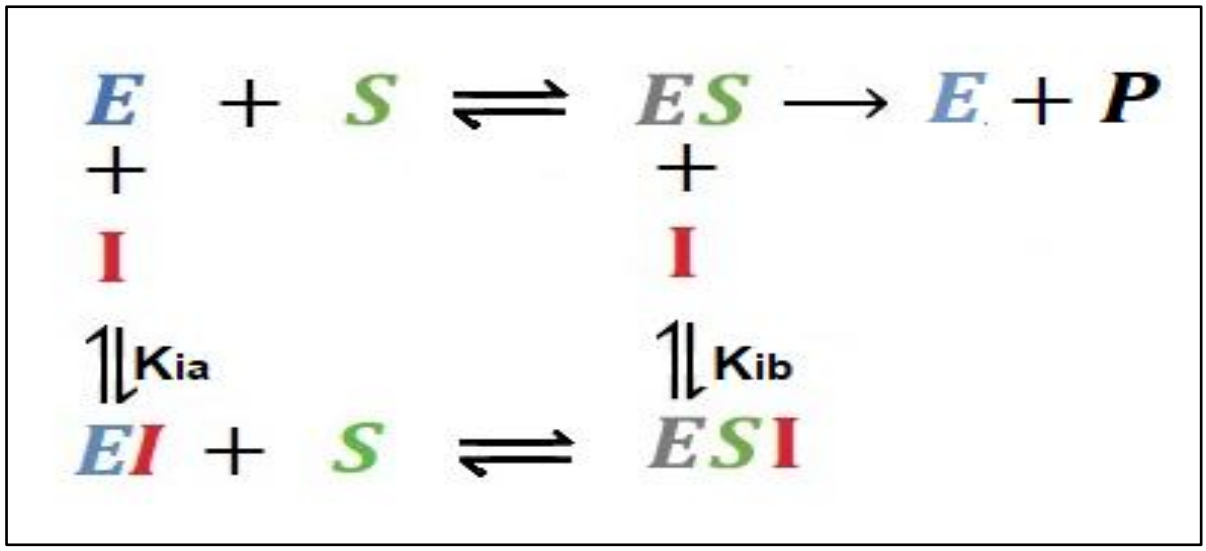

Figure 3. 3 The enzyme reaction mechanisms with an inhibitor.
E: enzyme, S: substrate, ES: enzyme-substrate complex, P: product, K_{ia}: inhibition constant for the free E, K_{ib}: inhibition constant for the ES complex. Image adapted from (Copeland, 2013, p. 58).

Enzyme inhibitors can be categorised as either reversible or irreversible (Kessel and Ben-Tal, 2018, p. 834). Reversible inhibitors typically form weak non-covalent bonds with enzymes and are characterised by the speed with which they dissociate from the enzyme. In contrast, irreversible inhibitors form strong covalent bonds with enzymes and are characterised by their slow dissociation or no dissociation from the enzyme. There are four main types of reversible inhibitors: competitive, non-competitive, uncompetitive and mixed-type inhibitors (Kessel and Ben-Tal, 2018, p. 834).

3.2.4 Competitive Inhibitors

Competitive inhibitors bind exclusively to the free E, forming an enzyme-inhibitor (EI) complex that consequently inhibits the formation of the ES complex (Figure 3. 4A) (Kessel and Ben-Tal, 2018, p. 838). Competitive inhibitors possess an affinity for binding to the free enzyme depending on the inhibitor, some will have high or low affinity, as indicated by the K_{ia} value. The competitive inhibitor competes with the substrate to bind reversibly to the active site of the enzyme, resulting in an alteration of the enzyme's affinity for the substrate. One approach to reduce the efficacy of the competitive inhibitor is by increasing the substrate concentration, thereby maintaining the rate of an enzyme reaction at V_{max}. Consequently, adding a competitive inhibitor to an enzyme reaction increases the K_m value, as depicted in Equation 3. 6 (Kessel and Ben-Tal, 2018, p. 835). The Michaelis-Menten equation with a competitive inhibitor can be described by replacing K_m with K_{mApp} as presented in Equation 3. 3. The impact of the inhibitor is apparent in the Lineweaver-Burk plot, depicted in Figure 3. 5A (Copeland, 2013, p. 61; Kessel and Ben-Tal, 2018, p. 838).

$$\boldsymbol{KmApp = Km(1 + \frac{[I]}{Kia})}$$

Equation 3. 6 Michaelis-Menten constant with a competitive inhibitor.
KmApp: Michaelis-Menten constant of an inhibited enzyme, Km: Michaelis-Menten constant of an uninhibited enzyme, [I]: inhibitor concentration, Kia: inhibition constant to a free enzyme

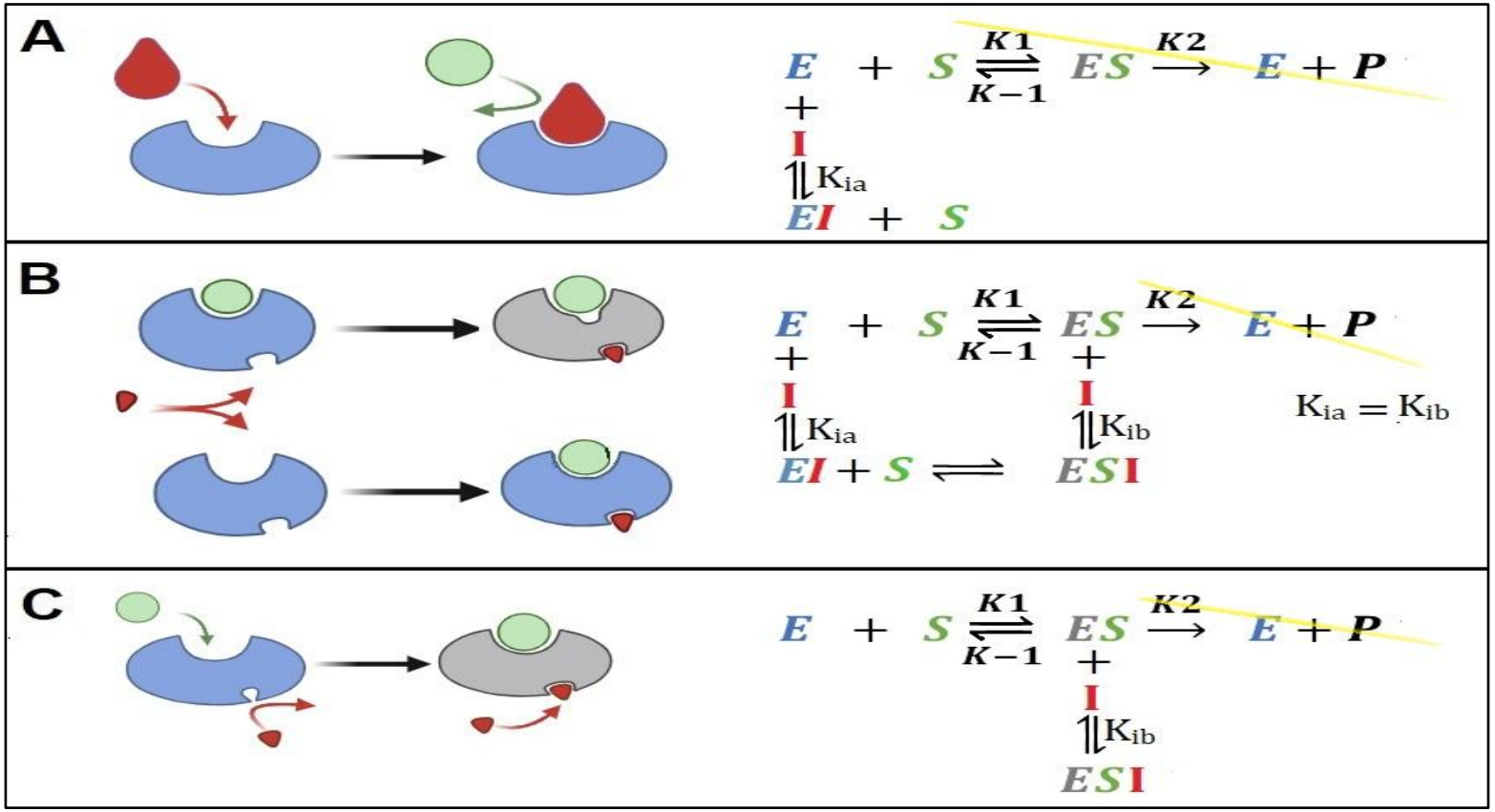

Figure 3. 4 Interactions of reversible inhibitors with the enzyme and their Michaelis-Menten equations. A, B and C depict competitive, non-competitive and uncompetitive inhibitors, respectively. E: enzyme, depicted as a blue circle with a pocket, S: substrate, depicted as a green ball, I: inhibitor, depicted as a red oval shape, ES: enzyme-substrate complex (ES) complex, depicted as a grey circle with a green ball in its pocket, P: product, K1: rate constant for ES formation, K-1: rate constant for the dissociation of ES complex adversely to E+S, K2: rate constant for the dissociation of ES complex to E+P. K_{ia}: inhibition constant for free E, K_{ib}: inhibition constant for the ES complex. Image adapted from (Copeland, 2013, p. 60) and created from biorender.com.

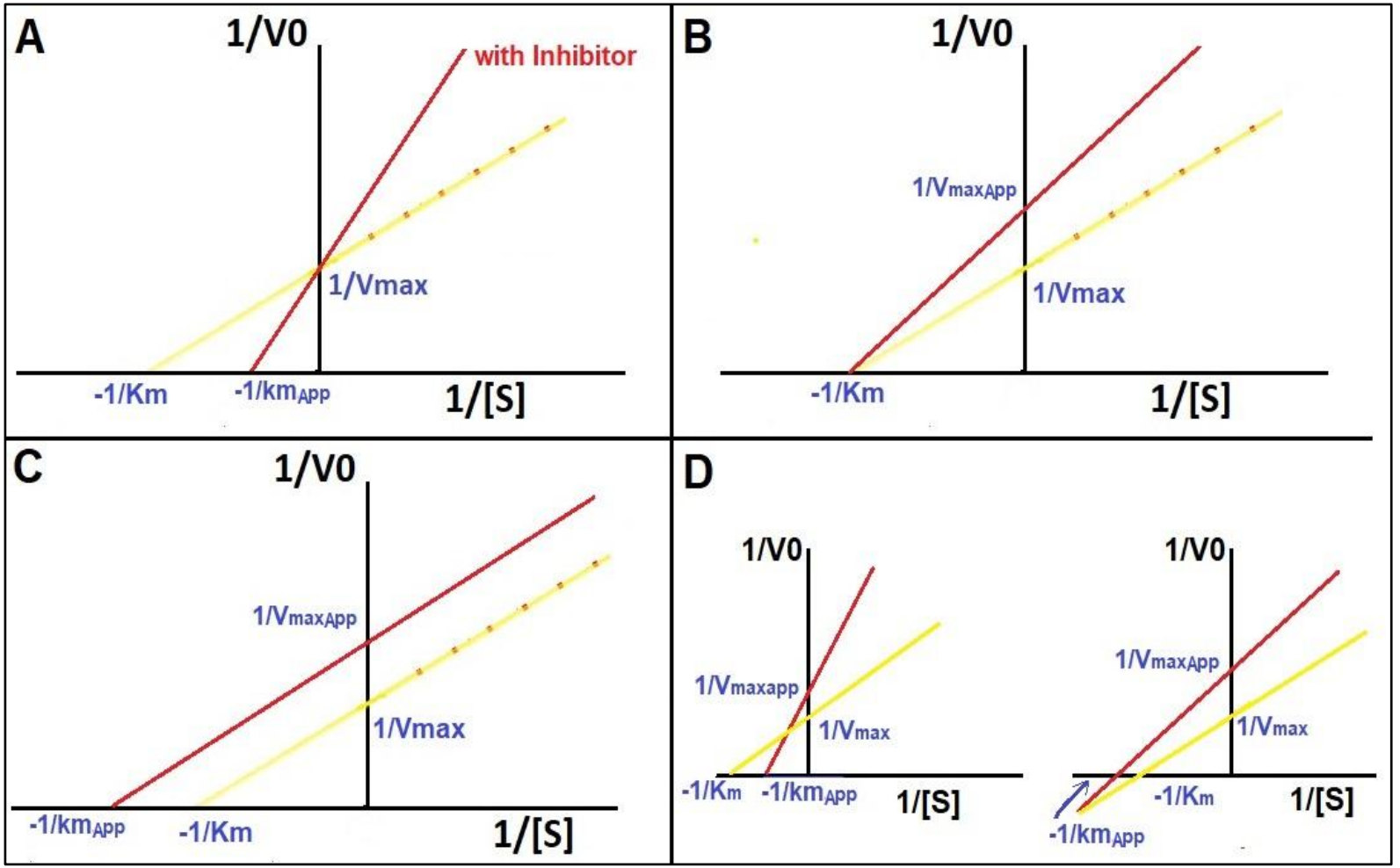

Figure 3. 5 Lineweaver-Burk plots of the reversible Inhibitors.

A, B, C and D depict a competitive, non-competitive, uncompetitive and mixed-type inhibitor, respectively. The reaction rates with and without inhibitors are denoted by red and yellow lines, respectively. The intercept on the y-axis is the reciprocal of the maximum reaction rate (V_{max}). The intercept on the x-axis is the reciprocal of the Michaelis-Menten constant (K_m) (Copeland, 2013, pp. 62-84; Ring, Wrighton and Mohutsky, 2014).

3.2.5 Uncompetitive Inhibitors

Uncompetitive inhibitors bind exclusively to the ES complex, forming an ESI complex (Figure 3. 4C) (Kessel and Ben-Tal, 2018, p. 842). Uncompetitive inhibitors have an affinity for binding to the substrate-bound enzyme depending on the inhibitor, some will have high or low affinity, as indicated by the K_{ib} value. Consequently, adding an uncompetitive inhibitor to an enzyme reaction decreases both the V_{max} and K_m values, as demonstrated in Equation 3. 7 and Equation 3. 8, respectively. The Michaelis-Menten equation with an uncompetitive inhibitor can be described by replacing V_{max} and K_m with V_{maxApp} and K_{mApp} in Equation 3. 3. The impact of the inhibitor is most clearly seen in the Lineweaver-Burk plot, as shown in Figure 3. 5C

$$\boldsymbol{KmApp} = \frac{\boldsymbol{Km}}{(\mathbf{1} + \frac{[\boldsymbol{I}]}{\boldsymbol{Kib}})}$$

Equation 3. 7 The Michaelis-Menten constant under uncompetitive inhibition.
K_{mApp}: Michaelis-Menten constant of an inhibited enzyme, K_m: Michaelis-Menten constant of an uninhibited enzyme, [I]: inhibitor concentration, K_{ib}: inhibition constant to a substrate-bound enzyme.

$$\boldsymbol{VmaxApp} = \frac{\boldsymbol{Vmax}}{(\mathbf{1} + \frac{[\boldsymbol{I}]}{\boldsymbol{Kib}})}$$

Equation 3. 8 The maximum rate of the enzyme under uncompetitive inhibition.
KmApp: Michaelis-Menten constant of an inhibited enzyme, Km: Michaelis-Menten constant of an uninhibited enzyme, [I]: inhibitor concentration, Kib: inhibition constant to a substrate-bound enzyme.

3.2.6 Non-competitive Inhibitors

Non-competitive inhibitors can bind to both the free E and the ES complex with the same affinity (Figure 3. 4B) (Kessel and Ben-Tal, 2018, p. 844). These inhibitors bind to locations other than the active site of the enzymes, referred to as the allosteric site (Kessel and Ben-Tal, 2018, p. 844). This form of inhibition does not compete with the

substrate for binding to the enzyme's active site, resulting in the preservation of the enzyme's affinity for the substrate (K_m remains unchanged). Furthermore, non-competitive inhibitors have equal affinity for binding to both the free enzyme and ES complex, indicated by similar K_{ia} and K_{ib} values. Consequently, adding a non-competitive inhibitor to an enzyme reaction decreases the V_{max} value (Equation 3. 9) (Kessel and Ben-Tal, 2018, p. 841). The Michaelis-Menten equation with a non-competitive inhibitor can be described by replacing V_{max} with V_{maxApp} in Equation 3. 3. The impact of the inhibitor is most clearly apparent in the Lineweaver-Burk plot, as shown in Figure 3. 5B (Copeland, 2013, p. 68; Kessel and Ben-Tal, 2018, p. 842).

$$\boldsymbol{VmaxApp} = \frac{\boldsymbol{Vmax}}{(\mathbf{1} + \frac{\boldsymbol{[I]}}{\boldsymbol{Kib}})}$$

Equation 3. 9 Maximum rate of the enzyme activity under non-competitive inhibition.
K_{mApp}: Michaelis-Menten constant of an inhibited enzyme, K_m: Michaelis-Menten constant of an uninhibited enzyme, [I]: inhibitor concentration, K_{ib}: inhibition constant when the inhibition constant of a substrate-bound enzyme.

3.2.7 Mixed-type Inhibitors

A mixed-type inhibitor can bind to both the free E and the ES complex, but its affinity for each is different (Kessel and Ben-Tal, 2018, p. 842). This type of inhibitor can bind to both the free enzyme's active site and the ES complex's allosteric site. Consequently, this inhibitor alters the enzyme's affinity for the substrate because it competes with the substrate for binding to the enzyme's active site. The difference in affinity for binding to the free enzyme and ES complex in mixed-type inhibitors is indicated by different K_{ia} and K_{ib} values. Therefore, adding a mixed-type inhibitor to an enzyme reaction can decrease the V_{max} and either increase or decrease K_m values, depending on the inhibitor affinity for the free or ES complex, as shown in Equation 3. 9 and Equation 3. 10, respectively (Ring, Wrighton and Mohutsky, 2014).

$$KmApp = \frac{Km\,(1 + \frac{[I]}{Kia})}{(1 + \frac{[I]}{Kib})}$$

Equation 3. 10 Michaelis-Menten constant under mixed-type inhibition.
K_{mApp}: Michaelis-Menten constant of an inhibited enzyme, K_m: Michaelis-Menten constant of an uninhibited enzyme, I: inhibitor concentration, K_{ia}: inhibition constant to a free enzyme, K_{ib}: inhibition constant to the substrate-bound enzyme.

The Michaelis-Menten equation (Equation 3. 3) with a mixed-type inhibitor can be described by replacing V_{max} with V_{maxApp} (Equation 3. 9) and K_m with K_{mApp} (Equation 3. 10). The impact of the inhibitor is most clearly apparent in the Lineweaver-Burk plot (Figure 3. 5D). The data in Figure 3. 5D provides insight into whether the inhibitor has a higher affinity for the free enzyme or the ES complex. For example, if the intersection is above the x-axis, it indicates a greater affinity for the free enzyme and that K_{mApp} is greater than K_m, but where the point of the intersection is located below the x-axis, it indicates a greater affinity for ES complex and that K_{mApp} is lower than the K_m (Ring, Wrighton and Mohutsky, 2014). A mixed inhibitor is classified as a non-competitive inhibitor if it demonstrates the same affinity for both the free E and the ES complex (K_{ia} equals K_{ib} value) (Copeland, 2013, p. 68).

3.2.8 Enzyme Inhibitors and Obesity

Orlistat is an approved anti-obesity medication that inhibits pancreatic lipase activity (Sjöström *et al.*, 1998). As mentioned in the previous chapter, X-ray crystallography studies have demonstrated that Orlistat is an irreversible inhibitor that covalently binds to the enzyme's active site (Heck, Yanovski and Calis, 2000). In the previous chapter (Chapter 2), 15 Kuwaiti seaweed samples, including homogenates, WE extracts, and ethanol pellets remaining following EEion, exhibited an inhibitory effect on pancreatic lipase activity. Therefore, the main objective of this chapter is to investigate their mode of inhibition.

3.3 Aims

The aim of this chapter was:

- To determine the mode of inhibition of pancreatic lipase in 15 Kuwaiti seaweed samples, including homogenates, WE and the EP, which exhibited the highest inhibitory effect on pancreatic lipase activity in the turbidity assay.
    - Through the use of Michaelis-Menten kinetics and Lineweaver-Burk plots.

3.4 Methods

An olive oil turbidity assay was utilised to examine the impact of Kuwaiti seaweeds on the enzymatic reaction rate of pancreatic lipase and different olive oil (substrate) concentrations in the presence of and without different Kuwait seaweeds and their extracts.

3.4.1 Material

The materials used in this chapter were similar to the chemicals listed in Chapter 2.

3.4.2 Equipment

The same types of equipment as in Chapter 2 were used.

3.4.3 Seaweed

The Kuwaiti seaweed samples, comprising of homogenates, WE and EP, exhibited the most significant inhibitory effect on pancreatic lipase activity as detailed in Chapter 2, were utilised in this chapter to investigate their mode of inhibition on pancreatic lipase. The samples employed in this chapter included:

- Homogenate samples: 0.6 mg/ml of *C. sericioides*, 1.3 mg/ml of *G. pusillum*, 1.0 mg/ml of *S. trinodis* N, 1.6 mg/ml of *S. trinodis* A, 1.8 mg/ml of *C. sinuosa*, 1.2 mg/ml of *I. stellata* and 2 mg/ml of *P. boergesenii*.
- WE samples: 0.4 mg/ml of *S. trinodis* N, 0.45 mg/ml of *S. trinodis* A, 1.4 mg/ml of *C. papillatum* and 2.9 mg/ml of *P. boergesenii*.
- EP samples: 0.25 mg/ml of *C. papillatum*, 1.5 mg/ml of *C. sericioides*, 3.2 mg/ml of *P. boergesenii* and 2.6 of *G. pusillum.*

3.4.4 Reagent Setup

Free fatty acids were extracted from olive oil using the method outlined in Chapter 2. Initially, 20 g of previously processed olive oil was combined with 100 ml of acetone to form a 20% (w/v) purified olive oil solution, which was then refrigerated at 4°C for subsequent experiments. Various substrate concentrations were derived from the 20% (w/v) stock solution to determine the kinetic parameters V_{max} and K_m.

In preparation, a 60 ml Tris buffer (as described in Chapter 2) was heated to 70°C and subsequently mixed with 6 ml of the 20% purified olive oil solution for 10 minutes, resulting in a 2% olive oil solution used as the substrate. This 2% stock substrate solution was then cooled to room temperature of 20-25°C and used within six hours of preparation in all experiments. Higher substrate concentrations were employed to achieve enzyme saturation at the active site and subsequently determine V_{max}.

Serial dilutions of the 2% olive oil stock solution were prepared to obtain concentrations of 2, 1, 0.5, 0.25, 0.125 and 0.063%. Each of these concentrations was combined with seaweed homogenates, WE, and EP to achieve the desired experimental concentrations for measuring V_{max} and K_m.

Pancreatic lipase 1.29 mg/ml with 18 µg/ml of colipase was prepared as previously mentioned in Chapter 2.

3.4.5 Procedures

The enzymatic reaction of pancreatic lipase, with and without Kuwaiti seaweeds and their extracts, was examined at different substrate concentrations using the following procedure:

- Without seaweed: Two 96-well microplates were used to pre-incubate all enzyme and substrate solutions (Figure 3. 6). On a 96-well plate (plate A), 10 µl of pancreatic lipase labelled ‘enzyme’ or WE labelled ‘blank’, which served as a negative control, were filled into the wells Row A-C and Row E-G, respectively (Figure 3. 6A). On a second plate (plate B), 240 µl of the substrate serial dilutions at concentrations 2, 1, 0.5, 0.25, 0.125 and 0.063% (v/v) were added to each respective column from 1 to 6 (Figure 3. 6B). Both plates were subsequently incubated for 10 minutes at 37°C. Following incubation, 200µl (V_1) of the serial dilutions (C_1) from plate B were added to plate A, resulting in a total solution of 210µl (V_2). Plate A was then immediately inserted into a spectrophotometer and read every 5 minutes at 405nm for 45 minutes. The final concentrations (C_2) of the substrate substrates after using: Equation 2. 2 were 1.9, 0.95, 0.48, 0.24, 0.12, and 0.06%, respectively. Finally, the axis interception pattern and crossing of the linear line in the Lineweaver-Burk plot were

examined to determine the pancreatic lipase reaction rate without Kuwaiti seaweeds and extracts, serving as the control.

- With seaweed: Two 96-well microplates were used to pre-incubate all enzymes and seaweed-substrate solutions Figure 3. 6. 10µl of pancreatic lipase labelled ‘enzyme’ and WE labelled ‘blank’, serving as a negative control, were added to the appropriate wells on the first 96-well plate (plate A), as shown in Figure 3. 6A. On a second plate (plate B), 240 µl of seaweed-substrate serial dilution solutions were added to each respective column (Figure 3. 6B). Both plates were subsequently incubated for 10 minutes at 37°C. Following incubation, 200µl of the seaweed-substrate serial dilution solutions from plate B was added to plate A. Plate A was then immediately inserted into a spectrophotometer and read every 5 minutes at 405 nm for 45 minutes. Finally, the axis interception pattern and crossing of the linear line in the Lineweaver-Burk plot were examined to determine the pancreatic lipase reaction rate with Kuwaiti seaweeds and extracts, serving as the inhibitors.

Figure 3. 6 96-well plate layout for kinetics assay.

Figure 3. 6A shows plate A, which contained 10µl of pancreatic lipase at 1.29 mg/ml combined with colipase (Row A-C) labelled 'enzyme', and 10µl of WE (Row E-G) labelled 'blank', which served as a negative control. Figure 3. 6B shows Plate B, which contained 240 µl of Serial dilutions of the olive oil substrate solutions from 2% down to 0.06%. The serial dilutions began at the higher concentrations in column 1 (2%) and down to 0.06% in column 6. After 10 minutes of incubation at 37°C, a 96-well plate A was inserted into the microplate reader. Next, 200 µl of Serial dilutions of the olive oil substrate solutions, from Figure 3. 6B were added to plate A and immediately read every 5 minutes at 405 nm for 45 minutes.

3.4.6 Calculation

The average absorbance of each substrate concentration was subtracted from its corresponding sample. Then, the absorbance of each substrate concentration without seaweed was used to recognise the maximum absorbance representing the maximum enzyme activity, which was at 35 minutes with the highest concentration. Therefore, the absorbance reading at 35 minutes was selected for the following experiments. The rate of reaction was calculated from the change in absorbance over time (35 minutes). The reaction rate of pancreatic lipase with different substrate concentrations in the presence of Kuwait seaweeds, examined using Lineweaver-Burk plots, indicated a mixed-type inhibition. Consequently, the K_{ia} and K_{ib} values were calculated using Equation 3. 9 and Equation 3. 10, respectively.

3.4.7 Statistical Analysis

Data from the spectrophotometer was extracted using Microsoft Excel to identify changes in turbidity throughout the 35 minutes. The data was then analysed using GraphPad Prism 9 software. Lineweaver-Burk plots were constructed using linear regression. K_{ia} and K_{ib} values were calculated by determining the V_{maxApp} (the reciprocal of the y-intercept) and K_{mApp} (the negative reciprocal of the x-intercept) from the Lineweaver-Burk plots. Each experiment was performed in three independent experiments, each with three biological replicates.

3.5 Results

Fifteen Kuwaiti seaweed samples with the highest inhibitory effect on pancreatic lipase activity, evidenced in Chapter 2, were selected to investigate how these seaweeds and extracts are able to inhibit pancreatic lipase. The mode of inhibition was determined by analysing the intercept pattern and crossing of linear lines for the reciprocal data of pancreatic lipase in the presence and absence of seaweed samples against substrate concentration through double reciprocal Lineweaver-Burk plots. The V_{max} and K_m values for inhibited and uninhibited enzymes can be calculated from Lineweaver-Burk plots by the reciprocal of the y-intercept and the negative reciprocal of the x-intercept, respectively.

3.5.1 The Lineweaver-Burk Plots with and without Green and Red Seaweed Homogenate

In Figure 3. 7A, the Lineweaver-Burk plot shows the y-intercept for the reaction rate of pancreatic lipase with and without the green seaweed homogenate derived from *C. sericioides*. The change in absorbance at 405nm over 35 minutes (ΔAbs/35 mins) in the presence of the *C. sericioides* homogenate was larger than without the seaweed homogenate, 26.07 vs. 13.19 ΔAbs/35 mins, respectively. The X-intercept of the Lineweaver-Burk plot for the reaction rate of pancreatic lipase with *C. sericioides* was nearly the same as without the seaweed homogenate (-5.96 vs. -5.92, respectively) (Figure 3. 7A). These findings indicate that the homogenate derived from the green seaweed *C. sericioides* decreases the V_{max} (1/Y-intercept) of pancreatic lipase reaction and keeps the K_m unchanged (-1/X-intercept), suggesting a non-competitive inhibition mode of action (Figure 3. 7A).

In Figure 3. 7B, the y-intercept for the reaction rate of pancreatic lipase with the homogenate derived from red seaweed *G. pusillum* was higher than without the seaweed homogenate, 22.23 vs. 13.19 Δ Abs/35 mins, respectively. Additionally, the X-intercept of the Lineweaver-Burk plot for the reaction rate of pancreatic lipase in the presence of the *G. pusillum* homogenate was bigger than without the seaweed homogenate (-5.46 vs. -5.92, respectively) (Figure 3. 7B).

These results indicate that the red seaweed homogenate derived from *G. pusillum* decreases the V_{max} (1/Y-intercept) and increases the K_m (-1/X-intercept) of pancreatic lipase reaction, suggesting a mixed inhibition mode of action (Figure 3. 7B).

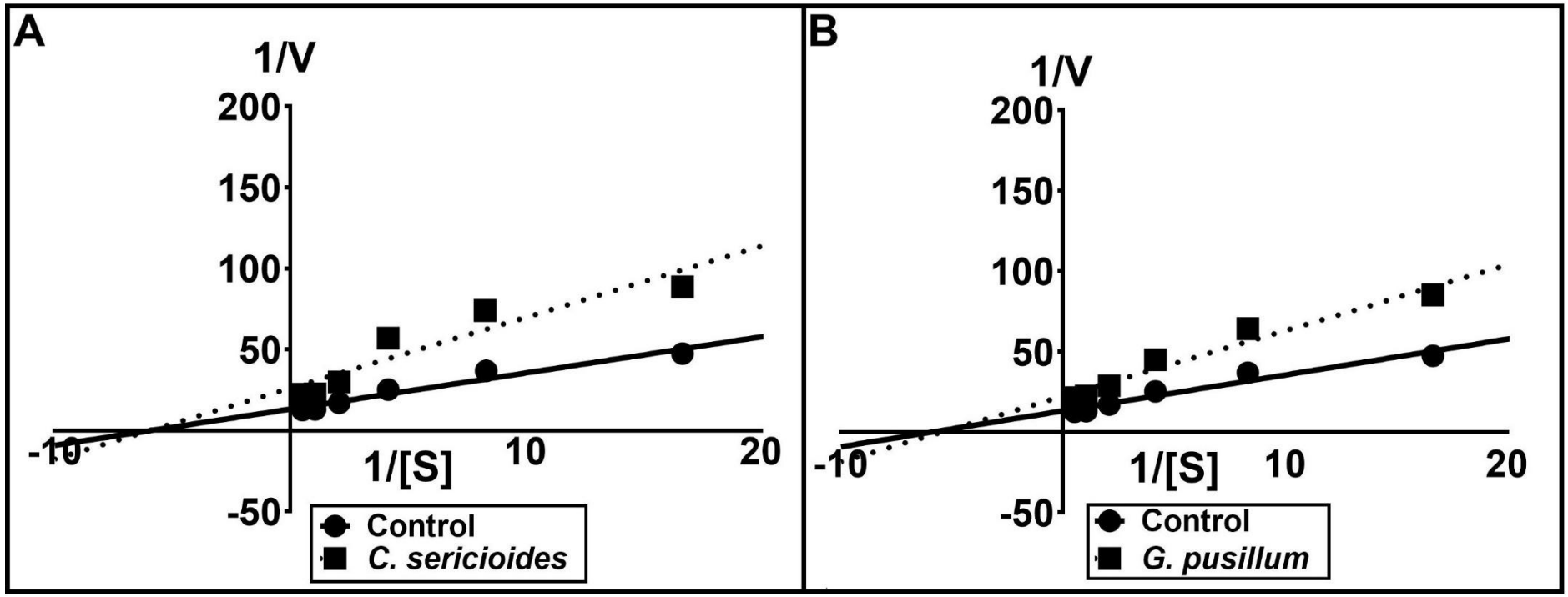

Figure 3. 7 Lineweaver-Burk plots for pancreatic lipase activity with and without green or red seaweed homogenate.
Figure 3. 7A and B depict pancreatic lipase with the addition of the green seaweed *Cladophora sericioides homogenate* at 0.57 mg/ml and the red seaweed *Gelidium pusillum* homogenate at 1.23 mg/ml, using dashed black lines, respectively. Pancreatic lipase controls are depicted using a solid black line, containing 0.06 mg/ml lipase and no Kuwait seaweeds or their extracts. All samples were added to various substrate [S] concentrations ranging from 0.06 to 1.9%. The velocity (the rate of turbidity change) was calculated by measuring the change in absorbance at 405nm over 35 minutes (ΔAbs/35 mins).

3.5.2 The Lineweaver-Burk Plots with and without Brown Seaweed Homogenate

The Lineweaver-Burk plots for different brown seaweed homogenates are illustrated in Figure 3. 8. In Figure 3. 8A, the plot shows the Y-intercept for the reaction rate of pancreatic lipase with and without the brown seaweed homogenate derived from *S. trinodis* collected in November. The ΔAbs/35 mins in the presence of the *S. trinodis* collected in November homogenate was larger than without the seaweed homogenate, 22.20 vs. 13.19 ΔAbs/35 mins, respectively. Similarly, the ΔAbs/35 mins in the presence of the *S. trinodis* collected in April homogenate was also larger than without the seaweed homogenate, 25.94 vs. 13.19 ΔAbs/35 mins, respectively (Figure 3. 8B).

The X-intercept of the Lineweaver-Burk plot for the reaction rate of pancreatic lipase with the *S. trinodis* homogenates collected in November was bigger than without the seaweed homogenate (-4.87 vs. -5.92, respectively) (Figure 3. 8A). However, the X-intercept of the Lineweaver-Burk plot for the reaction rate of pancreatic lipase with the *S. trinodis* homogenates collected in April was nearly the same as without the seaweed homogenate (-5.91 vs. -5.92, respectively). (Figure 3. 8B). These findings indicate that the homogenate derived from the brown seaweed *S. trinodis* collected in November decreases the V_{max} (1/Y-intercept) of pancreatic lipase reaction and increases K_m (-1/X-intercept), suggesting a mixed inhibition mode of action (Figure 3. 8A). Interestingly, the brown seaweed *S. trinodis* collected in April showed a decrease in the V_{max} (1/Y-intercept) and kept K_m (-1/X-intercept) unchanged of pancreatic lipase reaction, indicating a non-competitive inhibition mode of action (Figure 3. 8B).

Figure 3. 8C, D and E illustrate the Lineweaver-Burk plots with the brown seaweed homogenates derived from *C. sinuosa, I. stellata* and *P. boergesenii.* All the Y-intercepts for the reaction rate of pancreatic lipase were larger in the presence of the brown seaweeds when compared to the absence of seaweed homogenates, with *C. sinuosa* (30.97 vs. 13.19 ΔAbs/35 mins), with *I. stellata* (52.17 vs. 13.19 ΔAbs/35 mins), and with *P. boergesenii* (24.38 vs. 13.19 ΔAbs/35 mins) (Figure 3. 8C, D and E, respectively).

The X-intercepts of the Lineweaver-Burk plots for the reaction rate of pancreatic lipase in the presence of the brown seaweed homogenates derived from *C. sinuosa* and *P. boergesenii* were both bigger than without the seaweed homogenate (-5.38 vs. -5.92)

and (-4.92 vs. -5.92, respectively) (Figure 3. 8C and E, respectively). These findings indicate that the homogenates derived from the brown seaweeds *C. sinuosa* and *P. boergesenii* decrease the V_{max} (1/Y-intercept) of pancreatic lipase reaction and increase K_m (-1/X-intercept), suggesting a mixed inhibition mode of action (Figure 3. 8C and E, respectively). However, the X-intercept of the Lineweaver-Burk plot for the reaction rate of pancreatic lipase with the brown seaweed homogenate derived from *I. stellata* was smaller than without the seaweed homogenate (-7.40 vs. -5.92, respectively). These findings indicate that the brown seaweed homogenate derived from *I. stellata* decreases both the V_{max} (1/Y-intercept) and K_m (-1/X-intercept) of the pancreatic lipase reaction, as evident from the points where the plot crosses the x and y axes. These findings indicate a mixed inhibition for *I. stellata* (Figure 3. 8D).

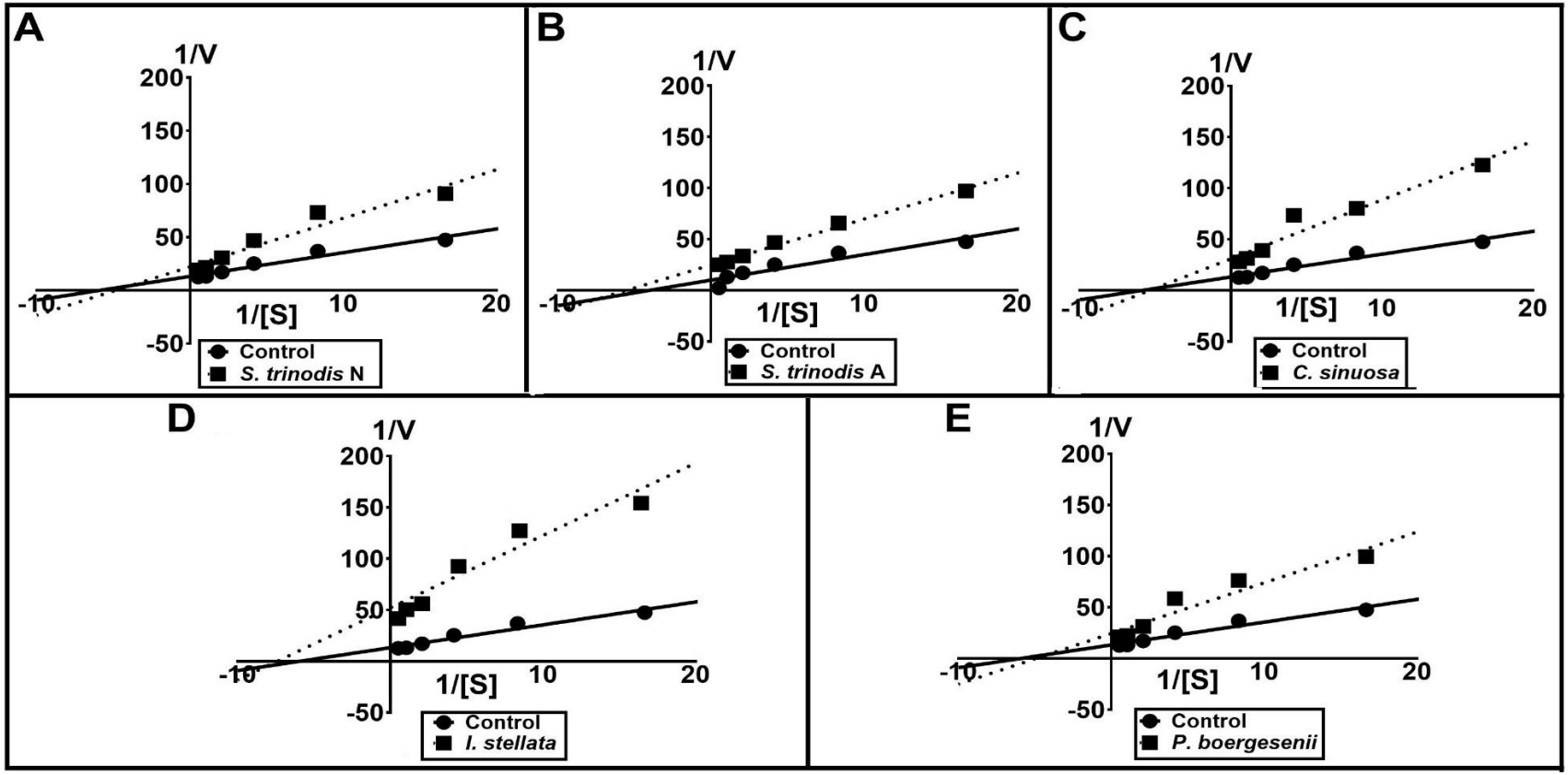

Figure 3. 8 Lineweaver-Burk plot for pancreatic lipase activity with and without brown seaweed homogenates.
Figure 3. 8A and B depict pancreatic lipase activity with the homogenates derived from *Sirophysalis trinodis* collected in November 2020 (N) and April 2021 (A) at a concentration of 0.95 and 1.5 mg/ml, respectively. Figure 3. 8C, D and E depict pancreatic lipase activity with the addition of the homogenate derived from *Colpomenia sinuosa*, *Iyngaria stellata* and *Padina boergesenii* at 1.7, 1.14 and 1.9 mg/ml, respectively. Pancreatic lipase controls included 0.06 mg/ml pancreatic lipase and no brown seaweed homogenate. All samples with and without seaweed homogenate are depicted using a dashed or solid black line, respectively. All samples were added to various substrate [S] concentrations ranging from 0.06 to 1.9%. The velocity (the rate of turbidity change) was calculated by measuring the change in absorbance at 405nm over 35 minutes (ΔAbs/35 mins).

3.5.3 The Lineweaver-Burk Plots with and without Seaweed WE

In Figure 3. 9A, the Lineweaver-Burk plot shows the y-intercept for the reaction rate of pancreatic lipase with and without the WE from green seaweed *C. papillatum*. The plot demonstrates that in the presence of the *C. papillatum* WE, the ΔAbs/35 mins is larger compared to the ΔAbs/35 mins without the WE (48.82 vs. 13.19 ΔAbs/35 mins) (Figure 3. 9A). Furthermore, the X-intercept of the Lineweaver-Burk plot for the reaction rate of pancreatic lipase with the *C. papillatum* WE was smaller than without the WE (-10.94 vs. -5.92, respectively). These findings indicate that the WE derived from *C. papillatum* decreases both the V_{max} (1/Y-intercept) and K_m (-1/X-intercept) of pancreatic lipase reaction, suggesting a mixed inhibition mode of action (Figure 3. 9A).

The Lineweaver-Burk plots for WE derived from the brown seaweed *S. trinodis* N and *S. trinodis* A are illustrated in Figure 3. 9B and C, respectively. The plots show that the reaction rate of pancreatic lipase containing WE from *S. trinodis* N and *S. trinodis* A were both larger compared to the reaction rate of pancreatic lipase without the WE (38.40 vs. 13.19 and 25.94 vs. 13.19 ΔAbs/35 mins, respectively). The X-intercept of the Lineweaver-Burk plots for the reaction rate of pancreatic lipase with the *S. trinodis* N WE was smaller than without the seaweed WE (-7.12 vs. -5.92) (Figure 3. 9B). However, the X-intercept of the Lineweaver-Burk plots for the reaction rate of pancreatic lipase with the *S. trinodis* A WE was almost the same as that without the seaweed WE (-5.91 vs. -5.92) (Figure 3. 9C). These findings indicate that the WE derived from the brown seaweed *S. trinodis* N decreases both the V_{max} (1/Y-intercept) and K_m (-1/X-intercept) of pancreatic lipase reaction, indicating a mixed inhibition mode of action (Figure 3. 9B). Whereas the data here indicate that the WE extract derived from the brown seaweed S. *trinodis* A decreases the V_{max} (1/Y-intercept) and keeps the K_m (-1/X-intercept) unchanged, indicating a non-competitive inhibition mode of action (Figure 3. 9C)

Figure 3. 9D illustrates the reaction rate of pancreatic lipase with and without the WE from brown seaweed *P. boergesenii*. The plot demonstrates that in the presence of the *P. boergesenii* WE, the ΔAbs/35 mins was higher than without the WE (53.21 vs. 13.19 ΔAbs/35 mins) (Figure 3. 9D). The X-intercept of the Lineweaver-Burk plot for the reaction rate of pancreatic lipase with *P. boergesenii* WE was lower than without the WE (-12.89 vs. -5.92, respectively). These findings indicate that the WE derived from

P. boergesenii decreases both the V_{max} (1/Y-intercept) and K_m (-1/X-intercept) of pancreatic lipase reaction, suggesting a mixed inhibition mode of action (Figure 3. 9D).

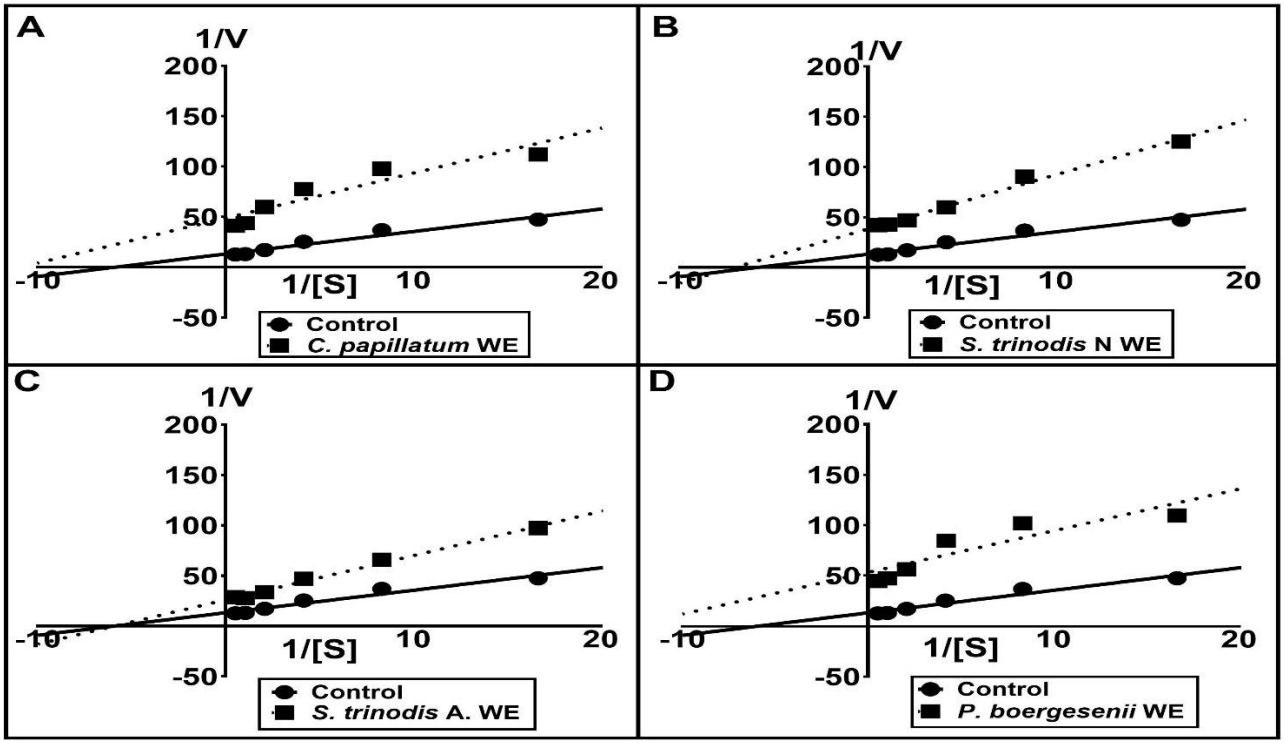

Figure 3. 9 Lineweaver-Burk plot for pancreatic lipase activity with and without WE extract from Kuwaiti seaweeds.

Figure 3. 9A-D depict pancreatic lipase activity with the deionised water extract (WE) from the green seaweed *Codium papillatum* at 1.3 mg/ml, the brown seaweed *Sirophysalis trinodis* collected in November 2020 (N) and April 2021 (A) (0.38 and 0.42 mg/ml, respectively) and *Padina boergesenii* at 2.8 mg/ml, respectively. Pancreatic lipase controls included 0.06 mg/ml pancreatic lipase and no brown seaweed homogenate. All samples with and without seaweed homogenate are depicted using a dashed or solid black line, respectively. All samples were added to various substrate [S] concentrations ranging from 0.06 to 1.9%. The velocity (the rate of turbidity change) was calculated by measuring the change in absorbance at 405nm over 35 minutes (ΔAbs/35 mins).

3.5.4 The Lineweaver-Burk Plots with and without Seaweed EP

The Lineweaver-Burk plot of the EP from Kuwaiti seaweed is shown in Figure 3. 10. Figure 3. 10A illustrates the reaction rate of pancreatic lipase with and without the EP from the green seaweed *C. sericioides*. The plot demonstrates that in the presence of the *C. sericioides* EP, the ΔAbs/35 mins was higher than without the EP (26.12 vs. 13.19 ΔAbs/35 mins) (Figure 3. 10A). The X-intercept of the Lineweaver-Burk plot for the reaction rate of pancreatic lipase with *C. sericioides* EP was smaller than without the EP (-8.57 vs. -5.92 mg/ml, respectively). These findings indicate that the EP derived from *C. sericioides* decreases both the V_{max} (1/Y-intercept) and K_m (-1/X-intercept) of pancreatic lipase reaction, suggesting a mixed inhibition mode of action (Figure 3. 10A).

Figure 3. 10B depicts the reaction rate of pancreatic lipase with and without the EP from the green seaweed *C. papillatum*. The y-intercept for the reaction rate of pancreatic lipase with the EP was larger than without the EP (46.86 vs. 13.19 ΔAbs/35 mins) (Figure 3. 10B). The X-intercept of the Lineweaver-Burk plot for the reaction rate of pancreatic lipase with *C. papillatum* EP was smaller than without the EP (-11.01 vs. -5.92, respectively). These findings indicate that the EP derived from *C. papillatum* decreases both the V_{max} (1/Y-intercept) and K_m (-1/X-intercept) of the pancreatic lipase reaction, suggesting a mixed inhibition mode of action (Figure 3. 10B).

Figure 3. 10C illustrates the reaction rate of pancreatic lipase with and without the EP from brown seaweed *P. boergesenii*. The plot demonstrates that in the presence of the *P. boergesenii* EP, the ΔAbs/35 mins was higher than without the EP (49.28 vs. 13.19 ΔAbs/35 mins) (Figure 3. 10C). The X-intercept of the Lineweaver-Burk plot for the reaction rate of pancreatic lipase with *P. boergesenii* EP was smaller than without the ethanol pellet (-11.55 vs. -5.92, respectively). These findings indicate that the EP derived from *P. boergesenii* decreases both the V_{max} (1/Y-intercept) and K_m (-1/X-intercept) of pancreatic lipase reaction, suggesting a mixed inhibition mode of action (Figure 3. 10C).

Figure 3. 10D depicts the reaction rate of pancreatic lipase with and without the EP from red seaweed *G. pusillum*. The plot demonstrates that in the presence of the *G. pusillum* EP, the ΔAbs/35 mins was higher than without the EP (43.49 vs. 13.19 ΔAbs/35 mins) (Figure 3. 10D). The X-intercept of the Lineweaver-Burk plot for the

reaction rate of pancreatic lipase with *G. pusillum* EP was smaller than without the EP (-9.41 vs. -5.92, respectively). These findings indicate that the EP derived from *G. pusillum* decreases both the V_{max} (1/Y-intercept) and K_m (-1/X-intercept) of pancreatic lipase reaction, suggesting a mixed inhibition mode of action (Figure 3. 10D).

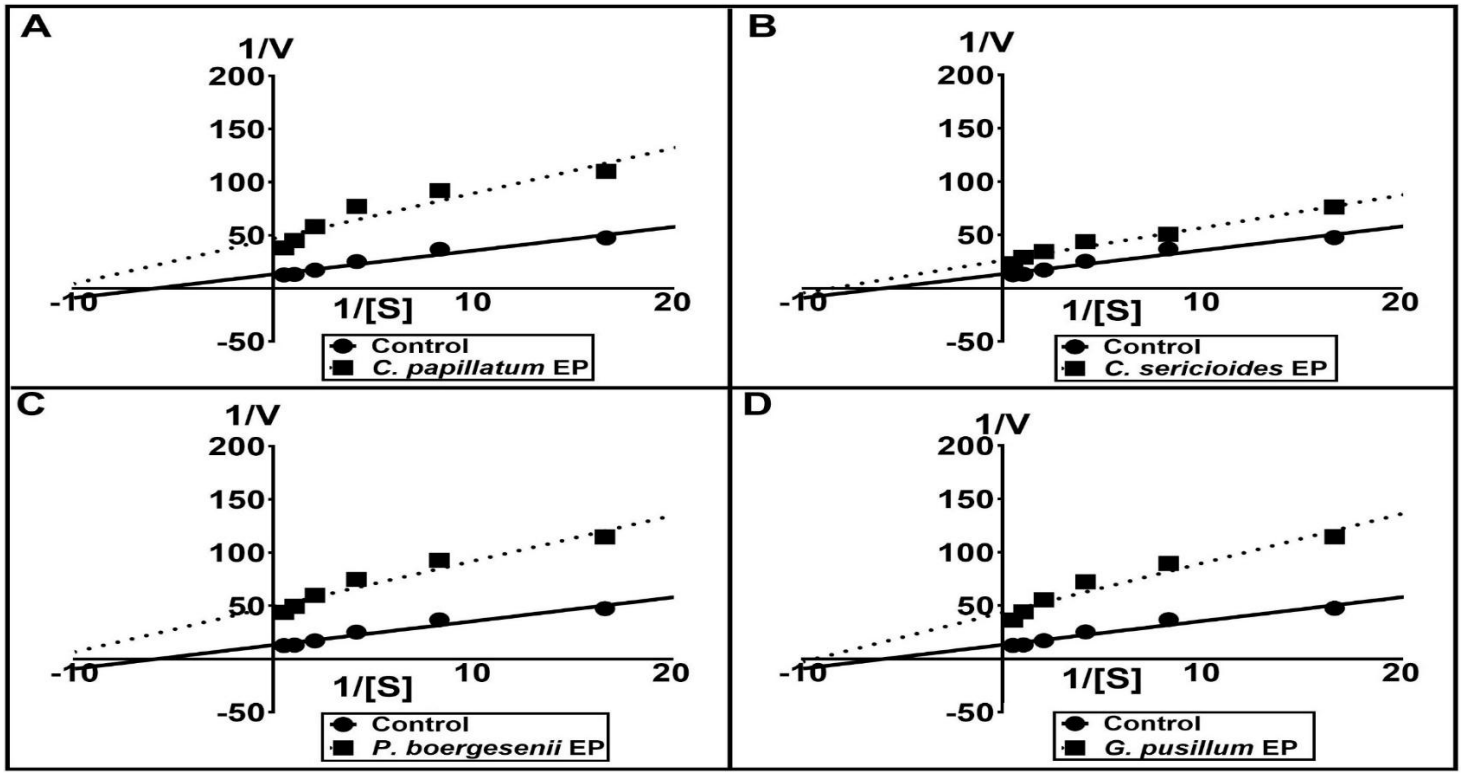

Figure 3. 10 Lineweaver-Burk plot for pancreatic lipase activity with and without ethanol pellets remaining after EE from Kuwaiti seaweeds.

Figure 3. 10A and B depict pancreatic lipase activity with the addition of the ethanol pellets (EP) from the green seaweeds *Cladophora sericioides* (*Clad* EP) and *Codium papillatum* (*Codi* EP) at 1.4 and 0.2 mg/ml. Figure 3. 10C and D depict the brown seaweed *Padina boergesenii* (*Padi* EP) at 3.0 mg/ml and the red seaweed *Gelidium pusillum* (*Geli* EP) at 2.5 mg/ml. Pancreatic lipase controls included 0.06 mg/ml pancreatic lipase and no brown seaweed homogenate. All samples with and without EP are depicted using a dashed or solid black line, respectively. All samples were added to various substrate [S] concentrations ranging from 0.06 to 1.9%. The velocity (the rate of turbidity change) was calculated by measuring the change in absorbance at 405nm over 35 minutes (ΔAbs/35 mins).

3.5.5 Kinetic Parameters of Inhibition Reaction by Kuwait Seaweed Extracts

The kinetic parameters of the reaction without Kuwaiti seaweed extracts, V_{max} and K_m, and with Kuwaiti seaweed extracts, V_{maxApp} and K_{mApp}, were determined from the Lineweaver-Burk plots. Pancreatic lipase inhibition exhibited by the Lineweaver-Burk plots for all seaweed samples was found to be a mixed-type inhibition except homogenate from green seaweed *C. sericioides* and homogenate and WE from brown seaweed *S. trinodis* A exhibited a non-competitive inhibition. Consequently, based on the definition of mixed-type inhibition, Kuwaiti seaweed and its extracts are likely to bind to both the free pancreatic lipase and the ES complex formed following pancreatic lipase and substrate interactions with different affinity ($K_{ia} \neq K_{ib}$). In addition, based on the definition of non-competitive inhibition, the homogenate from *C. sericioides* and homogenate and WE from brown seaweed *S. trinodis* A are likely to bind to both free pancreatic lipase and ES complex with the same affinity ($k_{ia} = K_{ib}$). Therefore, the inhibition constants for binding seaweed extracts to free pancreatic lipase (K_{ia}) and to the ES complex formed between pancreatic lipase and the substrate (K_{ib}) were calculated. The V_{max} and K_m of the uninhibited reactions were 0.076 ΔAbs/35 mins and 0.169, respectively.

3.5.5.1 Kinetic Parameters of Inhibition Reaction by Seaweed Homogenate

Table 3. 1 illustrates the calculated values of kinetic parameters for the inhibition reactions based on the green, red and brown seaweed homogenates. In the presence of seaweed homogenate, the V_{max} decreased to V_{maxApp}. However, the K_{mApp} values in the presence of different seaweed homogenates compared to K_m without seaweed homogenates showed variations, including decrease, increase or remain unchanged. The K_{mApp} was unchanged in the presence of homogenates from green seaweed *C. sericioides* and brown seaweed *S. trinodis* A, with an equal K_{ia} and K_{ib} (Table 3. 1). However, the K_{mApp} decreased in the presence of *I. stellata* homogenate, with K_{ia} bigger than K_{ib} (Table 3. 1). In contrast, the K_{mApp} values increased in all other seaweed homogenates, with K_{ia} lower than K_{ib} (Table 3. 1).

Therefore, the homogenates from *C. sericioides* and *S. trinodis* A exhibited the same affinity to free enzymes and ES complexes. However, the *I. stellata* homogenate showed a higher affinity to the ES complex. In contrast, *S. trinodis* N, *C. sinuosa* and *P. boergesenii* exhibited a high affinity to free enzymes.

Table 3. 1 The Kinetic parameters of inhibition reaction by seaweed homogenates.

Seaweed Type	Seaweed	V_{maxApp} ΔAbs/35 mins	K_{mApp} [S]%	K_{ia}	K_{ib}	MoI
Green	*Cladophora sericioides*	0.038	0.168	0.60	0.60	Non-competitive inhibition
Red	*Gelidium pusillum*	0.045	0.183	1.57	1.90	Mixed with ↑ affinity to free enzyme
Brown	*Sirophysalis trinodis* N	0.045	0.205	0.96	1.46	Mixed with ↑ affinity to free enzyme
	Sirophysalis trinodis A	0.039	0.169	1.70	1.70	Non-competitive inhibition
	Colpomenia sinuosa	0.032	0.186	1.14	1.34	Mixed with ↑ affinity to free enzyme
	Iyengaria stellata	0.019	0.135	0.56	0.41	Mixed with ↑ affinity to SE complex
	Padina boergesenii	0.041	0.20	1.63	2.36	Mixed with ↑ affinity to free enzyme

V_{maxApp}: apparent maximum reaction rate; K_{mApp}: Michaelis-Menten constant; K_{ia}: inhibition constant for binding seaweed homogenate to the free pancreatic lipase K_{ib}: inhibition constant for binding seaweed homogenate to pancreatic lipase-substrate complex; MoI: mode of inhibition; N; seaweed collected in November 2020; A: seaweed collected in April 2021; ↑: high; ΔAbs/35 mins: change in absorbance at 405nm over 35 minutes.

3.5.5.2 Kinetic Parameters of Inhibition Reaction by WE

The data presented in Table 3. 2 includes the calculated values of kinetic parameters from the inhibition reaction elicited by the WE. In the presence of the WE, the V_{max} decreased to V_{maxApp}. In addition, the K_{mApp} decreased for all WE, except for one from *S. trinodis* A remained unchanged (Table 3. 2). The K_{ia} values were higher than the K_{ib} values in the presence of all the WE except for one from *S. trinodis* A (Table 3. 2). The K_{ia} value was equal to the K_{ib} value in the presence of WE from *S. trinodis* A (Table 3. 2). These data indicate that all of the WE, except for the one from *S. trinodis* A, had a high affinity for the ES complex. Whereas the WE from *S. trinodis* A had an equal affinity for free enzyme and ES complex.

3.5.5.3 Kinetics Parameters of Inhibition Reaction by EP

Table 3. 3 illustrates the calculated kinetic parameters from the inhibition reaction by the EP. In the presence of the EP, the V_{max} decreased to V_{maxApp}, and the K_{mApp} also decreased for all EP (Table 3. 3). The K_{ia} value was higher than K_{ib} in the presence of all the EP (Table 3. 3). These data indicate that all the EP examined had a high affinity for the ES complex.

Table 3. 2 The Kinetic parameters of inhibition reaction by deionised water extract (WE)

Seaweed Type	Seaweed	V_{maxApp} ΔAbs/35 mins	K_{mApp} [S]%	K_{ia}	K_{ib}	MoI
Green	*C. papillatum*	0.020	0.091	1.40	0.52	Mixed with ↑ affinity to SE complex
Brown	*S. trinodis* N	0.026	0.140	0.28	0.21	Mixed with ↑ affinity to SE complex
	S. trinodis A	0.039	0.169	0.46	0.46	Non-competitive inhibition
	P. boergesenii	0.019	0.078	3.41	0.96	Mixed with ↑ affinity to SE complex

V_{maxApp}: apparent maximum reaction rate; K_{mApp}: Michaelis-Menten constant; K_{ia}: inhibition constant for binding seaweed homogenate to the free pancreatic lipase; K_{ib}: inhibition constant for binding seaweed homogenate to pancreatic lipase-substrate complex; MoI: mode of inhibition; N; seaweed collected in November 2020; A: seaweed collected in April 2021; ↑: high; ΔAbs/35 mins: change in absorbance at 405nm over 35 minutes.

Table 3. 3 The Kinetic parameters of inhibition reaction by ethanol pellets (EP)

Seaweed Type	Seaweed	V_{maxApp} ΔAbs/35 mins	K_{mApp} [S]%	K_{ia}	K_{ib}	MoI
Green	*C. sericioides*	0.038	0.117	4.08	1.53	Mixed with ↑ affinity to SE complex
	C. papillatum	0.021	0.091	0.28	0.09	Mixed with ↑ affinity to SE complex
Brown	*P. boergesenii*	0.020	0.087	2.18	0.46	Mixed with ↑ affinity to SE complex
Red	*G. pusillum*	0.023	0.106	2.43	1.13	Mixed with ↑ affinity to SE complex

V_{maxApp}: apparent maximum reaction rate; K_{mApp}: Michaelis-Menten constant; K_{ia}: inhibition constant for binding seaweed homogenate to the free pancreatic lipase; K_{ib}: inhibition constant for binding seaweed homogenate to pancreatic lipase-substrate complex; MoI: mode of inhibition; ↑: high; ΔAbs/35 mins: change in absorbance at 405nm over 35 minutes.

3.6 Discussion

Seaweeds have been the subject of intense research due to their associated health benefits. In addition, seaweeds have been revealed the potential as an anti-obesity therapy, primarily through their ability to inhibit pancreatic lipase. The aim of this chapter was to elucidate the mechanism of action of the seaweeds as potential pancreatic lipase inhibitors. Specifically, the enzyme-inhibiting mechanism of 15 seaweed homogenates and extracts studied in Chapter 2, which have been previously reported on their ability to inhibit pancreatic lipase, were identified. To identify the enzyme-inhibiting mechanism of the seaweed homogenates and extracts, the K_m and V_{max}, as well as the K_{mApp} and V_{maxApp}, of the enzymatic reactions both without and with inhibition were analysed. All Kuwaiti seaweed samples and their extracts were classified as mixed inhibitors using Lineweaver-Burk plots, except homogenate from green seaweed *C. sericioides* and homogenate and WE from brown seaweed *S. trinodis* A exhibited a non-competitive inhibition. Furthermore, inhibition constants K_{ia} and K_{ib} were calculated to evaluate the binding affinity of the inhibitor to free pancreatic lipase and/or pancreatic lipase-substrate complex, respectively. The variation observed here in the K_{ia} and K_{ib} values may be attributed to different seaweed species and extraction solvents, likely due to the presence of diverse compounds with varying affinities towards the free enzyme or the enzyme-substrate complex. In addition, the difference in the mode of inhibition between *S. trinodis* N and S. trinodis A suggests a seasonal variation effect.

Seaweed-derived bioactive compounds, including polysaccharides (Zhang *et al.*, 2021), polyphenols (Shannon, Conlon and Hayes, 2023) and carotenoids (Kurniawan *et al.*, 2023), have previously been shown to effectively inhibit pancreatic lipase activity. Consequently, further studies have been conducted in an attempt to identify the kinetic parameters of these compounds on pancreatic lipase. However, to date, there remains a scarcity of research definitively identifying the kinetic parameters of seaweeds. Caulerpenyne, a compound extracted from the Japanese green seaweed *Caulerpa taxifolia*, has been studied as a competitive inhibitor of pancreatic lipase using the double reciprocal Lineweaver-Burk plot of reaction rate vs. substrate (triolein) concentrations (Bitou *et al.*, 1999). Dieckole is another compound, classified as a polyphenol extracted from the Korean brown seaweed *Eisenia bicyclis* (Jung *et al.*,

2013). Using the double reciprocal Lineweaver-Burk plot, Jung *et al.* (2013) were able to demonstrate that Dieckole is a non-competitive inhibitor of pancreatic lipase, using p-nitrophenyl butyrate (pNPB) as its substrate. Finally, Fucoidan, a sulfated polysaccharide extracted from the Chinese brown seaweed *Laminaria japonica*, has been identified as a mixed inhibitor using the double reciprocal Lineweaver-Burk plot of reaction rate vs. substrate (4-nitrophenyl laurate (pNPL)) concentrations (Lu, Gu and Yu, 2024).

To date, several studies have examined the inhibitory effects of plant extracts on pancreatic lipase activity. In a recent study by Khatchapuridze, Ploeger and Gulua (2023), the authors observed that Mukuzani dry red wine from Georgia exhibited a mixed-type inhibition through the use of the double reciprocal Lineweaver-Burk plot of reaction rate vs. olive oil concentrations, with a K_{ia} value lower than the K_{ib} value. However, it is important to note that the aqueous and ethyl-acetate extracts from the Mukuzani wine exhibited uncompetitive inhibition (Khatchapuridze, Ploeger and Gulua, 2023). The variation observed in the mode of inhibition may be attributed to active compounds in the dried wine and in the aqueous and ethyl-acetate extracts, likely due to the presence of diverse compounds with varying affinities towards the free enzyme or the enzyme-substrate complex (Khatchapuridze, Ploeger and Gulua, 2023). In another study, Gholamhoseinian, Shahouzehi and Sharifi-Far (2010) revealed that methanolic extracts derived from several Iranian plants exhibited different inhibitory effects on pancreatic lipase through the use of the double reciprocal Lineweaver-Burk plot of reaction rate vs. triolein concentrations (Gholamhoseinian, Shahouzehi and Sharifi-Far, 2010). The mode of inhibition was also studied in plants with the highest inhibitory properties, including *Quercus infectoria*, *Eucalyptus galbie*, *Rosa damascena* and *Levisticum officinale*. All the mEEs obtained from the Iranian plants exhibited a non-competitive inhibition, except for *Levisticum officinale*, which exhibited a mixed-type inhibition (Gholamhoseinian, Shahouzehi and Sharifi-Far, 2010).

A substantial body of evidence has examined the impact of polyphenols on the activity of pancreatic lipase. Wu *et al.* (2013) reported that the polyphenol epigallocatechin gallate (EGCG) derived from green tea non-competitively inhibited pancreatic lipase using the double reciprocal Lineweaver-Burk plot of reaction rate vs. pNPB concentrations. This study also observed using fluorescence spectroscopy, circular dichroism (CD), isothermal titration calorimetry, and molecular docking that EGCG was

able to bind with pancreatic lipase through hydrogen bonds and hydrophobic interactions, though this binding did not occur at or near the active site of pancreatic lipase (Wu *et al.*, 2013). Similarly, Hu *et al.* (2015) reported that the polyphenol caffeoylquinic acid and its major isomers derived from coffee could competitively inhibit pancreatic lipase using the double reciprocal Lineweaver-Burk plot of reaction rate vs. pNPL concentrations. The authors noted that caffeoylquinic acid and its major isomers bound closely to, but not at, the active site of pancreatic lipase. Hu *et al.* (2015) also acknowledged that the structural differences among the isomers of coffee likely influenced the strength of hydrogen bonds and hydrophobic interactions, thereby affecting their inhibitory potential (Hu *et al.*, 2015).

In a study by Glisan *et al.* (2017), the authors reported that theaflavin derivatives with gallate obtained from black tea competitively inhibited pancreatic lipase using the Michaelis-Menten plot of reaction rate vs. pNPB concentrations. The authors noted through *in-silico* modeling that these polyphenols formed hydrogen bonds with an inhibitor pocket within the structure of pancreatic lipase that was close to the substrate-binding domain. By binding with this inhibitor pocket, the polyphenols were able to modify the enzyme's active site by protonating the crucial catalytic residue (His264), which subsequently led to the inhibition of enzymatic activity (Glisan *et al.*, 2017).

Further research on polyphenols, conducted by (Martinez-Gonzalez *et al.*, 2017) noted that four polyphenols, including caffeic acid, p-coumaric acid, quercetin and capsaicin extracted from hot peppers, exhibited a mixed-type inhibition using the double reciprocal Lineweaver-Burk plot of reaction rate vs. pNPL concentrations. The authors revealed using molecular docking analysis that the binding sites of caffeic acid, p-coumaric acid, and quercetin were close to the active site of pancreatic lipase with lower K_{ia} values compared to the K_{ib} values. In contrast, the capsaicin binding site on pancreatic lipase was not located close to the active site, and a K_{ia} value was higher than a K_{ib} value (Martinez-Gonzalez *et al.*, 2017). Interestingly, the authors reported that the quercetin K_{mApp} value was higher than the control K_m, whereas the K_{mApp} values of p-coumaric acid and caffeic acid were lower than the control K_m. These findings suggest that the inhibitory properties of these polyphenols may vary due to their structural characteristics, binding locations, and inhibitory potencies (Martinez-Gonzalez *et al.*, 2017; Wu *et al.*, 2013).

3.6.1 Kinetic Parameter of Seaweed Homogenates

The kinetic data presented here demonstrates that the addition of homogenates from the green and red seaweeds, *C. sericioides* and *G. pusillum*, respectively, effectively decreases the V_{max}. These findings suggest that both *C. sericioides* and *G. pusillum* homogenates effectively inhibit catalysis, whether linked to a free enzyme or the enzyme-substrate complex. The K_m value was also increased in the presence of *C. sericioides* and *G. pusillum* homogenates, indicating weaker substrate binding to the enzyme and a reduced affinity between the substrate and the enzyme. Additionally, the K_{ia} value was lower than the K_{ib} value in the presence of *C. sericioides* and *G. pusillum* homogenates, indicating that these homogenates had a higher affinity for binding to free enzymes compared to enzyme-substrate complexes, thereby enhancing the inhibitory effect on pancreatic lipase stronger.

Similarly, the addition of homogenate from the brown seaweeds *S. trinodis* N and A (collected in both November 2020 and April 2021), *C. sinuosa*, *I. stellata,* and *P. boergesenii* were observed to decrease the V_{max}. These findings indicate that the brown seaweed homogenates effectively inhibit catalysis, regardless of whether these seaweeds were associated with a free enzyme or an enzyme-substrate complex. The K_m values increased in the presence of *S. trinodis* N, *C. sinuosa* and *P. boergesenii*. An increased K_m value suggests weak substrate binding to the enzyme, and reduced affinity between the substrate and the enzyme. Furthermore, the K_{ia} was lower than the K_{ib} values in the presence of *S. trinodis* N, *C. sinuosa* and *P. boergesenii* homogenates, indicating a higher affinity of these seaweeds for binding to free enzymes compared to enzyme-substrate complex. However, in the presence of the homogenates from *S. trinodis* A and *I. stellata*, the K_m values were reduced, and the K_{ia} was larger than the K_{ib} values. These data indicate that the homogenates from *S. trinodis* A and *I. stellata* have a higher affinity for binding to enzyme-substrate complexes than free enzymes.

Seaweed homogenates contain a wide range of phytochemical compounds, with each seaweed species possessing its own distinct phytochemical profile (Leandro *et al.*, 2020). The findings presented here suggest that the components within these seaweed homogenates have the potential to influence the inhibitory properties of pancreatic lipase activity through distinct mechanisms of inhibition, as evidenced by variance in

kinetics data. Additionally, although beyond the scope of this thesis, it is likely that the homogenates studied here contain various pancreatic lipase inhibitors, which may have influenced the results. Moving forward, further research is needed in order to identify which compounds are present in the seaweeds studied here and which of these compounds has the highest inhibitory effect on pancreatic lipase whilst identifying their mechanisms of action.

3.6.2 Kinetic Parameters of WE

The kinetic data from the WE from the green seaweed *C. papillatum* and brown seaweeds *S. trinodis* N, *S. trinodis* A and *P. boergesenii*, all showed a decrease in V_{max}. Similar to the homogenates, these data suggest that WE effectively inhibit catalysis, regardless of whether these seaweeds were associated with free enzymes or enzyme-substrate complexes. The K_m values were reduced, and K_{ia} values were higher than those K_{ib} values in the presence of the WE.

Seaweed WE are likely to contain water-soluble compounds, including soluble polysaccharides, proteins and soluble polyphenols (Austin *et al.*, 2018). The data presented here, along with the potential water-soluble compounds contained within the WE, may account for the higher affinity for binding to the enzyme-substrate complex, as evidenced by the lower K_{ib} value.

3.6.3 Kinetic Parameters of EP

The kinetic data for the EP showed a reduction in Vmax in the presence of the green seaweed *C. sericioides* and *C. papillatum*, brown seaweed *P. boergesenii* and the red seaweed *G. pusillum*. Thus, the EP effectively inhibited catalysis regardless of whether the seaweeds were associated with a free enzyme or an enzyme-substrate complex. The reduction in K_m values and a higher K_{ia} compared to K_{ib} in the presence of the EP suggests that these seaweeds have a higher affinity for binding to the enzyme-substrate complex rather than the free enzyme.

Seaweed EP contains ethanol-soluble compounds such as polyphenols, carotenoids and steroids, which have previously been shown to significantly influence the inhibitory properties of plants and seaweeds (Austin *et al.*, 2018). The data presented here, combined with previous literature, suggests that these ethanol-soluble compounds

may contain components with a higher affinity for binding to the enzyme-substrate complex, as evidenced by the lower K_{ib} value.

3.6.4 The Temporal Effect of *S. trinodis* in Relation to Mode of Inhibition

It has been demonstrated that seasonal variation, including sunlight, temperature and salinity, has a significant effect on the physical and chemical properties of seaweeds (Ptak *et al.*, 2021; Yesilova, Balkis and Taskin, 2017). The Kuwait coastline exhibits seasonal variations similar to those around the world. It was found that Kuwait's coastal temperatures were higher and salinities were lower in April than in November (Devlin *et al.*, 2019). In this chapter, the mode of inhibition for the homogenate and WE from *S. trinodis* N exhibited a mixed-type inhibition. However, the mode of inhibition for the homogenate and WE from *S. trinodis* A exhibited a non-competitive inhibition. The difference in the mode of inhibition between *S. trinodis* N and *S. trinodis* A is likely due to the seasonal variations, which impact the structure and function of seaweed, as detailed in 2.5.4.

3.7 Conclusion

The kinetic parameters obtained in this chapter demonstrate that all Kuwaiti seaweeds and their aqueous extracts and ethanolic pellets examined could reduce the maximum reaction rate V_{max} of pancreatic lipase, thus decreasing its activity. The data here revealed that Kuwaiti seaweeds and their extracts possess varying affinities for free pancreatic Lipase or pancreatic lipase-substrate complexes. The variety of different inhibitory properties observed here is likely to be species-dependent, influenced by the extraction solvents and variety of compounds contained within the seaweeds and their extracts. In addition, the difference in the mode of inhibition between *S. trinodis* N and *S. trinodis* A, which were collected in November 2020 and April 2021, respectively, indicates a seasonal variation effect on physical and bioactive compound properties. These compounds were not controlled for but are likely to have modified the affinities towards either the free pancreatic lipase or pancreatic lipase-substrate complexes.

In summary, all Kuwaiti seaweeds and their aqueous and ethanolic extracts examined here exhibited a mixed-type inhibition of pancreatic lipase, except homogenate from green seaweed *C. sericioides* and homogenate and WE from brown seaweed *S.*

trinodis A, which exhibited a non-competitive inhibition. Understanding the mode of inhibition can provide insight into the regulation and inhibition of pancreatic lipase by Kuwaiti seaweed. Therefore, this can add economic value to Kuwaiti seaweed, which can be used as a natural resource for managing weight and metabolic health.

Chapter 4 The Effect of Kuwaiti Seaweed and their Aqueous and Organic Extracts on in-vitro Fat Digestion Using a Model Gut

4.1 Introduction

The prevalence of obesity continues to rise unabated. There are several factors that contribute to this epidemic, including excessive calorie intake and reduced physical activity (Musaiger *et al.*, 2012). Interestingly, obesity prevalence in Kuwait and other countries in the Gulf Cooperation Council (GCC) is higher than in other parts of the world, with the consumption of foods high in fat being linked (Khalil *et al.*, 2018).

Fat consumption, along with its digestion and absorption, is essential for physiological homeostasis, facilitating the absorption of fat-soluble vitamins, providing energy, protecting vital organs, and promoting cell growth (Field and Robinson, 2019). However, excessive fat consumption has been linked to a myriad of acute and chronic health conditions, including diabetes (Alkandari *et al.*, 2020), cardiovascular diseases (Abdelaziz *et al.*, 2025) and certain cancers (Gaskell *et al.*, 2024), particularly in the GCC countries, notably Kuwait (Weiderpass *et al.*, 2019). Furthermore, beyond, the health implications, the burden of obesity and its related comorbidities carries significant socioeconomic and financial implications, specifically on healthcare systems (Shephard, 2019).

Fat is an umbrella term encompassing all types of lipids, including triacylglycerides, phospholipids, glycolipids, and sterols. Triacylglycerides are the predominant form of fat present in the diet, accounting for 90-95% (Field and Robinson, 2019; Omer and Chiodi, 2024). Triacylglycerides are composed of three fatty acids bound to the glycerol backbone via ester bonds (Figure 4. 1) (Xiccato, 2010, pp. 56-57; Field and Robinson, 2019). The length of fatty acids in triglycerides varies depending on the number of carbon atoms (C), ranging from short (C3 to C6), medium (C8 to C14) to long (C16 or more) and can have a significant effect on its physical properties. Furthermore, the bonds between C can vary, where a single, double or more than two double bonds elicit saturated, monounsaturated or polyunsaturated fatty acids (Field and Robinson, 2019).

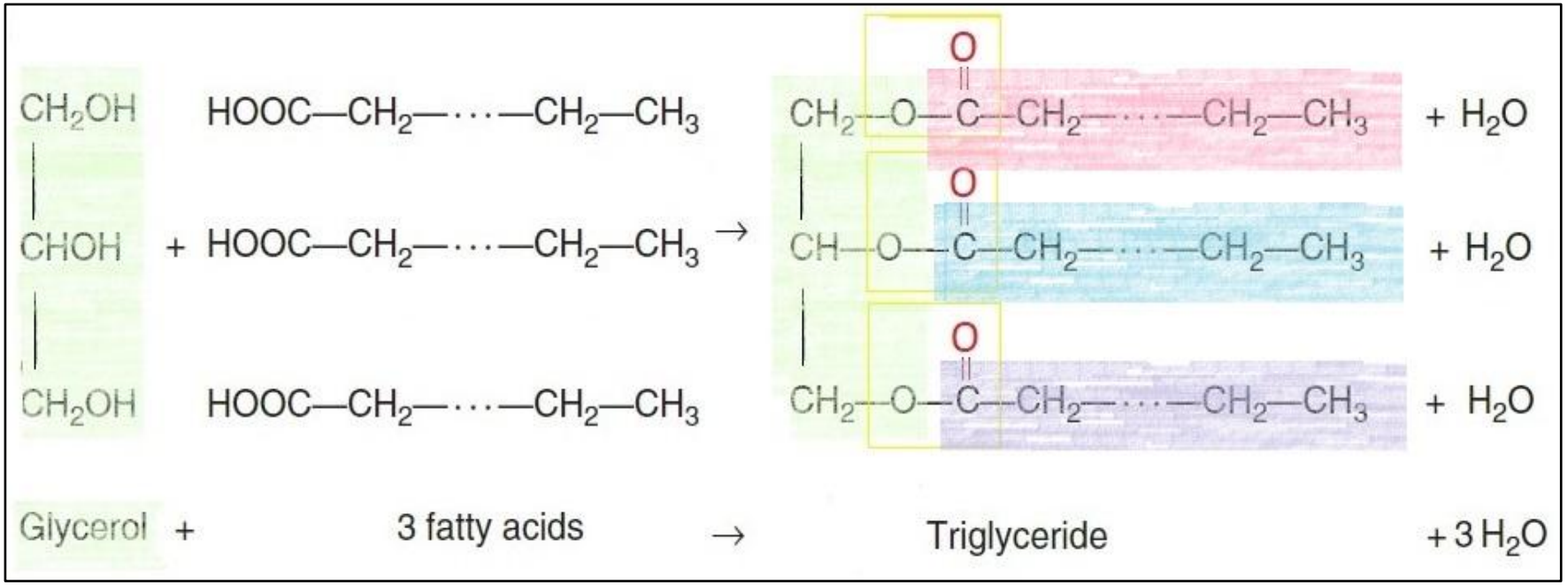

Figure 4. 1 Structure of triacylglyceride.

The schematic diagram depicts the formation of triacylglyceride through the binding of three fatty acids to glycerol backbone via a tri-ester bond, depicted in the yellow oblong. The glycerol molecule is attached to three fatty acids at different positions stereospecific number-1 (SN-1) (pink), SN-2 (blue) and SN-3 (purple). Fatty acid chains exhibit variations in the number of carbon atoms, categorised as short (2-6 carbons), medium (6-12 carbons), or long chain (13-21 carbons). Another classification is based on the type of bonds between carbon atoms (single saturated bonds and double unsaturated bonds). Adapted from (Xiccato, 2010, p. 57).

In an earlier study by Khalil *et al.* (2018), the authors underscored the criticality of the obesity level, associated comorbidities and socioeconomic and financial implications, which have escalated to a pandemic level in GCC countries, particularly in Kuwait. Reducing fat intake through calorie restriction is important for tackling obesity in addition to the other proposed strategies, such as a national intervention centred around education. However, the implementation of calorie reduction and dietary modification presents its own challenges and often has poor adherence (Khalil *et al.*, 2018). While pharmaceutical interventions for obesity are under development, drugs such as Orlistat to mitigate fat digestion offer an alternative approach. However, as with most medications, Orlistat is also associated with several adverse effects, rendering it suitable for short-term clinical use only (Jin *et al.*, 2021; Katimbwa *et al.*, 2022).

An alternative approach involves the use of naturally occurring compounds that possess the potential to reduce fat digestion and absorption without the aforementioned adverse effects of drugs such as Orlistat. Seaweed is one such compound, previously linked with multiple health benefits, and currently utilised as a natural source for reducing fat digestion (Austin *et al.*, 2018; Shannon, Conlon and Hayes, 2023). A recent review conducted by Trigo *et al.* (2023) identified the health benefits of seaweed on various parameters including blood glucose, blood pressure, anthropometrics, and blood lipids. Additionally, Astorga-España *et al.* (2017) previously highlighted the rich nutritional profile of seaweeds and their extracts when incorporated into high-saturated fat (meat-based products) or high-fibre foods (plant-based products). Seaweed has also been noted for its ability to enhance the nutritional value of food and elevate fibre and mineral content while concurrently reducing fat and salt levels and preserving food quality. Crucially, in GCC countries, seaweeds are also suitable for all dietary groups such as halal, vegan and kosher (Shannon and Abu-Ghannam, 2019). The properties pertaining to seaweed emphasised here underscore the potential of seaweed as a promising alternative for managing obesity and the associated comorbidities (Leandro *et al.*, 2020). It is, therefore, essential to assess the impact of seaweed on fat digestion.

4.1.1 Fat Digestion

Fat digestion is a complex and sequential process that requires the cooperation of bicarbonate, bile salts, and several lipases. This intricacy is further demonstrated by the requirement to transition between distinct pH levels, ranging from highly acidic in the stomach to alkaline in the small intestine, where most fat digestion occurs (Omer and Chiodi, 2024).

The digestion process begins in the mouth, where saliva and teeth begin the mastication process (Omer and Chiodi, 2024). Once in the stomach, the chyme is exposed to acidic conditions, gastric lipase and peristaltic contractions, which create a crude emulsion. Gastric lipase hydrolysing short or medium fatty acids of triacylglycerol from the ester bond at the stereotypic number-3 (SN-3), resulting in the release of a fatty acid and diglyceride into the duodenum (Xu *et al.*, 2021).

The passage of the chyme through the pyloric sphincter from the stomach and into the duodenum triggers the production of the hormones cholecystokinin (CCK) and secretin. Secretin is important for inhibiting further gastric acid secretions while initiating the release of pancreatic juice, which neutralises the acidity of the chyme and optimises the pH in the duodenum for lipase activity. Alongside pancreatic juice, pancreatic lipase and colipase are released into the duodenum to further facilitate fat digestion (Omer and Chiodi, 2024). Moreover, CCK is crucial for stimulating the gall bladder to secrete bile into the small intestine. Bile salts envelop and emulsify large fat globules, breaking them into smaller droplets, thereby increasing their surface area. This increased surface aids in the breakdown of triglycerides from the ester bond at positions SN1 and SN3 by pancreatic lipase and colipase, with the latter being essential for accessing the lipid-water interface of the chyme (Omer and Chiodi, 2024).

4.1.2 Seaweed and Fat Digestion In-Vivo

Seaweed has previously been recognised as a promising candidate for addressing obesity and diet-related diseases (Wan-Loy and Siew-Moi, 2016). Seaweed contains low-calorie content coupled with high dietary fibre content that has been cited for its potential to enhance gastrointestinal function by increasing satiety and reducing energy intake (Shannon and Abu-Ghannam, 2019). The data presented in Table 4. 1 provides a summary of in-vivo studies illustrating the beneficial effects of seaweed on

the digestive system. Specifically, these studies have demonstrated the ability of seaweed and its extracts to decrease appetite (El Khoury *et al.*, 2014), enhance gastrointestinal function (Hall *et al.*, 2012; Jensen *et al.*, 2012; Pelkman *et al.*, 2007), and reduce body weight (Abidov *et al.*, 2010; Jensen, Kristensen and Astrup, 2012; Hitoe and Shimoda, 2017).

It is important to acknowledge that, although the studies presented in Table 4. 1 show promise, there is a significant variation in the methodologies employed, as well as in the type of seaweed and its extracts consumption by participants, including beverages, supplements or mixed with food. The variations in study designs make any direct comparison difficult and represent an established limitation inherent in clinical trials. Further limitations encompass the high costs, duration, obtaining representative samples, confounding variables, and ethical and safety considerations associated with clinical trials. Consequently, research Into seaweed and its extracts has focussed on utilising in-vitro approaches, such as a model gut system, to address various obstacles encountered in clinical trials (Mulet-Cabero *et al.*, 2020).

Table 4. 1 In-vivo studies of seaweed and its extract as an anti-obesity agent.

In-vivo studies of seaweed and its extract as an anti-obesity agent.			
Authors	Seaweed	Aims & Methods	Results
Pelkman *et al.* (2007)	Alginate (Wayne, New Jersey)	-Alginate beverage was used to study its effect on food intake and satiety. - 29 healthy obese women were asked to drink 237ml alginate beverage (1g, 2.8g and control) twice a day for one week for each drink (before breakfast and after lunch).	Significant reduction in energy intake
Abidov *et al.* (2010)	Fucoxanthin (brown seaweed)	-Fucoxanthin and Xanthigen supplements were used by 151 obese women with high liver fat content to study the effect on body weight and body fat for sixteen weeks.	Significant reduction in body weight, body fat and liver fat content.
Hall *et al.* (2012)	Brown seaweed (*Ascophyllum nodosum*)- West Sussex, UK	-Study the effect of *Ascophyllum nodosum* seaweed-enriched bread on energy intake and appetite. -Twelve healthy obese males were asked to eat (4% per 400g wholemeal loaf) in a breakfast.	Significant reduction in energy intake after eating *Ascophyllum nodosum*-enriched bread
Jensen *et al.* (2012)	Alginate from (*Laminaria hyperborea* and *Lessonia Trabeculata*) (Denmark brown seaweeds)	-3% alginate beverage was drunk twice a day (before breakfast and lunch) to study its satiety effect on 20 healthy subjects.	Significant reduction of hunger and increased satiety

Jensen, Kristensen and Astrup (2012)	Alginate from (*Laminaria hyperborea* and *Laminaria digitata*) brown seaweeds	-To study the effect of an alginate supplement on body weight, 96 obese participants consumed it three times a day before each main meal for 84 days.	Significant reduction of body weight
El Khoury *et al.* (2014)	Alginate (*Laminaria hyperborean* from USA)	-Chocolate milk with (1.25% and 2.5%) alginate was administered to 24 healthy men, 2 hours before a pizza meal to investigate its effect on appetite, glucose, and insulin level.	More reduction in appetite, insulin, and glucose levels with 2.5% alginate chocolate milk
Hitoe and Shimoda (2017)	Fucoxanthin (Brown seaweed)	-Fucoxanthin capsules (1 and 3 mg) were used daily for 4 weeks to examine its effect on the body weight of 50 healthy adult Japanese participants with a BMI between 25 to 30kg/m^2.	Significant reduction of body weight, BMI and abdominal fat.

UK, United Kingdom; USA, United States of America; g, grams; ml, millilitre; BMI, body mass index; kg/m^2, kilogram/square meter.

4.1.3 Seaweed and Fat Digestion In-vitro

There are different digestion models, such as static, dynamic and semi dynamic model. The choice between these models is determined by the research issue and the research group's decision (Victor Calero *et al.*, 2024). In-vitro digestion models include a miniaturized revolutionary system (Victor Calero *et al.*, 2024), Info-Gest (Duijsens, Verkempinck and Grauwet, 2024), and the TNO Gastro-Intestinal Model (TIM) (Verwei *et al.*, 2016). Each of them has some benefits and drawbacks. The miniaturized device is an inexpensive high-throughput method employed for quick screening but does not possess the realistic nature needed for actual digestion (Victor Calero *et al.*, 2024). Info-Gest, as a reproducible standardised static model, is widely used in food and nutrition research since it is reproducible but not in a position to model dynamic phenomena of digestion (Duijsens, Verkempinck and Grauwet, 2024). TIM stands at the other end, representing a dynamic model simulating human digestion, inclusive of nutrient consumption and peristalsis, so it is the most physiological but also most costly and advanced (Verwei *et al.*, 2016). Whereas the miniaturized system and InfoGest are optimally suited to early research, the TIM is optimally suited to mechanistic research and pharmaceutical in-depth (Victor Calero *et al.*, 2024). However, none of the other available models allow continuous sampling and do not use whole bile so synthetic gut model, which was used in this study, is more physiologically relevant.

In this study, a synthetic gut model was employed to stimulate the sequential stages of digestion that occur within the gastrointestinal tract (GIT), encompassing salivary, gastric, and small intestinal phases, for investigating human digestion processes in vitro (Figure 4. 2). Since a synthetic model gut system has been invented and used in my supervisor's lab and in previous PhD studies, it was selected for this study. The model gut has previously demonstrated efficacy in replicating various digestion phases under controlled conditions, including the presence of enzymes, regulated temperature, and pH levels (Houghton *et al.*, 2014).

Previous studies have documented the potential inhibitory effect of diverse seaweed species and their extracts on pancreatic lipase activity using in-vitro approaches, as indicated in Table 2. 1. Various seaweed species from different global locations have been examined (Table 2. 1), all of which have exhibited impacts on seaweed properties and their inhibitory potential. Despite the in-vitro evidence showing the effectiveness

of seaweeds in inhibiting fat digestion, no studies have been conducted to date exploring the effects of Kuwaiti seaweed and its extracts on fat digestion.

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4.2 Aims

The primary purpose of this chapter is to investigate the effects of Kuwaiti seaweed on fat digestion in vitro using a synthetic model gut system. Consequently, the present study aimed to achieve the following objectives:

1. Utilise a model gut system replicating sequential digestion processes in the oral cavity, stomach, and small intestine. Utilising this model gut system enables the extraction of samples at various intervals. This process will ensure the following:
    a. Determine whether fat digestion proceeds at a rate comparable to in-vivo.
    b. Provide a direct comparison of fat digestion using a fat substrate alone, (serving as a positive control) vs. a fat substrate combined with various Kuwait seaweeds.
2. Determine whether Kuwait seaweed species can inhibit pancreatic lipase. This objective will yield the following outcomes:
    a. Identify whether Kuwait seaweeds that exhibited pancreatic lipase inhibited in an olive oil assay can translate into a reduction in fat digestion in a synthetic model gut.
    b. Identify which Kuwait seaweed can reduce fat digestion and determine whether differences exist among Kuwait species, including geographical variations.

4.3 Methods

The free glycerol reagent was used to quantify free glycerol in the model gut samples, facilitating the production of a measurable coloured dye with absorption at 550 nm. Since the artificial model gut system has been invented and used in my supervisor's lab and in previous PhD studies, it was selected for this study. Additionally, the chemicals and equipment required, such as a model gut equipment, spectrophotometer and standard reagents, were available in the lab, making this assay a viable option.

4.3.1 Materials

Enzymes utilised in the model gut were obtained from Sigma-Aldrich (UK): α-amylase (50 units/mg protein) from hog pancreas, pepsin from gastric mucosa (P7012) and pancreatin from the porcine pancreas (P7545). Gastric-like lipase was obtained from Amano enzyme (Inc AP12)

Fresh pig bile was homogenised and pre-warmed to 37°C before adding to the model gut (a gift from Thompson Abattoir, Bishop Auckland, UK). Olive oil was obtained from the Cooperative food (Newcastle Upon Tyne, UK). All other reagents utilised in this chapter were obtained from Sigma-Aldrich (USA). Kuwaiti seaweeds were kindly provided by the Marine Science Department at the Kuwait Institute for Scientific Research (KISR) and Kuwait University.

4.3.2 Equipment

The mixers for mixing reagents were purchased from IKA (UK). The Watson-Marlow peristaltic pump (Sci-Q 401U/D) and water baths (Grant GD 100) used in the model gut were set at 37°C and acquired from Fisher Scientific (UK). The 5415 R centrifuge from Stevenage, (UK) was used. All assays were measured at 550 nm using the EL808 96-well plate reader (Bio Teck, Bedfordshire, UK).

4.3.3 Seaweed

The seaweed homogenates, seaweed WE and EP exhibited the highest inhibitory effect on pancreatic lipase activity in the olive oil assay, as detailed in Chapter 2 were selected for use in the synthetic model gut. The samples employed in this chapter included:

- Homogenate samples: *C. sericioides*, *G. pusillum*, *S. trinodis* collected in November 2020 and April 2021, *C. sinuosa* and *I. stellata* and *P. boergesenii*.
- WE samples: *C. papillatum*, *S. trinodis* N, *S. trinodis* A and *P. boergesenii*.
- EP samples: *C. sericioides*, *C. papillatum*, *P. boergesenii* and *G. pusillum*.

4.3.4 Reagent Setup

The synthetic saliva, gastric juice, and small intestinal fluids for the synthetic model gut were prepared without enzymes as stock solutions, using deionised water and titrated to adjust pH using 6M HCL. All enzymes were added as required before each run of the model gut. The preparation of each synthetic digestive solution is described in detail here:

4.3.4.1 Synthetic Saliva Preparation

Artificial saliva comprised sodium bicarbonate at 62 mM, dipotassium hydrogen phosphate at 6 mM, sodium chloride at 15 mM, potassium chloride at 6.4 mM and calcium chloride dihydrate at 3 mM. The solution was then titrated to a pH of 7.4. Before each experiment, α-amylase was diluted in WE to 3 mg/ml. Subsequently, the 3 mg/ml α-amylase solution was diluted in artificial saliva to a concentration of 1µl/ml. For example: 50 µl of α-amylase was added to 50 ml of saliva juice).

4.3.4.2 Artificial Gastric Juice Preparation

Gastric juice comprised sodium chloride, potassium chloride, monopotassium phosphate and urea at 49.6, 9.4, 2 and 5 mM concentrations, respectively, before being titrated to a pH of 2. Before each experiment, gastric enzymes containing 0.04mg/ml of gastric lipase and 0.5 mg/ml of pepsin were prepared in artificial gastric juice. For

example, 24 and 300 mg of gastric lipase and pepsin, respectively were added to 600 ml of gastric juice.

4.3.4.3 Artificial Pancreatic Juice Preparation

Stock pancreatic juice was prepared by combining deionised water with specific quantities of sodium bicarbonate at 110 mM, dipotassium hydrogen phosphate at 2.5 mM, sodium chloride at 54.9 mM, calcium chloride at 1 mM and urea at 1.67 mM, before being titrated to a pH of 8. Before each experiment pancreatin at a concentration of 70 g/l was freshly added to stock pancreatic juice, mixed and then filtered through glass wool. For example, 42 g of pancreatin was added to 600 ml of pancreatic juice.

4.3.4.4 Fresh Porcine Bile

Porcine gall bladders were collected from the abattoir. Bile was extracted from the gallbladders, homogenised, and then frozen in aliquots of 25ml. Before each experiment, bile was defrosted and then incubated at 37°C in a water bath to mimic bile secretion into the small intestines. At the beginning of the intestinal phase of the mode gut, 25 ml of bile was added to each sample to begin the intestinal phase following 60 minutes in the gastric phase (Figure 4. 2).

4.3.5 Procedures

4.3.5.1 Water Bath Setup

A water bath was set to 37°C to incubate all synthetic solutions throughout the model gut process, with up to three samples being analysed at any one time (A, B, and C), as shown in Figure 4. 2. During the gastric and pancreatic phases of digestion, synthetic gastric and pancreatic solutions were continuously pumped into samples A, B and C at a rate of 0.5 ml/min using Watson Marlow Peristaltic pumps (Figure 4. 2). Following 60 minutes of the gastric phase, 25ml of bile was added to each of the three samples, A, B and C. Throughout each experiment, samples were agitated using overhead stirrers to stimulate peristaltic movement and mixing in the GIT.

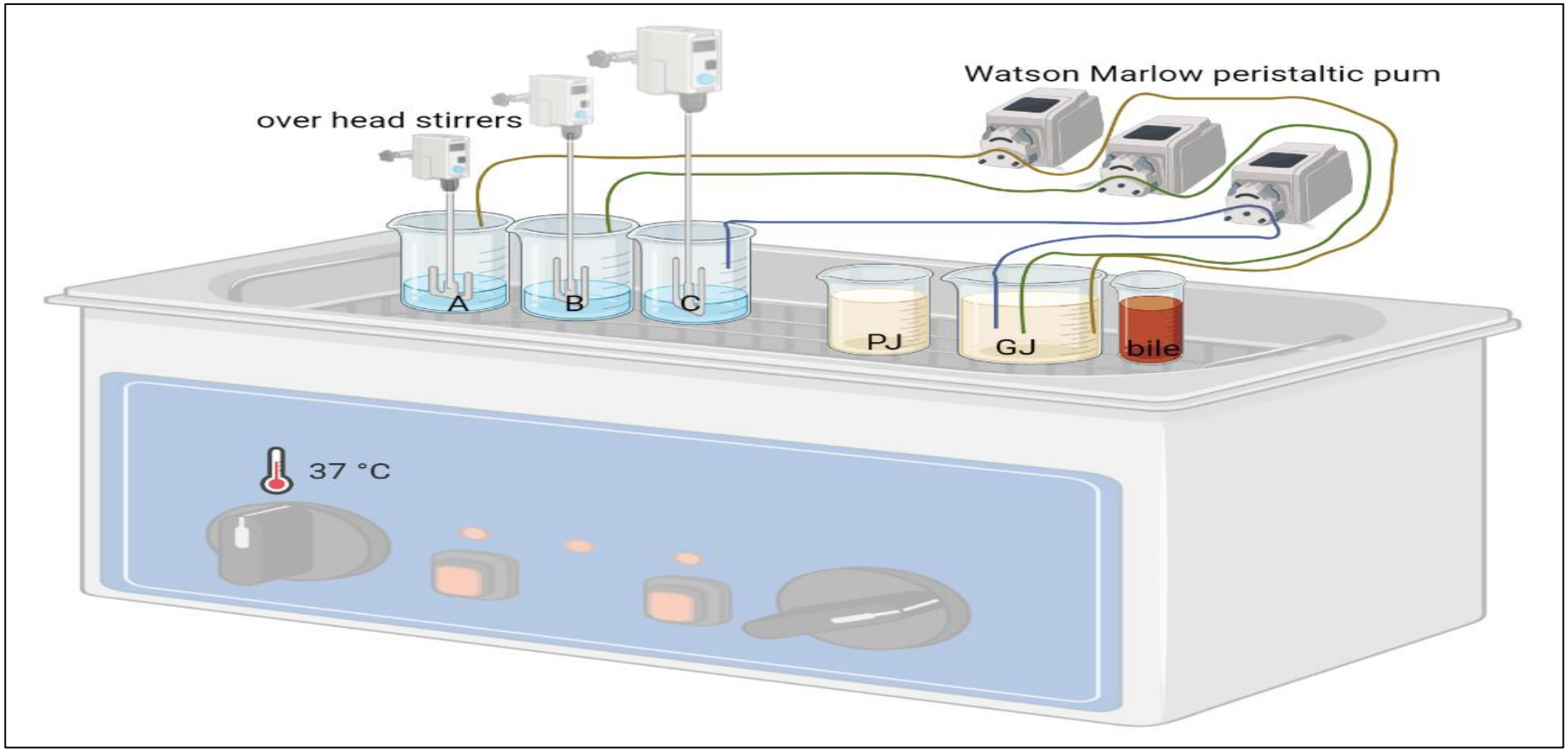


Figure 4. 2 The model gut arrangement.

Gastric juice (GJ) was pumped at 0.5 ml/min for 60 minutes (T60) to all samples depicted as A, B and C. At 61 minutes, the Watson-Marlow peristaltic pump was stopped from gastric juice. 25 ml of porcine bile was added to each sample (A, B and C) before pancreatic juice (PJ) was then pumped into all samples (A, B and C) at 0.5 ml/min for up to 180 minutes (T180). Throughout the model gut the overhead stirrers were used to stimulate peristaltic movement and mixing in the GIT. The laboratory equipment and model gut schematic diagram were created using Bio-render paint tools.

4.3.5.2 Seaweed and Control Samples

The Kuwaiti seaweed samples exhibiting the most significant inhibitory effects on pancreatic lipase in the olive oil assay, as detailed in 4.3.3, were subjected to testing in the artificial model gut. Olive oil was used as the fat substrate for fat digestion. Table 4. 2 provides detailed information on the four solutions employed in each of the experiments. To eliminate any potential interference in digestion, the absorbance values from the control solutions were subtracted from their corresponding fat substrate solution and seaweed with fat substrate solution. The seaweed control included 1 g of seaweed, 5 ml of deionised water and synthetic model gut saliva. The fat substrate control included 5 ml of WE and the synthetic model gut saliva (Table 4. 2).

Table 4. 2 Experimental solutions used in a synthetic model gut.

Experimental Solutions	Constituents
Seaweed only **(Seaweed control)**	1 g seaweed +5 ml deionised water + MG juices begin with saliva
Seaweed and olive Oil **(Seaweed)**	1 g seaweed + 5 ml oil+ 5 ml deionised water + MG juices begin with saliva
Olive oil only **(Substrate)**	5 ml oil + 5 ml deionised water + MG juices begin with saliva
MG juices only **(Substrate control)**	5 ml deionised water + MG juices begin with saliva

Four solutions were used for each experiment. g: grams; ml: millilitres; MG, model gut.

4.3.5.3 GI Phases

The saliva phase is the beginning of the model gut. In this phase, 5 ml of the synthetic saliva containing 5 µl of 3 mg/ml α-amylase and 5 ml deionised water were added to experimental solutions and incubated at 37°C while being continuously mixed for 10 minutes. Following the 10-minute incubation period, the gastric phase begins at 0 minutes (T0). At this point, 50 ml of gastric juice containing 0.5 mg/ml pepsin and 0.04 mg/ml gastric lipase was added to each experimental sample while mixing continued. The peristaltic pump then delivered gastric juice simultaneously to solutions A, B and C at a rate of 0.5 ml/min for 60 minutes (T60) (Figure 4. 2).

At 61 minutes (T61), 25 ml of porcine bile was added to each sample before synthetic pancreatic juice containing 70 g/L pancreatin was pumped into each sample simultaneously at a rate of 0.5 ml/min for up to 180 minutes (T180) (Figure 4. 2).

4.3.5.4 Samples Collection

A sample of 500 µl was extracted at time points 0, 15, 30, 60, 61, 75, 90, 120, 150, and 180 minutes from each sample. Each extracted sample was subsequently mixed with 10% trichloroacetic acid (TCA) in a 1:1 ratio in an Eppendorf tube to facilitate precipitation of undigested substrate and terminate enzyme action. Subsequently, all specimens were stored at 4°C overnight. Upon analysis, each sample was centrifuged at a rate of 10,000 revolutions per minute (rpm) (9,300xg) for 10 minutes. The supernatant from each sample was then analysed as described below.

4.3.5.5 Fat Digestion Analysis

Free glycerol served as a marker of in-vitro lipid digestion obtained throughout all gastrointestinal phases. Samples extracted from the model gut underwent processing using the free glycerol reagent, which facilitated changes in the absorbance measured at 550 nm using a plate reader. The intensity of the colour was proportional to the glycerol concentration in the sample. The free glycerol reagent was prepared by diluting the stock solution in 40 mL of deionised water. To quantify the concentration of glycerol in the extracted samples, a glycerol standard curve was established as a reference. A glycerol standard of 2 mM concentration was prepared in deionised water and subsequently serially diluted to 1, 0.5, 0.25, 0.125. 0.062, and 0.031 mM. Then, 5

µl of each sample described in 4.3.5.4, along with the standard curve samples, were dispensed into a 96-well microplate. Subsequently, 80 µl of the glycerol reagent was added to each sample and the standard serial dilutions in a 96-well microplate. The microplate was then incubated at room temperature for 20 minutes to facilitate colour development, after which it was subjected to reading at 550 nm.

The quantification of glycerol released was accomplished by multiplying the volume of the model gut system at each time point by the corresponding total amount of glycerol released. The mean value of the three replicates was used to calculate the glycerol released. The difference in the amount of glycerol released in the fat substrate alone vs. the fat substrate with Kuwait seaweed was used to evaluate the impact of Kuwaiti seaweed on in-vitro lipid digestion.

4.3.6 Statistical Analysis

All data obtained from the 96-well plate reader were extrapolated into Microsoft Excel. Regression analysis was conducted using the standard curves to generate the horizontal (x) and vertical (y) values to measure glycerol released in mg (milligram) to quantity the total fat digestion at each time point. GraphPad Prism 9 software was then utilised to analyse all data, wherein differences in lipid digestion alone were compared with lipid digestion in the presence of Kuwaiti seaweed. A two-way ANOVA with Bonferroni's analysis was performed to investigate a time x treatment effect. Additionally, the same analysis was carried out comparing lipid digestion alone vs. lipid digestion with *S. trinodis* collected in November and April. Each experiment was performed in three independent experiments, each with three biological replicates. The data are presented as mean ± standard deviation (SD). Statistically significant was set as a P value of ≤ 0.05.

4.4 Results

This chapter explored the potential inhibitory effects of Kuwaiti seaweed and its extracts on in-vitro fat digestion. Free glycerol served as a marker of fat digestion, with the glycerol concentration calculated using a standard curve of glycerol in deionised water (Figure 4. 3).

4.4.1 Glycerol Standard Curve

Glycerol was serially diluted in deionised water to serve as the positive control. The free glycerol standard curve was created using the free glycerol reagent. Figure 4. 3 illustrates a positive correlation between absorbance and glycerol concentration within the ranges of 0 to 0.5 mg/ml and 0.06 to 0.8 optical density (OD), respectively (R^2=0.99).

The concentration of glycerol in each sample extracted from the model gut during fat digestion alone and in conjunction with the fat substrate and Kuwaiti seaweed was subsequently quantified. Each experiment was conducted using its own calibration curve.

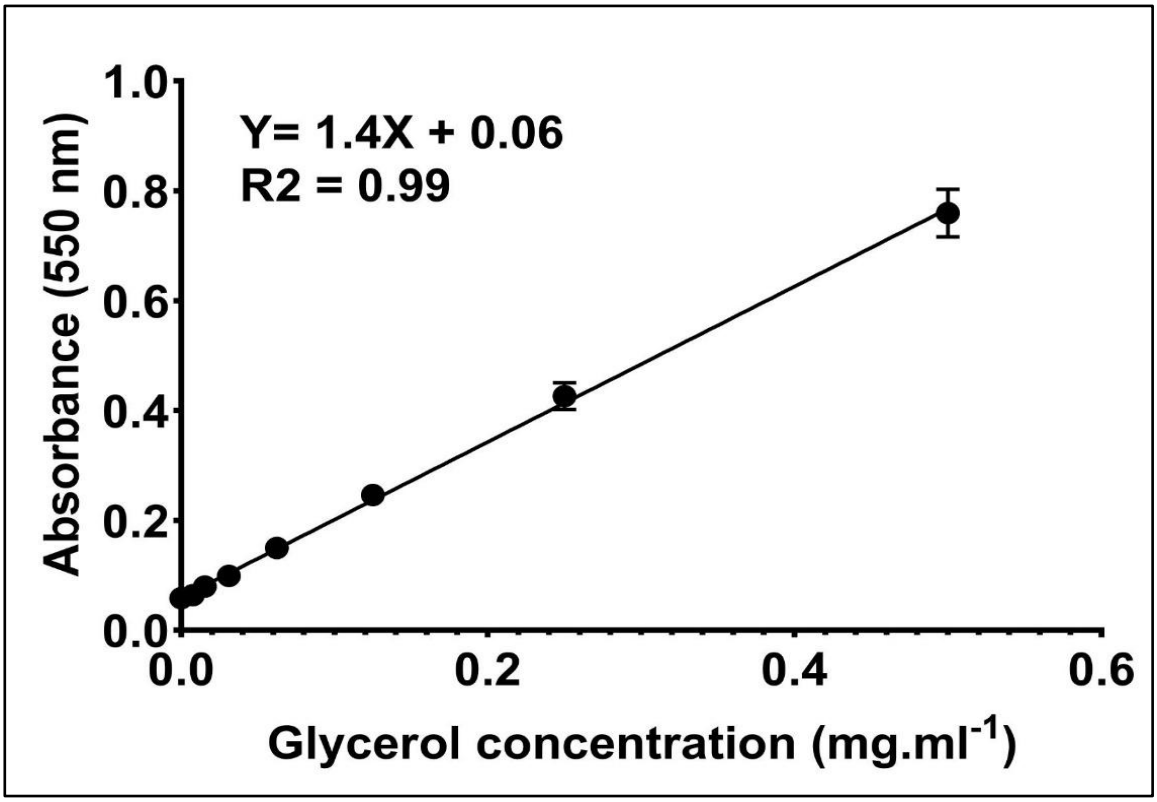


Figure 4. 3 Standard curve for free glycerol.

Glycerol was serially diluted in deionised water at concentrations ranging from 0.5 to 0 $mg.ml^{-1}$. 5 µl of each concentration of the standard curve was loaded onto a 96-well microplate in triplicate. 80 µl of free glycerol reagent was added to each sample and incubated at room temperature for 20 minutes before being measured at a wavelength of 550 nanometres (nm) on a plate reader. The mean of the triplicates was then used to plot the standard curve. Regression analysis provided the horizontal (x) and vertical (y) values to generate free glycerol in mg (milligram). Data presented as the mean ± standard deviation (n=3).

4.4.2 Effect of Seaweed Homogenate on Fat Digestion

The impact of 15 Kuwaiti seaweed samples, described in detail in 4.3.3, was examined. This chapter assessed homogenates from green, brown, and red species of Kuwait seaweeds for their potential to mitigate fat digestion.

4.4.2.1 Fat Digestion in Combination with Green or Red Seaweed Homogenates

The digestion profiles of the fat substrate (olive oil) alone and in conjunction with the green or red seaweed homogenates, *C. sericioides* and *G. pusillum* are depicted in Figure 4. 4A and B.

Figure 4. 4A and B depict that in the gastric phase (0 to 60 minutes), there was <1mg of glycerol released, gradually increasing beyond 75 minutes in all samples. In the small intestinal phase (61 to 180 minutes), a higher concentration of free glycerol was observed in the small intestinal phase of the model gut (Figure 4. 4A and B). The free glycerol concentration was significantly higher in the presence of *C. sericioides* at 150 minutes compared with olive oil substrate alone (26 ± 6 vs. 14 ± 6 mg, $p = 0.003$, respectively) (Figure 4. 4A).

A significantly larger free glycerol concentration was observed when the *G. pusillum* homogenate was combined with the olive oil substrate vs the olive oil substrate alone at 75 (19 ± 4 vs. 3 ± 2, $p = 0.05$, respectively), 90 (33 ± 6 vs. 19 ± 4, $p = 0.005$, respectively), 120 (51 ±11 vs. 26 ± 7, $p = 0.0003$, respectively) and 180 minutes (80 ±11 vs. 62 ± 10, $p = 0.02$, respectively) (Figure 4. 4B). These findings suggest that the addition of green or red seaweed homogenates enhances fat digestion.

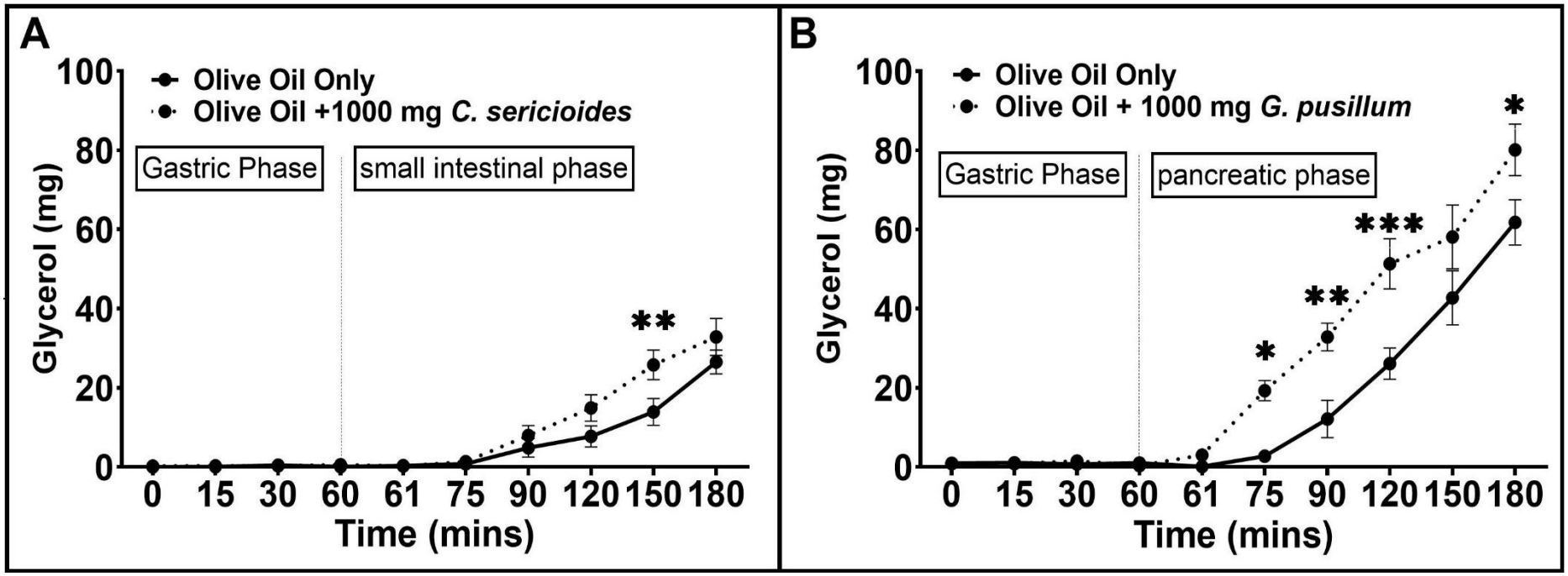


Figure 4. 4 Olive oil digestion alone vs. olive oil combined with green or red seaweed homogenates in the model gut.
Free glycerol amounts from olive oil alone (solid black line) and olive oil in conjunction with 1000 mg of the red and green seaweed homogenates *Cladophora sericioides* (Figure 4. 4A) and *Gelidium pusillum* (Figure 4. 4B), both denoted by dotted lines, respectively. To evaluate any time x treatment interactions in olive oil digestion alone vs. olive oil with seaweed homogenates, a two-way ANOVA with Bonferroni's analysis was performed. Data are presented as mean ± standard deviation (n=3). *, ** and *** denote a significant difference at $p < 0.05$, < 0.005 and < 0.0003.

4.4.2.2 Fat Digestion in Combination with Brown Seaweed Homogenates

The digestion profiles of the fat substrate (olive oil) alone and in conjunction with brown seaweed homogenates *S. trinodis* N, *S. trinodis* A, *C. sinuosa*, *I. stellata* and *P. boergesenii* are presented in Figure 4. 5A, B, C, D and E, respectively.

Figure 4. 5A and B display data from *S. trinodis* homogenate collected in November and April, respectively. In the gastric phase (0 to 60 minutes), <0.5 and 0.7 mg of glycerol were released, gradually increasing beyond 75 minutes in all samples. There were no significant differences in free glycerol between olive oil alone compared to olive oil in conjunction with *S. trinodis* N homogenate from November at any time points (Figure 4. 5A). However, the free glycerol was significantly lower at 150 minutes (7 ± 2 vs. 19 ± 5, p = 0.005) and 180 minutes (15 ± 4 vs. 28 ± 6, p = 0.003) for olive oil substrate alone compared to olive oil in conjunction with *S. trinodis* A homogenate collected in April, respectively (Figure 4. 5B).

Figure 4. 5C, D and E illustrate free glycerol from the digestion of olive oil alone compared to olive oil in conjunction with *C. sinuosa, I. stellata and P. boergesenii* homogenates, respectively. There were no significant differences in free glycerol in the gastric phase (0 to 60 minutes) when comparing olive oil alone with olive oil and *C. sinuosa, I. stellata and P. boergesenii* homogenates (Figure 4. 5C, D and E, respectively).

The free glycerol concentration was significantly higher in the olive oil and *C. sinuosa* compared to olive oil alone at 180 minutes (59 ± 16 vs. 40 ± 11, p = 0.02, respectively). Additionally, the free glycerol concentration was significantly higher in the olive oil and *I. stellata* compared with olive oil alone at 180 minutes 19 ± 5 vs. 7 ± 2, p = 0.002, respectively) (Figure 4. 5D). Finally, the free glycerol concentration was significantly higher in the olive oil and *P. boergesenii* compared to olive oil alone at 120 (48 ± 8 vs. 19 ± 6, p = 0.0002, respectively) and 150 minutes (48 ± 7 vs. 31 ± 9, p = 0.04, respectively) (Figure 4. 5E). These findings suggest that the addition of some brown seaweed homogenates enhances fat digestion.

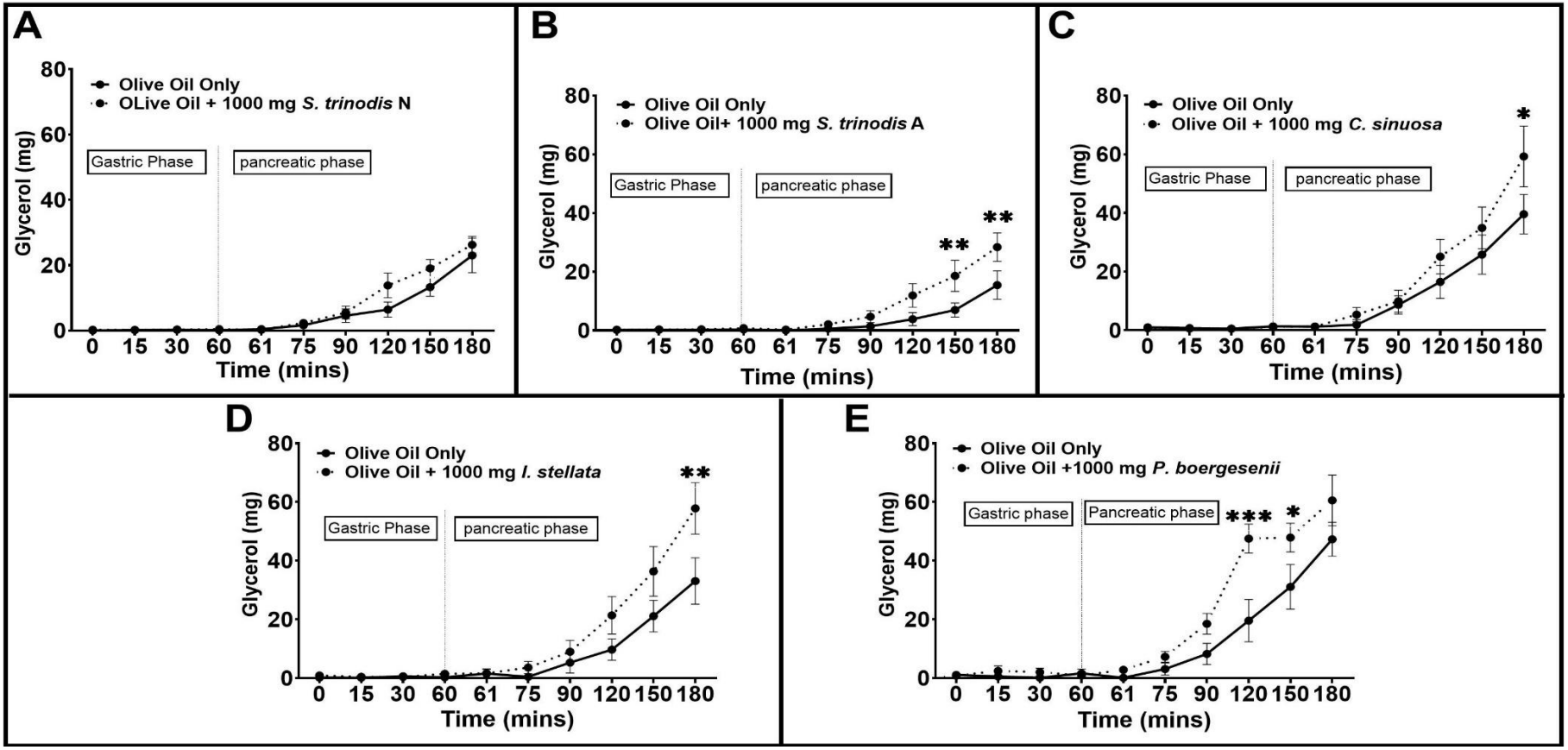


Figure 4. 5 Olive oil digestion alone vs. olive oil combined with brown seaweed homogenates in the model gut.
Free glycerol amounts from olive oil alone (solid black line) and olive oil in conjunction with 1000 mg of brown seaweed homogenates *Sirophysalis trinodis* collected in November 2020 (N) (Figure 4. 5A), *Sirophysalis trinodis* (A) collected in April 2021 (Figure 4. 5B), *Colpomenia sinuosa* (Figure 4. 5C) *lyngaria stellata* (Figure 4. 5D) and *Padina boergesenii* (Figure 4. 5E) all denoted by dotted lines. To evaluate any time x treatment interactions in olive oil digestion alone vs. olive oil with seaweed homogenates, a two-way ANOVA with Bonferroni's analysis was performed. Data are presented as mean ± standard deviation (n=3). *, ** and *** denote a significant difference at $p < 0.05$, < 0.005 and < 0.0003, respectively.

4.4.3 Effect of Seaweed WE on Fat Digestion

In this section, the effects of olive oil alone compared to olive oil in combination with seaweed WE extracts, specifically *C. papillatum*, *S. trinodis* N, *S. trinodis* A, and *P. boergesenii*, were evaluated, as depicted in Figure 4. 6A, B, C and D, respectively. Across all WE seaweed extracts combined with olive oil and olive oil-only controls, the level of free glycerol was not significantly different in the gastric phase, with free glycerol levels not exceeding 2mg in the gastric phase, before gradually increasing beyond 75 minutes in all samples (Figure 4. 6).

Beyond 135 minutes, the WE from *C. papillatum* showed no significant differences in free glycerol, with the greatest difference observed at 180 minutes when compared with the olive oil alone (Figure 4. 6A).

Free glycerol concentrations in WE from *S. trinodis* N and A (collected in November and April) combined with olive oil and olive oil alone was less than 2mg in the gastric phase, before gradually increasing beyond 75 minutes in all samples (Figure 4. 6B and C). The WE from *S. trinodis* N elicited no significant differences in free glycerol concentration when compared to olive oil alone between 61 to 180 minutes (Figure 4. 6B). In addition, the WE from *S. trinodis* A elicited no significant differences (Figure 4. 6C).

The WE from *P. boergesenii* elicited a no significant differences in free glycerol when compared to olive oil alone (Figure 4. 6D).

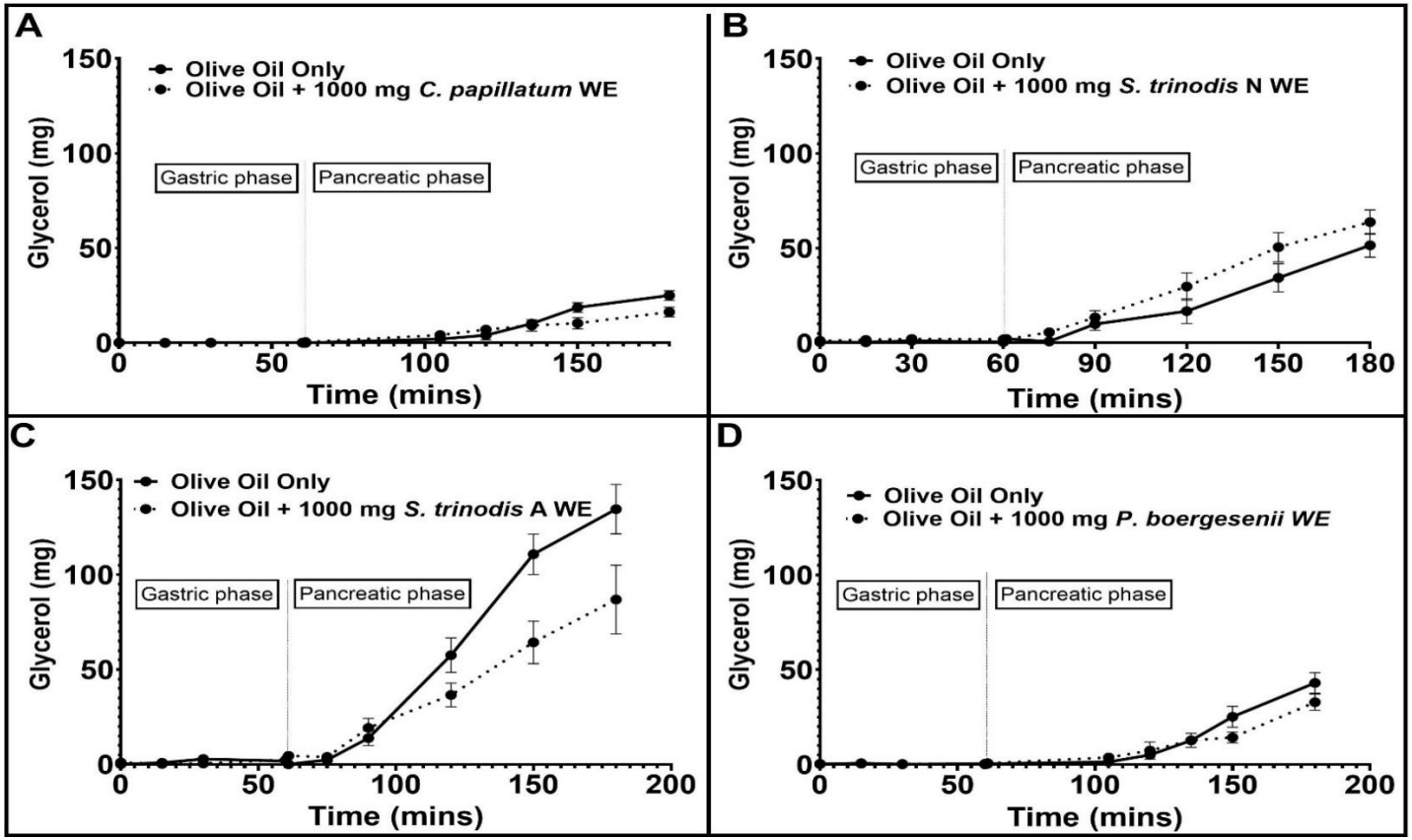


Figure 4. 6 Olive oil digestion alone vs. olive oil combined with seaweed WE in the model gut. Free glycerol amounts from olive oil alone (solid black line) and olive oil in conjunction with 1000 mg of deionised water extract (WE) seaweed extracts from the green seaweed *Codium papillatum* (Figure 4. 6A), brown seaweed *Sirophysalis trinodis* collected in November (N) (Figure 4. 6B) and April (A) (Figure 4. 6C) and *Padina boergesenii* (Figure 4. 6D). To evaluate any time x treatment interactions in olive oil digestion alone vs. olive oil with seaweed WE, a two-way ANOVA with Bonferroni's analysis was performed. Data are presented as mean ± standard deviation (n=3).

4.4.4 Effect of Seaweed EP on Fat Digestion

In this section, the effects of olive oil alone compared to olive oil in combination with EP obtained from four seaweeds, specifically *C. sericioides*, *C. papillatum*, *P. boergesenii*, and *G. pusillum* were evaluated (Figure 4. 7A, B, C and D, respectively).

Across all EP combined with olive oil and olive oil only controls, the level of free glycerol was not significantly different in the gastric phase, with free glycerol levels not exceeding 2mg in the gastric phase (0 to 60 minutes), before gradually increasing beyond 75 minutes in all samples (Figure 4. 7).

Figure 4. 7A displays free glycerol amounts when the EP from green seaweed *C. sericioides* was added to olive oil. A significant higher amounts of free glycerol were observed in the presence of the EP from *C. sericioides* compared to olive oil alone throughout the small intestinal phase at 120 (39 ± 6 vs. 20 ± 5, p=0.01, respectively) and 180 minutes (61 ± 7 vs. 83 ± 9, p = 0.003, respectively) (Figure 4. 7A),

When comparing free glycerol release in the model gut for olive oil alone with olive oil and an EP from green seaweed *C. papillatum*, there were no significant differences in free glycerol at any time point throughout the model gut (61 to 180 minutes) (Figure 4. 7B).

Figure 4. 7C and D illustrate the fat digestion profiles of the olive oil substrate alone compared with olive oil digestion with a EP from *P. boergesenii* or *G. pusillum* (Figure 4. 7C and D, respectively). There were no significant differences in free glycerol at any time point (Figure 4. 7C and D).

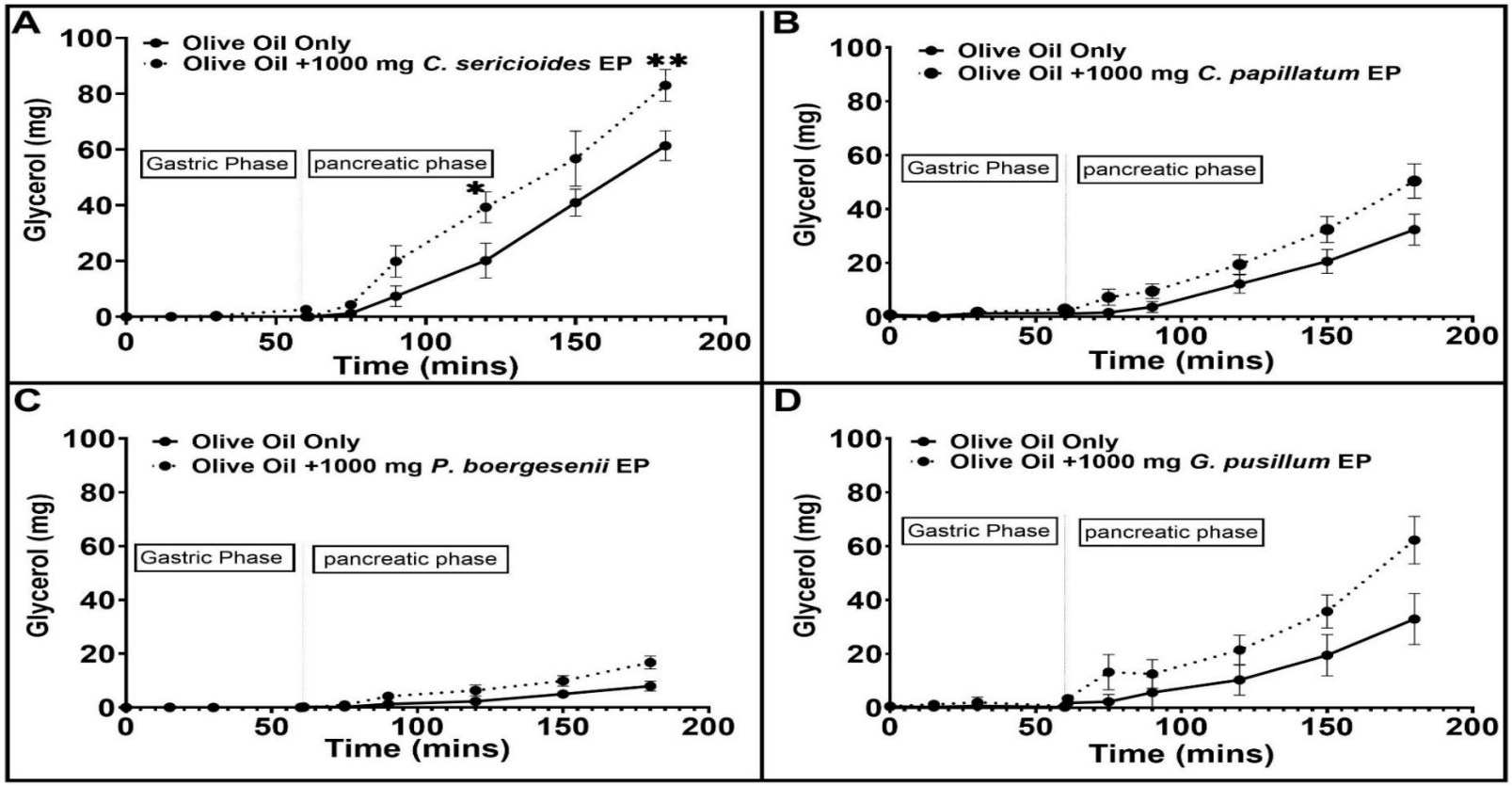


Figure 4. 7 Olive oil digestion alone vs. olive oil combined with seaweed ethanol pellet (EP) in the model gut.

Free glycerol amounts from olive oil alone (solid black line) and olive oil in conjunction with 1000 mg of the EP from green seaweed *Cladophora sericioides* (Figure 4. 7A), and the EP from green seaweed *Codium papillatum* (Figure 4. 7B), brown seaweed *Padina boergesenii* (Figure 4. 7C) and red seaweed *Gelidium pusillum* (Figure 4. 7D). To evaluate any time x treatment interactions in olive oil digestion alone vs. olive oil with seaweed EP, a two-way ANOVA with Bonferroni's analysis was performed. Data are presented as mean ± standard deviation (n=3). * and ** denote a significant difference at $p<0.05$ and $p<0.005$, respectively.

4.4.5 Effect of Temporal Variation on In-vitro Fat Digestion in Brown Seaweed

The temporal effects of seaweed homogenates and WE extracts from the brown seaweed *S. trinodis* were examined by comparing the amount of free glycerol released during fat digestion with seaweed collected in November 2020 and April 2021 (Figure 4. 8). No significant temporal effects on fat digestion were observed in the gastric (0 to 60 minutes) or small intestinal phases (61 to 180 minutes) between the seaweed homogenates (Figure 4.8A) and WE collected in November and April (Figure 4. 8B).

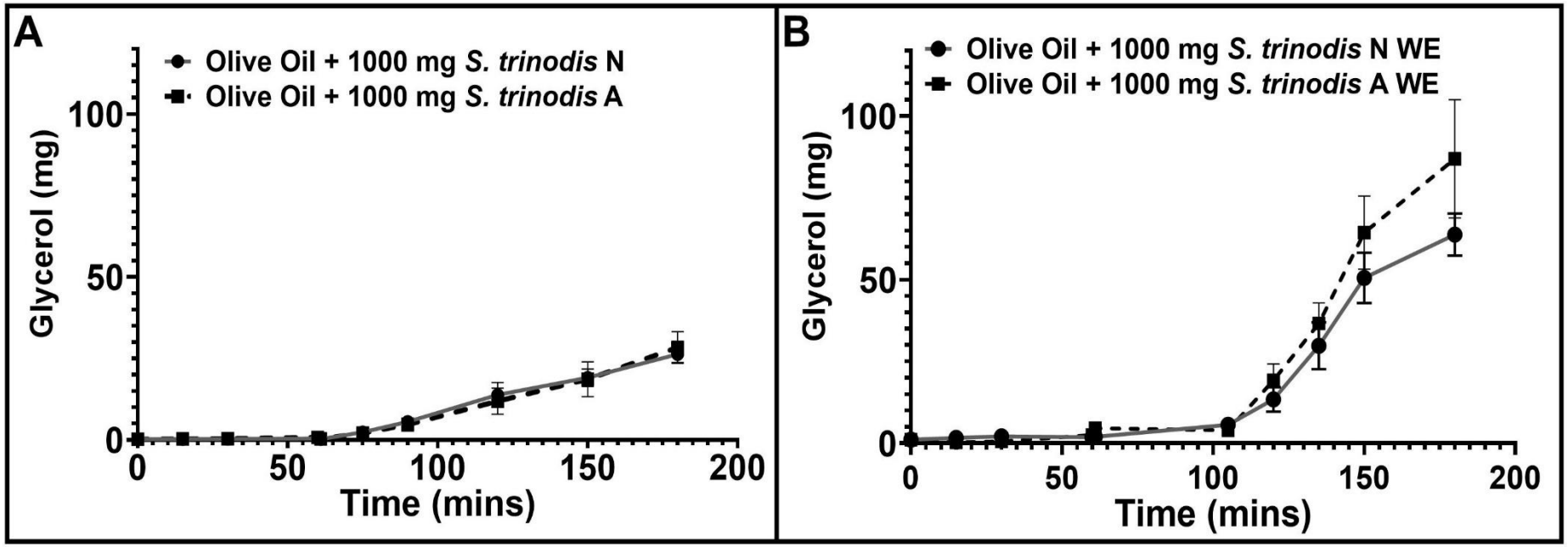


Figure 4. 8 A temporal variation on fat digestion in seaweed homogenate and deionised water extract (WE) from the brown seaweed *Sirophysalis trinodis*.

Free glycerol amounts from olive oil in conjunction with 1000 mg brown seaweed *Sirophysalis trinodis* collected from Kuwait coastline in November (N) (solid grey line) were compared with the amounts of free glycerol from olive oil in conjunction with 1000 mg brown seaweed *Sirophysalis trinodis* collected from Kuwait coastline in April (A) (dotted black line). Figure 4. 8A shows the difference in homogenates. Figure 4. 8B shows the differences in WE. To evaluate any time x treatment interactions in olive oil digestion with *S. trinodis* N vs. olive oil with *S. trinodis* A, a two-way ANOVA with Bonferroni's analysis was performed. Data are presented as mean ± standard deviation (n=3).

4.5 Discussion

The escalating rates of obesity, accompanied by associated comorbidities, and substantial socioeconomic burdens, underscore the pressing need to combat the obesity epidemic. While changing lifestyle is still the cornerstone of managing obesity, emerging strategies are being explored, including novel pharmacotherapies and naturally occurring compounds. Seaweeds represent a promising resource that could contribute to obesity treatment, given their diverse health-promoting properties, including the ability to inhibit pancreatic lipase and reduce fat digestion (Wan-Loy and Siew-Moi, 2016). Research on seaweeds is limited by the challenges of conducting clinical trials, even with promising outcomes. Employing synesthetic models that replicate digestion holds the potential to identify promising compounds that could subsequently undergo clinical evaluation while providing insight into the mechanisms underlying seaweed efficacy.

In-vitro digestion models provide a simplified and controlled system to reliably replicate the intricate dynamics of fat digestion. The primary advantage of using a model gut system lies in its reproducibility and ability to mitigate common challenges often encountered in human studies, such as high cost, time-intensive procedures, and ethical considerations (Mulet-Cabero *et al.*, 2020). Therefore, the objective of this research was to examine the effect of the Kuwaiti seaweed samples and their extracts, identified from Chapter 2, on fat digestion within a model gut that replicates digestion in the upper GIT. The primary finding of this chapter was that seaweed homogenates and their extracts did not inhibit fat digestion despite the earlier findings outlined in Chapter 2.

4.5.1 Fat Digestion in the Gastric Phase

Free glycerol, released from the fat substrate (olive oil), served as a marker of fat digestion in this study. This chapter demonstrates that the model gut effectively replicates fat digestion in the gastric phase, with a low concentration of free glycerol observed between 0-60 minutes. These findings align with previous research indicating that fat digestion in the gastric phase typically yields low levels of free glycerol, a phenomenon often considered of lesser significance in total fat digestion (Chater *et al.*, 2016; Zhao *et al.*, 2024). However, it is worth noting that the primary role of fat digestion

in the stomach may not be the liberation of free glycerol. Gastric digestion is believed to serve as a precursor to mechanical emulsification, referred to as pre-digestion (Xu *et al.*, 2021). Pancreatic Lipases activate in the presence of insoluble and emulsified substrates (Corstens *et al.*, 2017). In this phase, fat globules within the chyme are broken down into smaller droplets, facilitating lipid emulsification, a critical step in creating a lipid-water interface. The lipid-water interface is essential for optimal pancreatic lipase and colipase function in the small intestines (Xu *et al.*, 2021). Furthermore, the acidic environment present in the stomach is able to maintain the fatty acids in their non-ionised form, making them more likely to remain adequately emulsified and facilitate digestion in the small intestine. In addition, gastric lipase is responsible for the digestion of short to medium chain triacylglycerides. However, lipolysis of emulsified lipids occurs mostly in the small intestines due to pancreatic lipase activity (Xu *et al.*, 2021). The data presented herein indicate that fat digestion in the gastric phase of the model gut closely mirrors physiological fat digestion, confirming the model gut efficacy for studying lipid digestion.

4.5.2 Fat Digestion in the Small Intestinal Phase

The small intestine is where the majority of fat digestion occurs, characterised by an increase in the concentration of free glycerol, as observed in the current study. There was a gradual rise in the concentration of free glycerol during the digestion of olive oil across all experiments. These findings are consistent with those observed by Chater *et al.* (2016), who also observed a progression increase in fat digestion throughout the small intestinal phase. These results align with the anticipated physiological process of lipid digestion and absorption in the GIT (Omer and Chiodi, 2024; Xu *et al.*, 2021). Similar to the gastric phase, the data presented here confirm that the model gut serves as a reliable and reproducible system for replicating lipid digestion in humans.

4.5.3 The Effect of Kuwaiti Seaweeds and their Extracts on Fat Digestion

In the current study, there were no inhibitory effects of Kuwaiti seaweed homogenates, WE, and EP on fat digestion in the model gut. Surprisingly, these findings contrast sharply with the inhibitory effects observed on pancreatic lipase activity in the olive oil assay detailed in Chapter 2. One potential explanation may be due to the experimental

setup, particularly regarding the model gut. The olive oil assay used to investigate pancreatic lipase activity in Chapter 2 is a controlled assay using a substrate (olive oil) free from fatty acids, designed to eliminate confounding factors and optimise conditions to ensure optimal pancreatic lipase activity. In contrast, the model gut reflects mechanical and biochemical conditions similar to in-vivo digestion, containing various enzymes, fluctuating pH levels, bile salts and continuous stirring. It is possible that, individually or collectively, these factors may have impaired the efficacy of Kuwait seaweeds and their extracts in inhibiting pancreatic lipase activity. The current study revealed an increase in free glycerol release following the addition of Kuwait seaweeds and extracts to fat digestion in the in-vitro model gut. This increase in free glycerol, indicative of enhanced lipid digestion, may be attributed to preventing the saturation of reaction products (fatty acids) and altering substrate/product equilibrium (Bisswanger, 2014). This raises the possibility that the seaweed compounds may bind with free fatty acids within the substrate, removing them from the reaction, and hence increasing the pancreatic lipase reaction rate. Therefore, it is important to conduct an in-vitro model gut study to examine the effect of Kuwaiti seaweeds and their extracts on fat digestion using a substrate (olive oil) free from fatty acids.

The findings in this study are contrary when compared to those of Chater *et al.* (2016), who reported a reduction in fat digestion upon the addition of Hebridean seaweed homogenate, EE and EP to fat digestion in an in-vitro model gut system. Identifying specific reasons for the disparate results within this study and with the study of Chater *et al.* (2016) is challenging. A potential explanation for the lack of pancreatic lipase inhibition in Kuwait seaweeds and their extracts compared to Hebridean seaweed samples from Chater *et al.* (2016) might be explained by the presence of seaweed's species-specific bioactive compounds. Compounds like polyphenols and polysaccharides or other factors, likely to be present in Kuwait seaweeds and their extracts, may interact with each other or with other molecules present in the model gut, resulting in either positive or negative impact on lipid digestion (Kardum and Glibetic, 2018; Salgado *et al.*, 2007).

Seaweed homogenates encompass a broad spectrum of phytochemical compounds, whereas the WE and ethanol pellet extracts would solely contain water-soluble compounds (such as soluble polysaccharides and polyphenols) and those insoluble in ethanol (insoluble polysaccharides, alginate and fucoidan), respectively (Holdt and

Kraan, 2011). The composition and concentration of these bioactive compounds in seaweed can vary depending on factors such as species, geographical location, and timing of harvest (Cotas *et al.*, 2020; Venkatesan *et al.*, 2015).

An alternative explanation for the enhanced fat digestion observed here is by formation of polymers that are challenging to isolate or reduce the stability of active compounds (Obluchinskaya, Pozharitskaya and Shikov, 2022; Salgado *et al.*, 2007). Interestingly, previous research has noted the presence of polymers of phenolic compounds with polysaccharides (alginate) extracted from the Brazilian brown seaweed *p. gymnospora* (Salgado *et al.*, 2007). Furthermore, a recent study by Obluchinskaya, Pozharitskaya and Shikov (2022), reported that the bioactivity of fucoidan, a sulphated polysaccharide extracted from four Russian brown seaweeds, was significantly linked to polyphenols. Previous studies have demonstrated that low concentrations of polyphenol-rich extract from Hebridean brown seaweed (*A. nodosum*), Norfolk blackcurrants and Finland rowan berries activated pancreatic lipase activity (Austin *et al.*, 2018; McDougall, Kulkarni and Stewart, 2009). Another possible explanation may be related to the pH variability within the model gut. The pH within the model gut can range from approximately 2 in the gastric phase to over 7 in the small intestinal phase, exposing seaweed and its extracts to a range of pH levels. Previous literature has demonstrated that alginate possesses the ability to form gels under acidic pH conditions, typically below 3.5 (Draget, Skjåk-Bræk and Stokke, 2006). Hoad *et al.* (2004) demonstrated using magnetic resonance imaging (MRI) that alginates with a high guluronic acid content formed gels in the stomach, the formation of which also depended upon the molecular weight of the compounds. The data in this study suggest that Kuwaiti seaweed is more likely to produce polymers in the model gut that could enhance impact fat digestion.

Different dietary fibres have been shown to interact with bile acids and enhance their transit to the colon (Naumann *et al.*, 2019). Another study showed that different dietary fibre characteristics interacted differently with emulsion droplets (Beysseriat, Decker and McClements, 2006). This study raises the question of whether the characteristics of seaweed fibre play a role in this finding.

This finding is also contrary to previous studies, which have observed that bioactive compounds, including tea polyphenols, affect lipid digestion by various mechanisms

other than pancreatic lipase inhibition including modification of lipid emulsification and micelle formation (Ganesan, Tiwari and Rajauria, 2019; Shishikura, Khokhar and Murray, 2006). However, seaweed polyphenols may exhibit different properties, quantity, and quality from those extracted from tea or other species. In addition to differences properties from species to species, even the extraction of purified bioactive compounds instead of using whole dried fruit has been shown to have different effects on obesity (Prior *et al.*, 2008). Prior *et al.* (2008) showed that purified blueberry anthocyanin, a secondary active compound responsible for berries' colours, showed a reduction of weight gain in mice with a high-fat diet compared to mice fed whole-dried blueberry with the same diet. These findings cast some doubt on the properties of the bioactive compounds found in seaweed that may either have a positive or negative impact on fat digestion. Therefore, further research is needed to identify the bioactive compounds.

This inconsistency of increase in free glycerol may be due to interference by seaweed bioactive compounds in the glycerol assay. Therefore, further work is required to establish the viability by removing the interference. The absorbance from the negative sample (seaweed in WE samples in the glycerol assay) was then subtracted from its corresponding sample from the model gut.

Previous research has shown that polyphenols, when associated with various dietary fibres, form complexes, characterised by poor digestibility, allowing them to pass into the lower GIT (Saura-Calixto and Díaz-Rubio, 2007). If this is the case in the Kuwait seaweeds and their extracts, this may award benefits to lower GIT health. For example, the fermentation of dietary fibres in the lower GIT may reduce the risk of colon pathophysiology and enhance overall colon health (Ding *et al.*, 2020). Gel formation or precipitation with the use of Kuwait seaweeds in this study may explain the lack of fat digestion inhibition. Although not demonstrated in the present study, further research is warranted to investigate the gel-forming properties of Kuwait seaweeds and their structural characteristics. Furthermore, studying the Kuwait seaweeds under various pH conditions is essential to determine if alterations in solution pH contribute to the observed lack of fat digestion inhibition observed here. These investigations may identify novel therapeutic properties of Kuwait seaweeds, such as those evident with Gaviscon and alginates. Furthermore, understanding the potential of Kuwait seaweeds to inhibit pancreatic lipase in an in-vitro model gut, and whether the seaweeds require

specific treatments to protect them from the acidic conditions of the stomach to ensure optimal activity in the small intestines and inhibition of pancreatic lipase, as observed in Chapter 2.

4.5.4 The Temporal Effect of *S. trinodis* on Fat Digestion

The physical and phytochemical properties of seaweeds can be influenced by various factors, particularly seasonal variations such as sunlight exposure, temperature, and salinity levels in their growth conditions (Ptak *et al.*, 2021; Yesilova, Balkis and Taskin, 2017). In an earlier study by Devlin *et al.* (2019), the authors noted that the Kuwait coastline experiences significant seasonal fluctuations, with increased temperatures and reduced salinity levels in April compared to November (Devlin *et al.*, 2019). Seaweeds must adapt to these challenging climates and seasonal changes, leading to structural modifications that can affect their functionality. Seaweed lives in a changing environment, which includes strong waves and rising and falling tides, so seaweed develops a thick cell wall to resist these fluctuations. In addition, some seaweeds exist in an environment where temperatures and UV rays change, so seaweeds need to increase the rate of production of protective pigments and antioxidants to adapt to living in this environment (Konstantin *et al.*, 2023).

In the current study, we compared *S. trinodis* homogenates and WE collected in November and April, previously performed in Chapter 2, where WE from *S. trinodis* collected in November and April were observed to show a significant difference in their level of pancreatic lipase inhibition. In contrast, no significant difference in pancreatic lipase inhibition was observed with their homogenates. However, the November and April *S. trinodis* homogenates and WE both significantly inhibited pancreatic lipase. In this study, a modest non-significant reduction was observed in fat digestion with the WE from *S. trinodis* collected in April. However, there were no significant reductions in fat digestion in April or November for either of the seaweed homogenates and their WE in samples collected from the model gut. The lack of change in fat digestion observed here when comparing April and November may be attributed to the previously mentioned possibility of binding of seaweed bioactive compounds with free fatty acids.

4.6 Conclusion

The objective of this chapter was to investigate the effects of seven Kuwaiti seaweeds and their extracts on fat digestion in an in-vitro model gut, potentially by modulating the activity of pancreatic lipase, as previously demonstrated in Chapter 2. No significant reductions were observed in fat digestion in any of the seaweeds or their extracts. This lack of significant reduction may, in part, be attributed to the complexity of the model gut compared to the olive oil assay used in Chapter 2. Furthermore, it was noted no significant temporal on fat digestion when comparing homogenate and WE from the brown seaweed *S. trinodis* N and *S. trinodis* A (collected in November and April, respectively). It is possible, therefore, that the seaweed compounds may bind with free fatty acids within the substrate, removing them from the reaction, and hence increasing the pancreatic lipase reaction rate via increasing fat digestion. A further in-vitro model gut study of Kuwaiti seaweeds and their extracts on fat digestion using a substrate (olive oil) free from fatty acids is therefore suggested.

In summary, the findings presented here highlight the challenges of transitioning from a controlled experiment, such as the olive oil turbidity assay, to a complex model such as the model gut, and how this transition may impact the efficacy of seaweeds in inhibiting fat digestion. Moving forward, it is important to conduct extensive separation and characterisation of the Kuwait seaweed structure, potentially through the use of high-performance liquid chromatography (HPLC) and Mass Spectrometry (MS). Furthermore, gaining a deeper understanding of how these various characteristics influence the bioactive properties of the seaweeds is essential. Conducting modified model gut experiments with the addition of the seaweed samples after the gastric phase may also provide mechanistic insight into the effects of the gastric phase. Additionally, this approach would aid in the development of effective strategies for delivering these compounds in the future or may be employed in other industrial applications.

Chapter 5 Differences in Total Polyphenol Content of Kuwait Seaweed Based on Seaweed Species and Seasonal Variation.

5.1 Introduction

Polyphenols are a class of bioactive phytochemicals that play crucial roles in plant's organoleptic characteristics, including defending plants from biotic and abiotic stresses, which relate to living or previously alive organisms and non-living physical and chemical elements in the ecosystem, respectively (Rana *et al.*, 2022). Polyphenols are among the most abundant secondary compounds in nature and are widely distributed in plant-based diets, including fruits (Wu *et al.*, 2022b), cereals (Han, Yilmaz and Gulcin, 2018), vegetables (Radünz *et al.*, 2024) and seaweed (Gheda *et al.*, 2021). It is important to acknowledge that the density and quality of polyphenol compounds can be affected by many factors, including plant families and species, geographical location, and the growing and harvesting season (Cvitanovic *et al.*, 2018, p. 8). Typically, polyphenols are natural organic compounds containing one or more hydroxyl groups (-OH) that can be bound to one aromatic ring, described as simple phenols and phenolic acids, or phenol units, referred to as polyphenols (Rana *et al.*, 2022).

Although the exact mechanism of action of how polyphenols elicit these health benefits remains unknown, the specific structure of polyphenols is likely to be important. For example, polyphenols are said to exert strong antioxidant effects through their ability to neutralise free radicals by donating electrons or hydrogen molecules (Silva *et al.*, 2018; Han, Yilmaz and Gulcin, 2018). Furthermore, polyphenols have been shown to act as radical scavengers in lipid peroxidation chain reactions and chelate metals such as iron, reducing Fenton reactions and oxidation (Emad *et al.*, 2022; Wu *et al.*, 2022b). While the precise mechanism of action of polyphenols remains a topic of ongoing research, there is an extensive body of research linking polyphenols to health and quality of life, potentially due to their beneficial biological activity (Cvitanovic *et al.*, 2018, pp. 3-5; Rana *et al.*, 2022). Polyphenols have been associated with improving clinical outcomes and potentially delaying the progression of some diseases (Cory *et al.*, 2018). Examples of these disorders include neurodegenerative diseases (Singh *et*

al., 2018), cancer (Ding *et al.*, 2020), cardiovascular disease (Hua, Shi and Zhou, 2022), diabetes (Jin *et al.*, 2018), and obesity (Yuan *et al.*, 2018). Specifically, different types of polyphenols from fruits (Sosnowska, Podsędek and Kucharska, 2022), vegetables (Martinez-Gonzalez *et al.*, 2017), teas (Glisan *et al.*, 2017), wine (Khatchapuridze, Ploeger and Gulua, 2023) and seaweed (Shannon, Conlon and Hayes, 2023) have been shown to inhibit pancreatic lipase. However, it is important to acknowledge that further standardisation is required for the quality and dosage of polyphenols, polyphenol markers and study endpoints.

5.1.1 Polyphenol's Classification

Polyphenols are classified according to their chemical structures, which are based on the number of benzene rings and -OH groups. They can then be further classified based on their molecular architecture (Cvitanovic *et al.*, 2018, pp. 5-8) (Figure 5. 1). Phenolic acids are the basic forms of polyphenols containing a singular benzene ring attached to one carboxylic group (-COOH) (Figure 5. 1A and B). Phenolic acids can be obtained from all parts of a plant, and their presence is what gives some plants their sour, astringent, and bitter taste, depending on the phenolic acid concentration. It has been established that phenolic acids possess antioxidant, antimicrobial, anti-inflammatory and anti-diabetic properties, which has led to increased interest in their role in health and disease (Buelga, Paramas and Manzano, 2023).

Phenolic acids can be further categorised into two classes based on their functional groups, which relate to the number of carbon atoms. Benzoic and Cinnamic acid-derived substances are considered the simplest polyphenol groups with a molecular structure of -COOH and -CHCHCOOH, respectively (Figure 5. 1A and B). They can be found either as free acids or bound with other molecules, such as carbohydrates, organic acids, and other phenolic compounds. Generally, phenolic acids can be found in tea, coffee beans, black radishes, red fruits, berries and onions. Consequently, these foods and beverages are frequently associated with health benefits (Buelga, Paramas and Manzano, 2023; Cvitanovic *et al.*, 2018, p. 6; Rana *et al.*, 2022).

Flavonoids constitute another class of polyphenols, containing two aromatic rings (rings A and B) bound by an oxidated heterocyclic ring (C ring) (Figure 5. 1C). Currently, there are estimated to be approximately eight thousand different flavonoids, providing

a diverse array of different colours, odours, and tastes of plants (Cvitanovic *et al.*, 2018, p. 7). Furthermore, flavonoids have been shown to possess antioxidants and antimicrobial properties in humans (Gorniak, Bartoszewski and Kroliczewski, 2019; Silva *et al.*, 2018), while also being important for photoreceptors, visual attractors and feeding repellents (Pietta, 2000; Sahu and Mishra, 2022). Flavonoids can be further categorised into six subgroups based on the carbon bonds in the C ring, which can be unsaturated, oxidated and attached to the B ring. The most common major subgroups of flavonoids are flavones, flavanols, flavanones, flavonols, anthocyanins, and isoflavones. These groups are present in plants, especially root vegetables such as broccoli, green leaves, celery, blueberries, cocoa and citrus (Panche, Diwan and Chandra, 2016).

Stilbene compounds form another diverse group of polyphenolic compounds present in grapes, blueberries, cranberries, cocoa and peanuts (Duta-Bratu *et al.*, 2023). Stilbene are characterised by their two-benzene-ring structure connected by a double bond (C=C) (Figure 5. 1D). Although the distribution of stilbene is limited in comparison to other polyphenolic compounds (Cvitanovic *et al.*, 2018, p. 8), convincing data suggest that stilbenoid compounds may protect against aged-release diseases and confer neuroprotective, cardioprotective and anti-inflammatory effects (Reinisalo *et al.*, 2015).

Lignans are further subtypes of polyphenolic compounds that contain two phenylpropene units (Figure 5. 1E). Although present throughout plants, lignans are typically concentrated in the cell walls and are commonly found in cereal, oilseed and flaxseed (Jang, Kim and Cho, 2022). Lignans have previously been characterised as phytoestrogens and have been shown to exhibit antioxidant, anti-inflammatory and anticancer properties (Jang, Kim and Cho, 2022).

Phlorotannin forms another group of polyphenol compounds, found in high concentrations in brown seaweed (Maheswari and Babu, 2022). More specifically, phlorotannins are macromolecules derived from phloroglucinol (Figure 5. 1F), present in algae as free molecules within physodes or bound to other molecules in cell walls. Interestingly, phlorotannins can be further classified into six subgroups based on their molecular topology, which is important because different forms of phlorotannins

possess varying biological properties, conferring antioxidant, anti-Alzheimer, anti-allergy, antitumorigenic and antidiabetic properties (Cotas *et al.*, 2020).

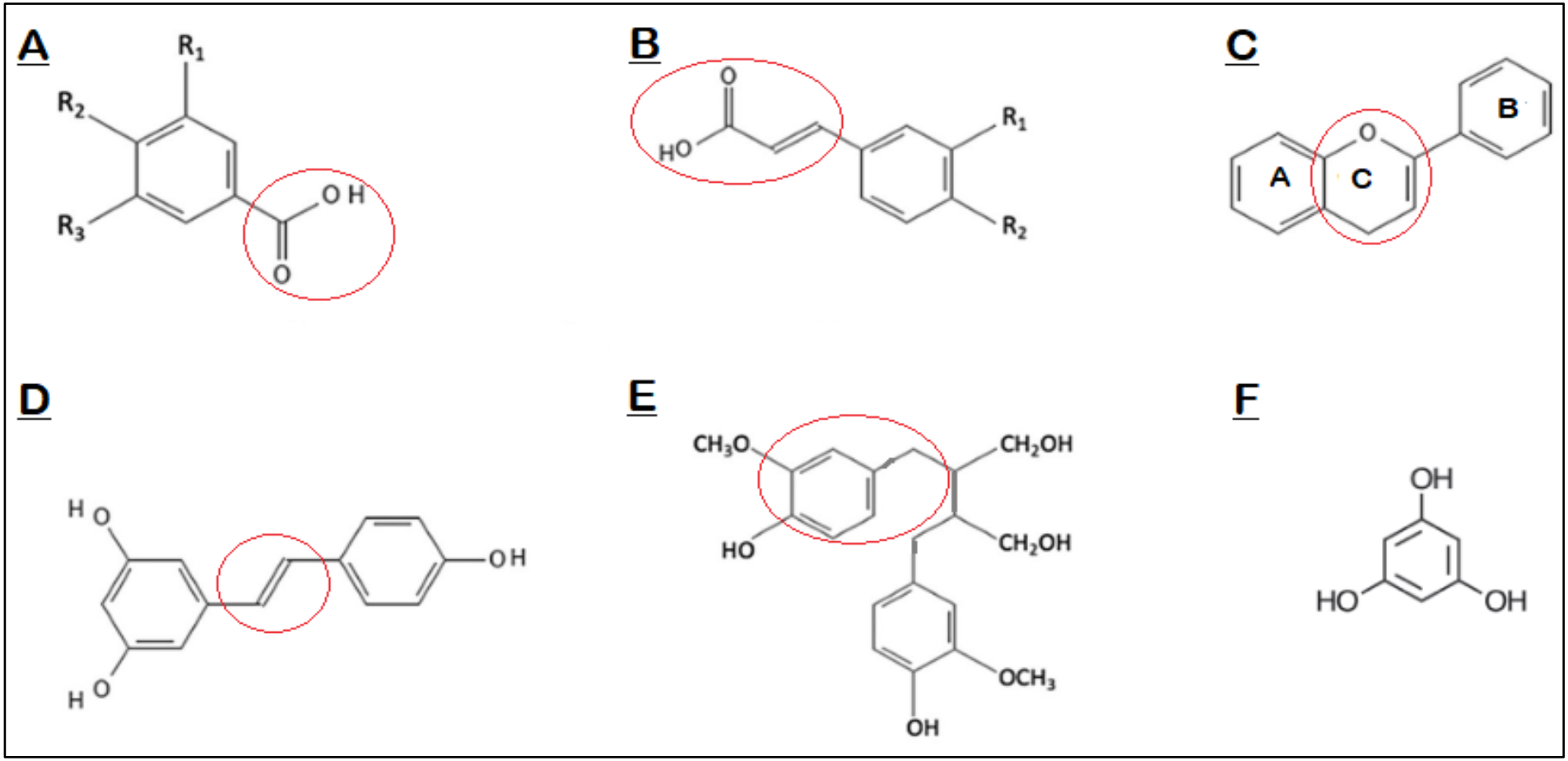


Figure 5. 1 Chemical structure of polyphenols.
Polyphenols have been classified into five groups based on their chemical structure. The red circles denote the groups that distinguish them from other phenolic groups. A: hydroxybenzoic acid, B: hydroxycinnamic acid (both phenolic acids), C: flavonoids where A and B denote aromatic rings and C denotes an oxidated heterocyclic ring), D: stilbenes, E: lignans and F: phlorotannins. Each polyphenol possesses a unique molecular structure and biological properties that are derived from R groups. Image adapted from (Rana *et al.*, 2022).

5.1.2 Seaweed Polyphenols

Edible seaweed has long been a staple food source in many Eastern countries, including China, Japan, and Korea, and is now a well-established food hydrocolloid, including alginate, carrageenan, and agar (Fathiraja *et al.*, 2022). Furthermore, researchers are now investigating seaweed for its phytochemical properties, specifically phenolic compounds due to the myriad of biological activities associated with them (cosmetics, nutraceuticals and pharmaceuticals) (Shannon and Abu-Ghannam, 2019). Several seaweeds including green, red, and brown forms have been shown to contain different polyphenolic compounds (Zhang *et al.*, 2006; Zhao *et al.*, 2023). However, some phenolic classes, such as flavonoids, bromophenols, phenolic terpenoids, mycosporine-like amino acids and non-typical phenolic compounds, have been reported to be present in all seaweed species. In contrast, phlorotannins are the most common phenolic compounds found in brown seaweed (Cotas *et al.*, 2020; Santos *et al.*, 2019). It is, therefore, crucial to identify the bioactivity of phenolic compounds found in seaweed.

5.1.2.1 Seaweed Polyphenols and their Bioactivities

Phenolic acids are simple phenolic compounds present in all seaweed species (Figure 5. 1A and B). Similarly, flavonoids are also present in all seaweed species (Figure 5. 1C); however, to date, there is limited research surrounding the concentrations of flavonoids in seaweed when compared to terrestrial plants (Zhao *et al.*, 2023). One of the most recent compounds extracted from all algae species, although highly concentrated in red algae, is bromophenols. Bromophenols contain benzene rings bound to an -OH and bromine (-Br) (Santos *et al.*, 2019; Zhao *et al.*, 2023). The interest in bromophenols has grown in recent years due to their antioxidant (Javan, Javan and Tehrani, 2013), anti-diabetic (Luo *et al.*, 2019), anti-bacterial (Jacobtorweihen *et al.*, 2024) and anti-cancer properties (Wang *et al.*, 2015). In contrast, phenolic terpenoids have primarily been reported in brown and red seaweeds. Phenolic terpenoids, although not as thoroughly researched as other phenolic classes, have also been reported to possess antioxidant (Mohandas and Kumaraswamy, 2018), anti-inflammatory (Hua, Shi and Zhou, 2022), and anti-bacterial activity (Guimarães *et al.*, 2019).

Mycosporine-like amino acids are photoprotective phenolic compounds commonly found in various aquatic organisms (Jofre *et al.*, 2020; Zhao *et al.*, 2023). In seaweed, mycosporine-like amino acids are commonly found in red species, and similar to other phenolic classes, they possess biological activities, including photoprotective antioxidant (Nishida *et al.*, 2020), anti-inflammatory (Hartmann *et al.*, 2015), anti-cancer (Becker *et al.*, 2016), wound-healing properties and anti-aging effects (Orfanoudaki *et al.*, 2020). The non-typical phenolic compounds are considered phenylpropanoid derivatives and are mostly found in brown and red seaweed (Cotas *et al.*, 2020). Of all the phenolic compounds, phlorotannins, predominantly found in brown seaweed, are the compound that has been most extensively studied (Gheda *et al.*, 2021). The most common classification of phlorotannins is based on the links between phloroglucinol units through phenyl (C-C), aryl-ether (C-O-C) and dibenzodioxin linkages, depicted using red circles in Figure 5. 2. Fucols (phenyl linkage), phlorethols (aryl-ether bond), fucophlorethols (phenyl and aryl-ether linkage) and eckols (benzodioxin linkage) are the most common subgroups of phlorotannins (Figure 5. 2) (Getachew, Jacobsen and Holdt, 2020; Maheswari and Babu, 2022).

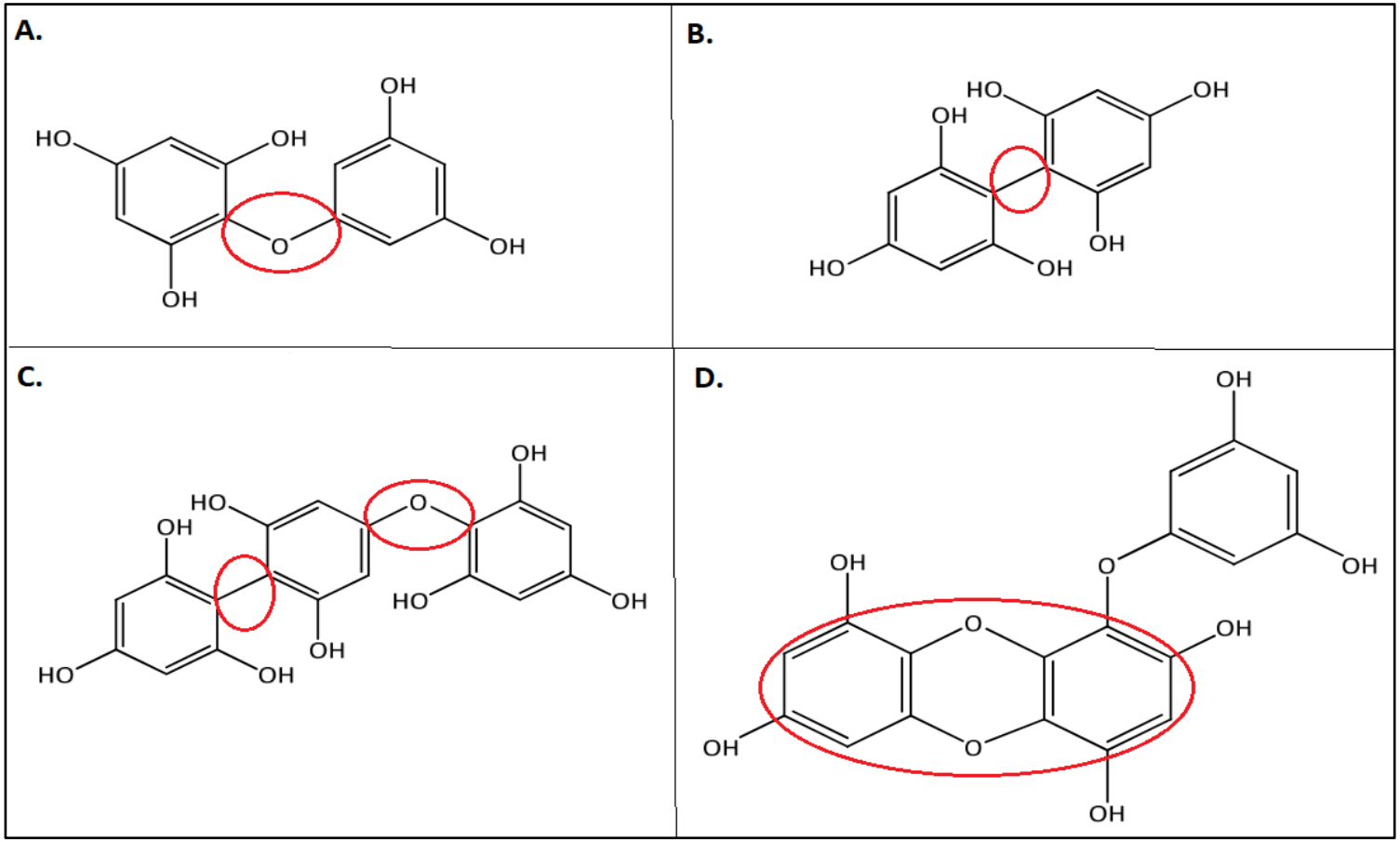


Figure 5. 2 Structural linkage in phlorotannins groups.

The chemical structure of different phlorotannin groups is depicted using a red circle. A: phlorethols, where the red circle denotes an aryl-ether bond; B: fucols, where the red circle denotes phenyl linkages; C: fucophlorethols, where the red circle denotes phenyl and aryl-ether linkage; and D: Eckols, where red circle denotes a dibenzo-dioxin linkage. Image adapted from (Getachew, Jacobsen and Holdt, 2020).

5.1.2.2 Seaweed Polyphenols Extraction, Quantification and Identification

Seaweed has been shown to be a rich source of polyphenolic compounds, including flavonoids, bromophenols, phenolic terpenoids, mycosporine-like amino acids, and non-typical phenolic compounds (Cotas *et al.*, 2020). Although, seaweeds have a myriad of uses, including being stable parts of diets, cosmetics, fertilisers, industrial gums, and chemicals. Recently, functional dietary compounds derived from seaweed have received considerable interest due to their potential health benefits. Consequently, research has focused on identifying which seaweeds confer optimal health benefits by studying their molecular composition and any bioactive compounds contained within (Wells *et al.*, 2017). Furthermore, optimising extraction methods of the bioactive compounds from seaweeds is essential and remains an area of much debate. However, it is clear that suboptimal extraction approaches can have a significant impact on the quality and quantity of polyphenol compounds (Sapatinha *et al.*, 2022). Some factors that can impact the quality and quantity of bioactive compounds include the species of seaweed being processed, the timing of sample collection (temporal), the geographical location of the seaweed harvested (spatial) and the polyphenol groups that may be contained within the seaweed (Kamal *et al.*, 2023).

The extraction of bioactive compounds from seaweeds can be complex due to the various steps involved in their extraction. For example, the extraction of polyphenols in seaweed involves sequential steps that include pre-treatment, referred to as sample preparation, extraction, and methods to examine the samples (Zhang *et al.*, 2022). Following the harvest process of the seaweed, a rinsing process is used to remove any impurities before drying the seaweed. The drying process typically involves air drying or freeze drying. Following the drying process, the seaweed is ground into a powder to enhance contact with solvents to optimise the extraction yield, which can be subsequently prepared for extraction (Cotas *et al.*, 2020). Understanding the extraction process from seaweed is important because any modification of these sequential steps can ultimately affect the quantity and quality of the polyphenolic compounds being extracted.

Commonly, two methods are used to extract polyphenolic compounds, known as the traditional or the modern method (Alara, Abdurahman and Ukaegbu, 2021; Getachew,

Jacobsen and Holdt, 2020). The traditional technique for extracting polyphenolic compounds uses solvents. The solvents used are typically pure organic solvents or binary aqueous mixtures, with the most commonly used solvents being acetone and ethanol (Gheda *et al.*, 2021; Lee *et al.*, 2020). The efficiency of the extraction process depends on factors including the temperature, duration used and the ratio between the seaweed material and solvent (Santos *et al.*, 2019). In contrast, modern extraction techniques include enzyme-assisted (Ihua *et al.*, 2019), ultrasound-assisted (Dang *et al.*, 2017), microwave-assisted (Yuan *et al.*, 2018), pressurized liquid (Otero, López-Martínez and García-Risco, 2019) and supercritical liquid extraction methods (Liu *et al.*, 2011).

Once the extraction process is complete, the extracted product can be assessed using various techniques to evaluate the quality and quantity of the polyphenols (Razem *et al.*, 2022). One approach is colorimetric assay techniques, with the most common approach being the Folin-Ciocalteu assay. Colorimetric array detectors are based on the light that passes through a given solution, from which the concentration can be calculated. This method is sensitive and selective when quantifying polyphenol concentration (Razem *et al.*, 2022; Zhang *et al.*, 2006). An alternative to colorimetric assays is chromatographic procedures, based on the principle of separating molecules in solution based on their physical properties (Zhao *et al.*, 2021). Chromatography is valid and reliable when characterising and quantifying phenolic compounds (Zhang *et al.*, 2022; Zhao *et al.*, 2021).

5.1.2.3 Total Polyphenol Contents (TPC)

When calculating the total polyphenol content (TPC) from a given extraction sample, various approaches can be used, with the most common approach being the Folin-Ciocalteu method (Rangel *et al.*, 2013). The Folin-Ciocalteu reagent is composed of phosphomolybdic and phosphor-tungstic acid (yellow colour), which is reduced by the presence of phenolic compounds in an alkaline solution, producing a blue colour that can be detected using a wavelength of 750 nm. The change in the colour from yellow to blue elicits an absorbance value that is directly proportional to the phenolic content in the sample. This absorbance value can then be compared to a reference sample, often referred to as a positive and negative control (Zhang *et al.*, 2006). Phloroglucinol is widely used as a positive control when characterising polyphenols due to its

extensive characterisation and concentration of polyphenolic compounds present in seaweed (Ford *et al.*, 2019; Zhang *et al.*, 2006).

5.1.3 Seasonal and Temporal Variation in Seaweed Polyphenol Quality and Quantity

There is a diverse range of seaweeds found throughout the world, exposing them to varying growth conditions, including sunlight, temperature, salinity and nutrient availability (Biancacci *et al.*, 2021; Park *et al.*, 2023). Seasonal variation and the timing of the harvest have been shown to affect polyphenol content extracted from seaweeds (Kamal *et al.*, 2023). In a recent study by Fellah *et al.* (2017), the effect of seasonal variation on polyphenol concentration was confirmed. The authors observed a significantly different polyphenol concentration in three species of seaweed from Algeria based on the harvest date (Fellah *et al.*, 2017). Interestingly, Fellah *et al.* (2017) observed that in red seaweed Sphaerococcus coronopifolius and brown seaweed Halopteris scoparia, the highest concentrations of polyphenols were found in samples harvested during autumn. Similarly, Kumar, Sahoo and Levine (2015) also observed variations in polyphenol concentration extracted from the Indian brown seaweed Sargassum wightii, originating from Tamil Nadu throughout 2009-2010. A peak concentration was observed in January, while the lowest was observed in September (Kumar, Sahoo and Levine, 2015).

The geographical location in which seaweed grows has also been shown to affect the quality and quantity of polyphenols extracted from the seaweed. In European coastlines, Tanniou *et al.* (2014) reported variations in polyphenol concentration in sargassum muticum, with the highest TPC observed in samples collected from Portugal, Norway and France. In Southeast Asia, Hees *et al.* (2017) reported significant variations in phenolic concentrations extracted from brown seaweed at seven different locations along the Western Australia coastline. Similarly, Saraswati *et al.* (2020) studied Indonesian brown seaweed, Sargassum cristaefolium, collected from four different coastal locations on Java Island. The authors reported varying phenolic content at all four coastal locations, with the highest and lowest TPC observed from samples collected at Sayang Heulang and Ujung Genteng, respectively (Saraswati *et al.*, 2020). These data demonstrate variations in both the timing of harvest and geographical locations, which must be accounted for when studying the biochemical

contents of seaweeds and comparing different seaweeds due to the vast array of confounding factors (Connan and Stengel, 2011; Saraswati *et al.*, 2020; Tabassum, Xia and Murphy, 2016).

5.1.4 Kuwait's Seaweed polyphenols

As previously discussed, geographical location can impact the composition and polyphenol content of the seaweed being studied. In Western Asia, the coastline surrounding Kuwait has been shown to affect the chemical composition of the seaweeds due to the biotic and abiotic marine environment surrounding Kuwait, resulting in seaweed species-specific characteristics (AlYamani *et al.*, 2014). To date, there is limited research studying the potential bioactive components of Kuwait seaweed. In a recent study by Habeebullah *et al.* (2019), 26 different seaweed species obtained from the coastline of Kuwait using water, 50 and 100% EEs were analysed to assess the TPC and antioxidant properties. This study has shown that TPC contents varied in seaweed species with the highest TPC and antioxidant activity present in brown species (Habeebullah *et al.*, 2019). The same authors subsequently studied seven brown seaweeds from the Kuwait coastline and evaluated the antioxidant activity of polyphenols derived from the seaweed (Habeebullah *et al.*, 2020). The authors reported that the highest TPC and antioxidant activity were observed in *Sargassum boveanum*, *Sargassum angustifolium*, and *Feldmannia irregularis* (Habeebullah *et al.*, 2020). The TPC reported in Kuwaiti Sargassum boveanum, Sargassum angustifolium, and Feldmannia irregularis was found to be higher than in the same species of seaweed located around the coastlines of Korea (Heo *et al.*, 2003) and France (Sánchez-Camargo *et al.*, 2016).These studies emphasize the chemical diversity among different seaweeds and the extraction methods used for their extraction, which can have economic and therapeutic benefits.

5.2 Aims

The aims of this chapter are:

- To determine the TPC in Kuwaiti seaweeds and its aqueous and ethanolic extracts.
- To assess the impact of seasonal variation on TPC from the seaweed *S. trinodis*, collected from Salmiya Beach in Kuwait in both November 2020 and April 2021.
    - To determine the optimal season for harvesting *S. trinodis* to achieve the highest TPC.
- To examine the correlation between the TPC in the Kuwaiti seaweed samples, including homogenate, aqueous and ethanolic extracts, and their inhibitory effect on pancreatic lipase (measured by IC50) (Chapter 2).

5.3 Methods

The Folin-Ciocalteu reagent was used to assess the TPC from selected seaweeds by detecting the formation of a blue-coloured product at a wavelength of 750 nm, as it is widely used. The blue product is formed in the presence of polyphenols from the seaweed due to the reduction and oxidation reaction between the phosphomolybdic and phosphor-tungstic acids contained in the Folin-Ciocalteu assay. The addition of sodium carbonate solution creates alkaline conditions, which further facilitates this reaction and accentuates the colour change. To quantify the TPC extracted from seaweeds, phloroglucinol was used as a positive control (Zhang *et al.*, 2006).

5.3.1 Materials

Folin-Ciocalteu reagent (Sigma 9252) and sodium carbonate were obtained from Sigma-Aldrich (UK). Phloroglucinol dihydrate was purchased from Sigma-Aldrich (UK). Absolute ethanol and methanol were obtained from Fisher Scientific (UK). Kuwaiti seaweeds were kindly donated to this research project by the KISR and Kuwait University Marine Science Department. The seaweed *Fucus vesiculosus* (*F. vesiculosus*) from the Outer Hebrides was kindly donated by LEHVOSS Gee Lawson, Congleton (UK).

5.3.2 Equipment

The 96-well plate reader (EL808 Bio Teck) was used at 550 nm (Bedfordshire, UK). A pH meter with a 213 microprocessor was used to measure pH (Hana Instruments, Leighton Buzzard, UK).

5.3.3 Seaweed

Using the results generated in Chapter 2 (Table 2. 5), the top 15 Kuwait seaweed samples with the highest inhibition on lipase activity were selected to assess their TPC. The seaweed homogenates were green seaweed (*C. sericioides*), red seaweed (*G. pusillum*) and brown seaweed (*S. trinodis* N, *S. trinodis* A, *C. sinuosa*, *I. stellata* and *P. boergesenii*). The WE samples were WE of *C. papillatum*, *S. trinodis* N, *S. trinodis* A and *P. boergesenii*. The EP were EP of *C. sericioides*, *C. papillatum*, *P. boergesenii* and *G. pusillum*.

F. vesiculosus is well established for its bioactive properties, primarily due to its abundant polyphenolic content (Catarino *et al.*, 2019). Therefore, the TPC of Kuwaiti seaweeds were compared to those of *F. vesiculosus*.

5.3.4 Reagent Setup

To identify the TPC, 0.5 g of whole seaweed (homogenate) was aliquoted and pre-incubated in a 15 ml tube with 10 ml of a 1:1 mixture of methanol and water (pH 2) for one hour on the roller in the dark at room temperature before being centrifuged at 12,000xg for 10 minutes. Following centrifugation, the supernatant was transferred into a new 15ml tube (tube 1). The pellet (of whole seaweed) was then incubated in 10ml of a 7:3 mixture of acetone and water (pH 2) for one hour on the roller in the dark at room temperature before being centrifuged at 12,000xg for 10 minutes. The supernatant was then transferred into tube 1 and mixed by vortex. 100 µl of the mixed supernatants (in tube 1) was then diluted with deionised water (1:10) and added to a new 2 ml Eppendorf tube, used as a diluted sample. A blank solution was prepared using the same steps as described here with the omission of seaweed.

The seaweed extracts (WE extract and ethanol pellets) were prepared as previously described (Chapter 2). The same steps as previously described for whole seaweed, were used for preparing the ethanol pellet. 1-5 mg of seaweed WE extract was mixed with 1 ml of WE in a 2 ml Eppendorf tube (by vortex). The samples were then centrifuged for 10 minutes at 12000xg. A second extraction was then prepared by diluting 100 µl of previously mixed supernatants with deionised water at a ratio of 1:10 in a new 2 ml Eppendorf tube. This diluted solution was used as a diluted sample. A blank solution was prepared using the same steps as described here with the omission of the extract.

Phloroglucinol was used as a positive control at a concentration of 1mg/ml. To achieve this concentration, phloroglucinol was added to deionised water, before creating a serial of seven dilutes ranging from 1 to 0.016 mg/ml to use as a standard curve (Figure 5. 3). The standard curve of phloroglucinol was used as a reference to calculate the content of polyphenol compounds in the Kuwaiti seaweed.

5.3.5 Procedures

A minimum of three replicates, each consisting of 20 µl were analysed, including diluted samples, blank (negative control), and positive control solutions. In triplicate, each sample was added to a 96-well microplate and 100 µl of the Folin-Ciocalteu reagent was added to each of the samples before being incubated in a dark for 5 minutes at room temperature. 80 µl of a 7.5% sodium carbonate solution was then added to each well containing a sample. The microplate was then covered and incubated in a dark at room temperature for 2 hours to facilitate colour development. Following incubation, the microplate was read at a wavelength of 750 nm.

5.3.6 Calculation

The TPC was calculated using Equation 5.1. The mean and standard deviation (SD) absorbance was calculated from the triplicate samples from each test sample, including the blank (negative) and Phloroglucinol (positive) control samples. The absorbance from the blank sample (negative control) was then subtracted from its corresponding sample. The mean value for each sample was subsequently entered into Equation 5.1 before being expressed in milligrams (mg) of phloroglucinol equivalent (PGE) per gram (g) of dry seaweed.

$$\boldsymbol{TPC}\ \% = \left(\frac{(\boldsymbol{mean\ result\ of\ sample}(\mathrm{mg})\times \boldsymbol{SV}\times \boldsymbol{DF})}{\boldsymbol{weight\ of\ sample}\ (\boldsymbol{g})}\right) \times \mathbf{100}$$

Equation 5. 1 Total polyphenol content (TPC) calculation formula.
SV: solvent volume; DF: dilution factor; mg: milligrams; g:gram.

5.3.7 Statistical Analysis

All raw data were extracted from the spectrophotometer and uploaded onto a Microsoft Excel spreadsheet. Subsequently, a standard curve was constructed using the positive control (Phloroglucinol) to quantify the TPC extracted from seaweeds as phloroglucinol equivalents (PGE). The data were then extracted and analysed using GraphPad Prism 9 software. To evaluate the differences between TPC in all seaweed samples, a one-way ANOVA with post-hoc Tukey's test and an unpaired t-test were used. Spearman correlation analysis to evaluate the association between Kuwaiti seaweed TPC and their ability to inhibit pancreatic lipase (measured by IC50).

Each experiment was performed in three independent experiments, each with three biological replicates. Unless otherwise stated, all data are presented as mean ± standard deviation (SD).

5.4 Results

The TPC of various seaweed species and different extraction solvents significantly varied. *F. Vesiculosus* was used as a positive control to compare its TPC with that of Kuwait seaweed and its extracts. The correlation between Kuwaiti seaweed TPC and pancreatic lipase inhibition (using IC50 values) was examined.

5.4.1 Phloroglucinol Standard Curve

Phloroglucinol was serially diluted as the positive control, to plot a standard curve for TPC (Figure 5. 3). The TPC generated from the Kuwaiti seaweed was then quantified using the PGE. An individual standard curve of Phloroglucinol was created for each 96-well microplate.

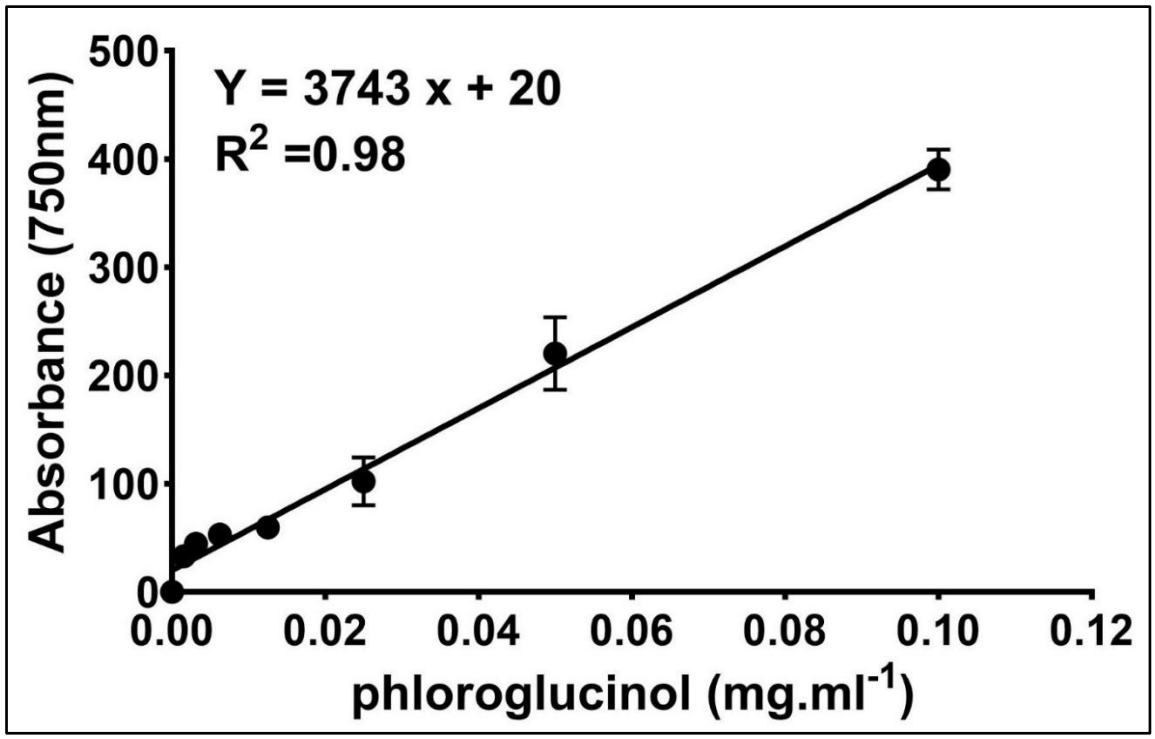


Figure 5. 3 Standard curve for phloroglucinol.
Phloroglucinol was serially diluted as the positive control, to plot a standard curve for total phenolic content (TPC). Regression analysis was performed, generating the horizontal (X) and vertical (Y) values to predict TPC in mg (milligrams) phloroglucinol equivalent (PGE)/grams (g) of dry seaweed. Data is presented as the mean ± standard deviation (SD) from n=3.

5.4.2 TPC of Seaweed Homogenates

The TPC of Seven seaweed homogenates was determined. A non-significant difference in the TPC was observed between the brown seaweeds *P. boergesenii* and *S. trinodis* extracted in April, with a TPC of 0.8 (± 0.14) and 0.5 (± 0.12) mg PGE/ g dry seaweed, respectively (P=0.07) (Figure 5. 4). However, the TPC of the brown seaweed (*P. boergesenii*) was the highest compared with the TPC from other brown, red and green seaweeds (P≤0.05) (Figure 5. 4). The TPC of other brown seaweeds was 0.5 (± 0.14), 0.4 (± 0.09) and 0.4 (± 0.08) mg PGE/ g dry seaweed for *I. stellata*, *C. Sinuosa* and *S. trinodis* extracted in November, respectively (Figure 5. 4). There was no significant difference in the TPC for these brown seaweed (*I. stellata*, *C. Sinuosa* and *S. trinodis* extracted in November) (P>0.05) (Figure 5. 4).

The TPC of red seaweed (*G. Pusillum*) was 0.6 (± 0.06) mg PGE/ g dry seaweed (Figure 5. 4). There was no significant difference in the TPC for brown seaweeds (*I. stellata*, *C. Sinuosa* and *S. trinodis* extracted in November and April) and red seaweed (*G. Pusillum*) (P>0.2) (Figure 5. 4).

The TPC of the green seaweed *C. sericioides* was significantly lower than brown seaweeds (*I. stellata*, *S. trinodis* extracted in April and *P. boergesenii*) and red seaweed (*G. Pusillum*), with a TPC of 0.2 (± 0.06) (P≤0.03), as shown in Figure 5. 4.

However, the data presented in Figure 5. 4 reveals that there was no significant difference in the TPC for the green seaweed (*C. sericioides*) and brown seaweeds (*S. trinodis* extracted in November and *C. Sinuosa*) (p>0.2) (Figure 5. 4).

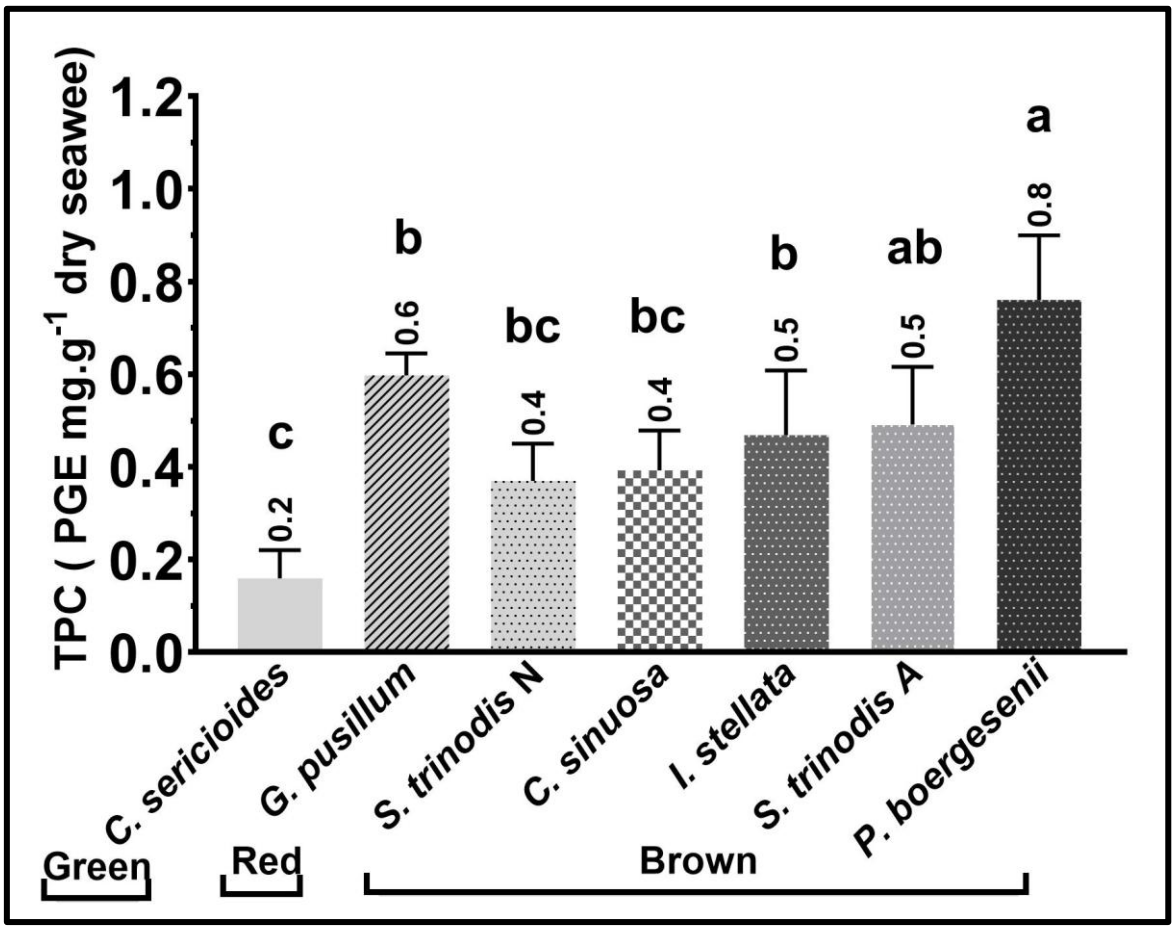


Figure 5. 4 Total Phenolic Content (TPC) from green, brown and red seaweed homogenates.

The TPC of homogenate from green seaweed (*Cladophora sericioides*), red seaweed (*Gelidium Pusillum*) and brown seaweed (*Sirophysalis trinodis* N, *Colpomenia Sinuosa, Iyengaria stellata, Sirophysalis trinodis* and *Padina boergesenii)* were calculated in milligrams (mg) phloroglucinol equivalent (PGE)/grams (g) of dry seaweed. A one-way ANOVA was performed with post-hoc Tukey's test to evaluate the differences between TPC in all seaweed homogenates. Data is presented as mean ± standard deviation (SD) (n=3). Values with different letters show statistically significant differences ($P < 0.05$). *S. trinodis* N was collected in November 2020; *S. trinodis* A was collected in April 2021

5.4.3 TPC of Seaweed WE

The TPC of four WE from green (*C. papillatum*) and brown seaweeds (*P. boergesenii* and *S. trinodis* N and *S. trinodis* A was determined. There were significant differences in the TPC when comparing the WE from different seaweed species (Figure 5. 5). The highest TPC present in the WE was observed in *S. trinodis* N, extracted in November, with a TPC of 4.0 (± 0.2) ($P<0.0001$) (Figure 5. 5). The TPC of the WE extracted from brown seaweed *P. boergesenii* was significantly higher than that extracted from brown seaweeds *S. trinodis* A (extracted in April) and green seaweed (*C. papillatum*), with a TPC of 2.6 (±0.6), 1.4 (± 0.1) and 0.9 (± 0.3), respectively ($p <0.004$).

There were no significant differences in the TPC of WE when comparing *C. papillatum* and *S. trinodis* A (extracted in April) ($p>0.05$) (Figure 5. 5).

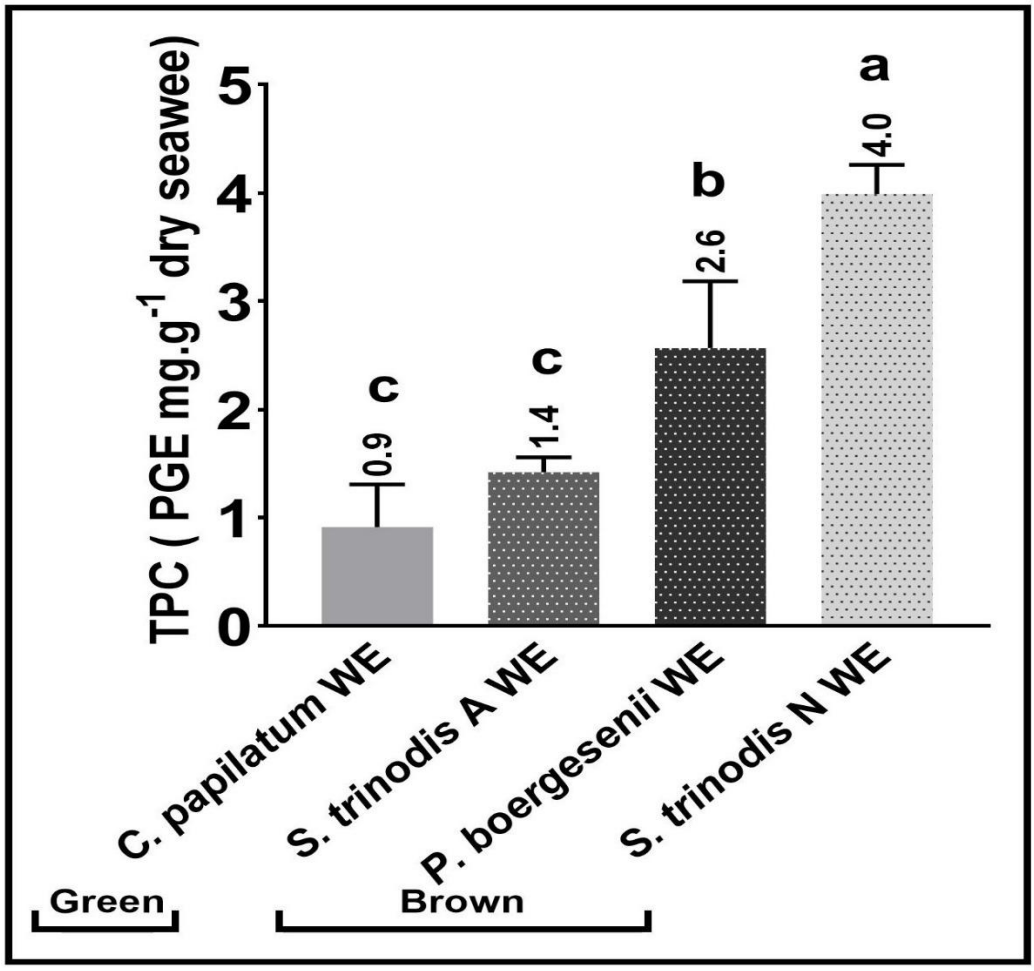


Figure 5. 5 Total phenolic content (TPC) of seaweed deionised water extract (WE).

The TPC of WE was calculated in mg of phloroglucinol equivalent (PGE)/grams (g) of dry seaweed. The WE was extracted from green (*Codium papillatum*) and brown Kuwaiti seaweed species (Sirophysalis trinodis N, *Sirophysalis trinodis* A and *Padina boergesenii*). A one-way ANOVA was performed with post-hoc Tukey's test to evaluate the differences in TPC between all the seaweed species extracts. Data is presented as mean ± standard deviation (SD) (n=3). Values with different letters show statistically significant differences ($P < 0.05$. S. trinodis N was collected in November 2020; S. trinodis A was collected in April 202.

5.4.4 TPC of Seaweed EP

A non-significant difference in the TPC was observed between the EP extracted from green seaweed (*C. sericioides* and *C. papillatum*) ($P>0.05$) (Figure 5. 6). The TPC of EP extracted from brown seaweed (*P. boergesenii*) and red seaweed (*G. Pusillum*) ethanol was not significantly different ($P>0.05$) (Figure 5. 6). The TPC of EP extracted from green seaweed (*C. sericioides* and *C. papillatum*) was significantly lower than that from brown seaweed (*P. boergesenii*) and red seaweed (*G. Pusillum*) ($P<0.006$) (Figure 5. 6).

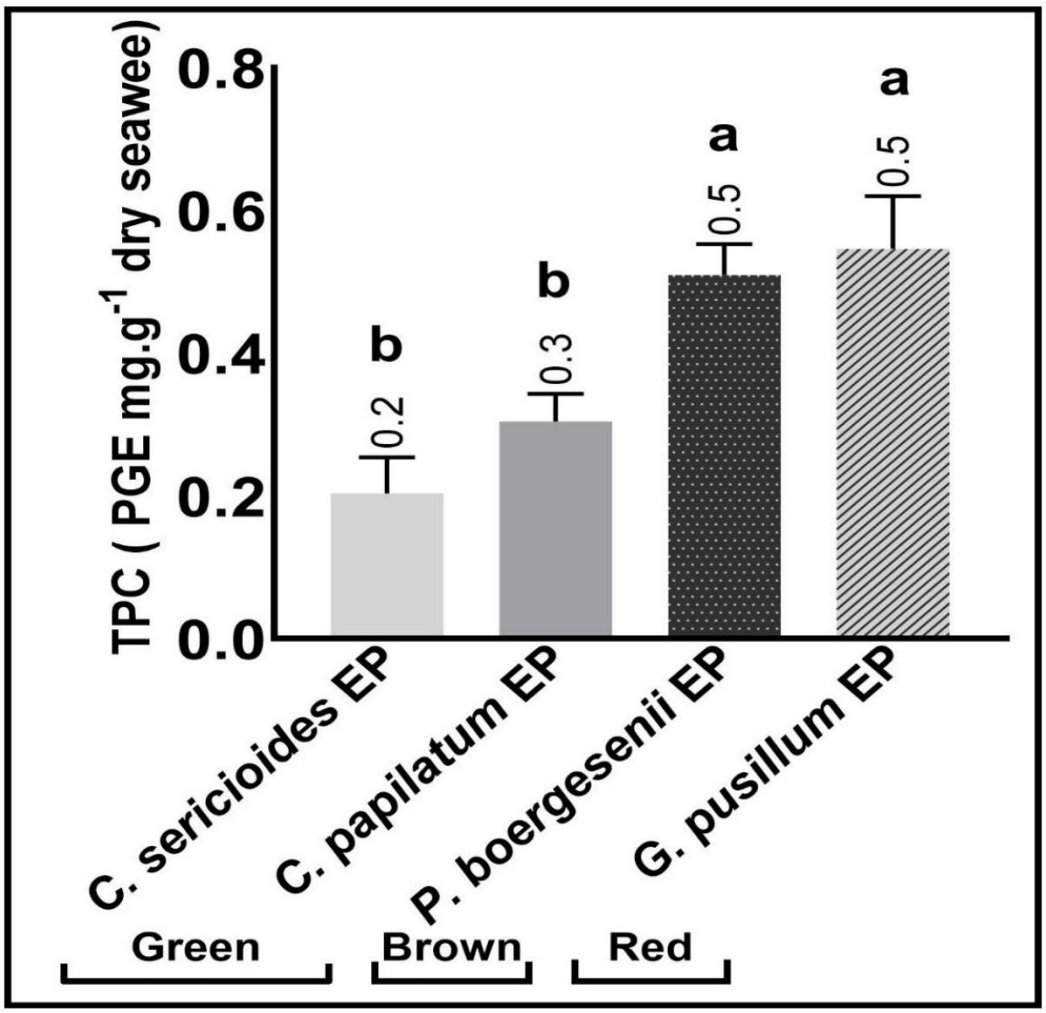


Figure 5. 6 Total phenolic content (TPC) of seaweed ethanol pellet (EP). The TPC of Kuwaiti seaweed EP from green seaweed (*Cladophora sericioides* and *Codium papillatum*), brown seaweed (*Padina boergesenii*), and red seaweed (*Gelidium Pusillum*) were analysed. The TPC was calculated following 100% ethanol for *Cladophora sericioides* or 50% ethanol extraction, for all other species of seaweed and calculated in milligrams (mg) phloroglucinol equivalent (PGE)/grams (g) dry seaweed. In addition. A One-way ANOVA was performed with post-hoc Tukey's test to evaluate the differences in TPC between all seaweed species extract. Data are presented as mean standard deviation (±SD) (n=3). Values with different letters show statistically significant differences ($P < 0.05$).

5.4.5 TPC differences between Kuwaiti Seaweed and their Extracts

5.4.5.1 TPC Variations of Green Seaweeds and their Extracts

No significant difference was observed in the TPC between *C. sericioides* homogenate and its EP ($P>0.05$) (Figure 5. 7A). The polyphenol content in the WE from *C. papillatum* was significantly higher than that in the EP (Figure 5. 7B).

5.4.5.2 TPC Variations of Brown Seaweeds and their Extracts

The TPC from the brown seaweed *S. trinodis* was consistently higher in the WE when compared to the homogenate (Figure 5. 8). The TPC of the WE was significantly higher than that of the homogenate in *S. trinodis* when extracted in November and April ($P< 0.002$), respectively (Figure 5. 8A and B, respectively). The WE from *P. boergesenii* had a significantly higher polyphenol concentration when compared to the homogenate and its EP ($p<0.002$), respectively (Figure 5. 8C). However, the TPC was not significantly different between the homogenate and the EP extracted from *P. boergesenii*.

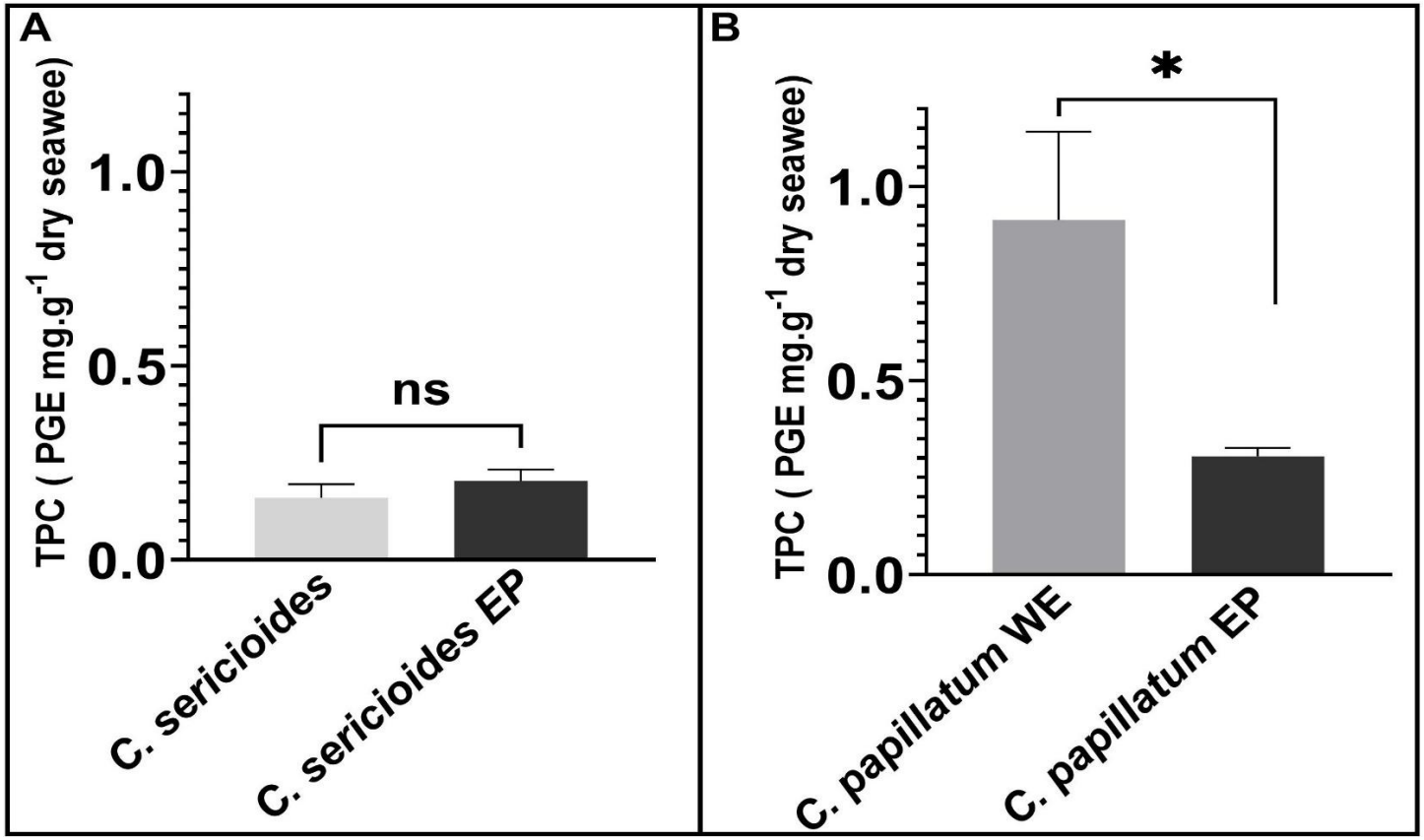


Figure 5. 7 Total phenolic content in green seaweeds following different extraction solvents.
Figure 5. 7A shows TPC variation in seaweed homogenate and seaweed pellets after 100% ethanol extraction (EP) from *Cladophora sericioides*, analysed using an unpaired t-test. Figure 5. 7B shows variation in TPC of deionised water extract (WE) and seaweed EP from *Codium papillatum*, analysed using an unpaired t-test. Data are presented as the mean ± standard deviation (SD) (n=3). * denotes a significant difference at $p<0.05$. PGE: phloroglucinol equivalent; ns: non-significant.

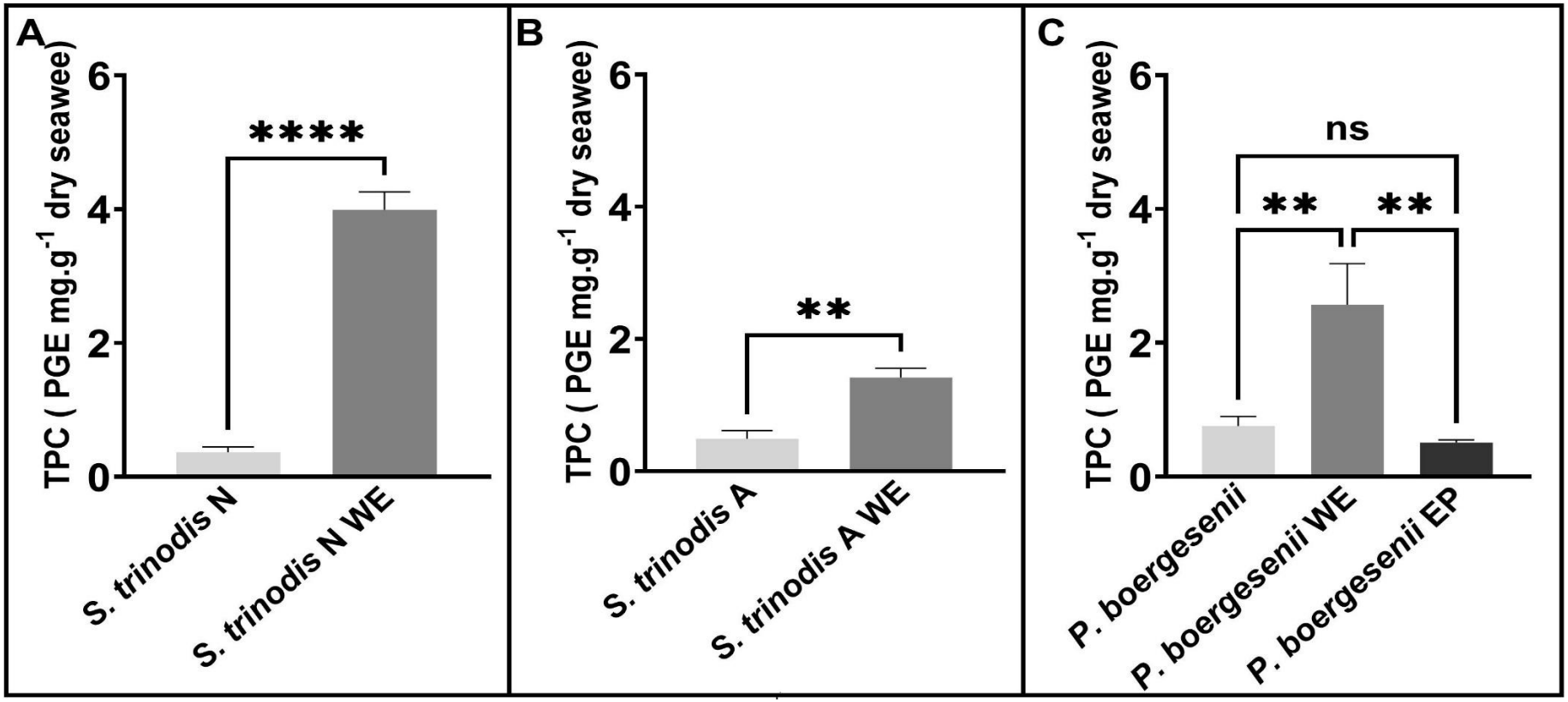


Figure 5. 8 Total phenolic content (TPC) in brown seaweeds following different extraction solvents.

Figure 5. 8A shows TPC variation between the homogenate and deionised water extraction (WE) of *Sirophysalis trinodis* extracted in November (N) analysed using an unpaired t-test. Figure 5. 8B shows TPC variation between the homogenate and WE extract of *Sirophysalis trinodis* extracted in April (A) analysed using an unpaired t-test. Figure 5. 8C shows TPC variations between the homogenate, WE and pellets after 50% ethanol extraction (EP) in *Padina boergesenii* analysed using a one-way ANOVA. Data are presented as mean ± standard deviation (SD) (n=3). ** and **** denote significant differences at p ≤0.002 and 0.0001, respectively. PGE: phloroglucinol equivalent.

5.4.5.3 TPC Variation of Red Seaweed and its Extracts

There was no significant difference between the red seaweed (*G. pusillum*) homogenate and the EP (P >0.05) (Figure 5. 9).

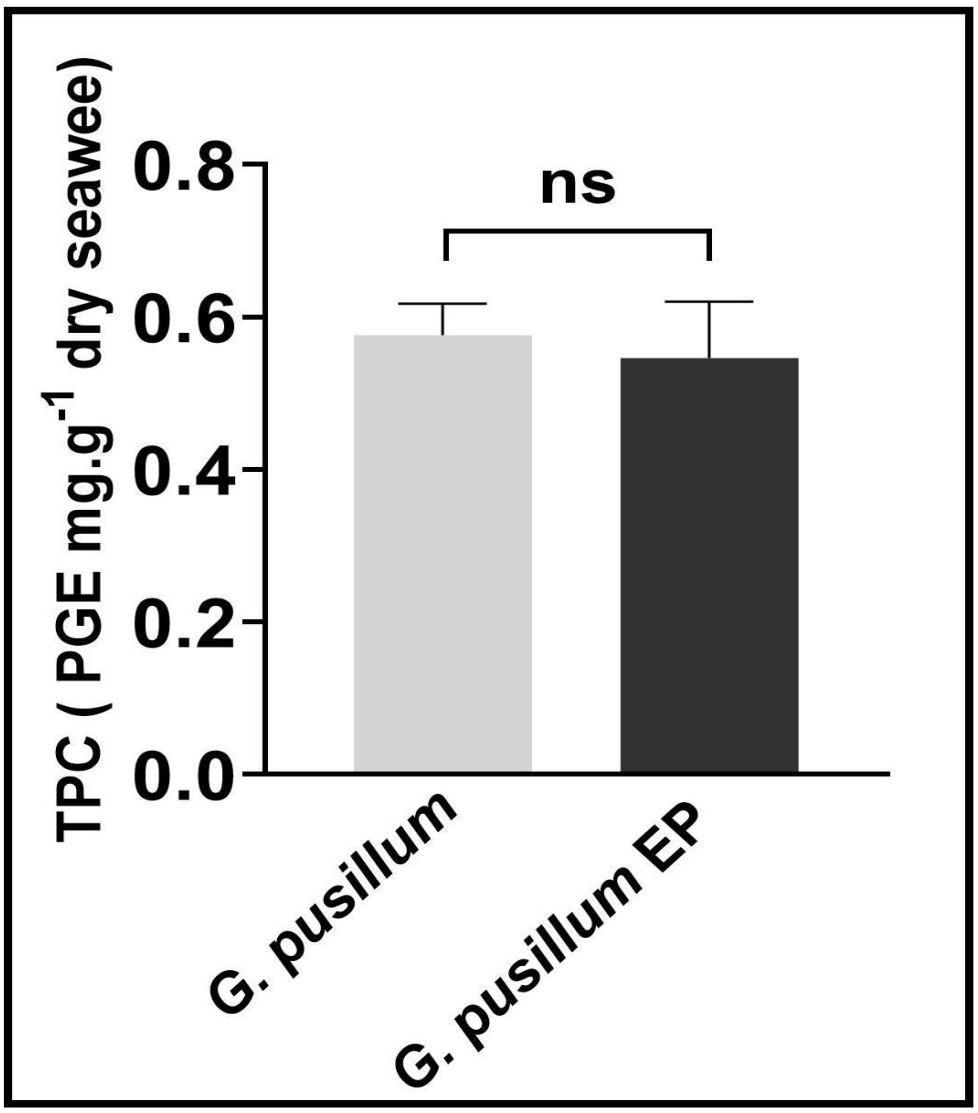


Figure 5. 9 Total phenolic content (TPC) differences in red seaweed following different extraction solvents.
Comparison of TPC in the red seaweed (*Gelidium Pusillum*) from the homogenate and the pellet remaining after 50% ethanol extraction (EP), which was analysed using an unpaired t-test. Data are presented as mean ± standard deviation (SD) (n=3). PGE: phloroglucinol equivalent; ns: non-significant.

5.4.5.4 Seasonal Variation in TPC from the Brown Seaweed *S. trinodis*

There was no significant difference in TPC between homogenate samples when comparing extraction from *S. trinodis* in November 2020 and April 2021 ($p>0.05$) (Figure 5. 10A). However, a significant difference was observed in the TPC when comparing the WE between November 2020 and April 2021, ($P= 0.0001$) (Figure 5. 10B).

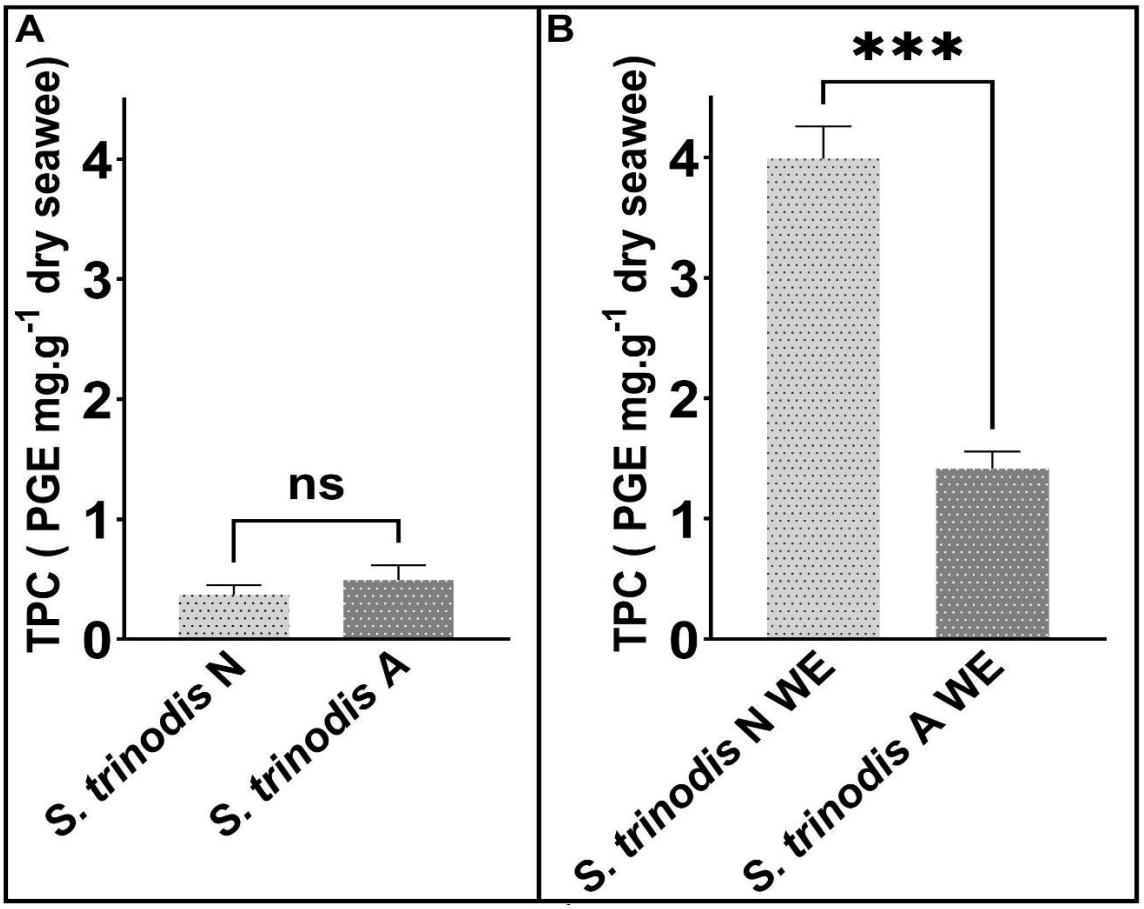


Figure 5. 10 Seasonal variation in total phenolic content (TPC) in brown seaweed *S. trinodis*.

Figure 5. 10A shows TPC variation in the brown seaweed *Sirophysalis trinodis* homogenate collected in November 2020 (N) and April 2021 (A), analysed using an unpaired t-test. Figure 5. 10B shows TPC variation in the brown seaweed *Sirophysalis trinodis* deionised water extract (WE), analysed using unpaired t-test tests. Data are presented as mean ± standard deviation (SD) (n=3). *** denotes a significant difference at $P < 0.0001$. PGE: phloroglucinol equivalent; ns: non-significant.

5.4.6 Comparison of *F. Vesiculosus* TPC and Kuwait Seaweeds

F. vesiculosus is well established for its bioactive properties, primarily due to its abundant polyphenolic content. This investigation found that the TPC of the Kuwaiti seaweed homogenates was significantly lower compared to the homogenate of Fucus Vesiculosus, which had a TPC of 2.1 mg PGE/ g dry seaweed ($p<0.0001$) (Figure 5. 11A).

The TPC present in the WE from the brown seaweed *S. trinodis* N was significantly higher than the TPC in the *F. Vesiculosus* homogenate (Figure 5. 11B) ($p<0.0005$). However, the TPC from the *F. Vesiculosus* homogenate was significantly higher than the WE from *C. papillatum* ($p<0.05$) (Figure 5. 11B). Figure 5. 11B shows non-significant differences between *F. Vesiculosus* homogenate and the WE from brown seaweeds, *S. trinodis* A and *P. boergesenii* ($p>0.05$).

The TPC present in the EP was significantly lower than that in *F. Vesiculosus* homogenate ($p<0.0001$) (Figure 5. 11C).

5.4.7 Evaluation of any Correlation between TPC and Pancreatic Lipase IC50

To explore potential associations between the TPC in Kuwaiti seaweed samples (homogenate, WE and the EP) and its IC50 values of pancreatic lipase inhibition, Spearman correlation coefficient tests were conducted, as shown in Figure 5. 12. Notably, no significant association were found between pancreatic lipase IC50 values and the TPC in Kuwaiti seaweed homogenate, WE and the EP ($P>0.05$) (Figure 5. 12A, B and C, respectively).

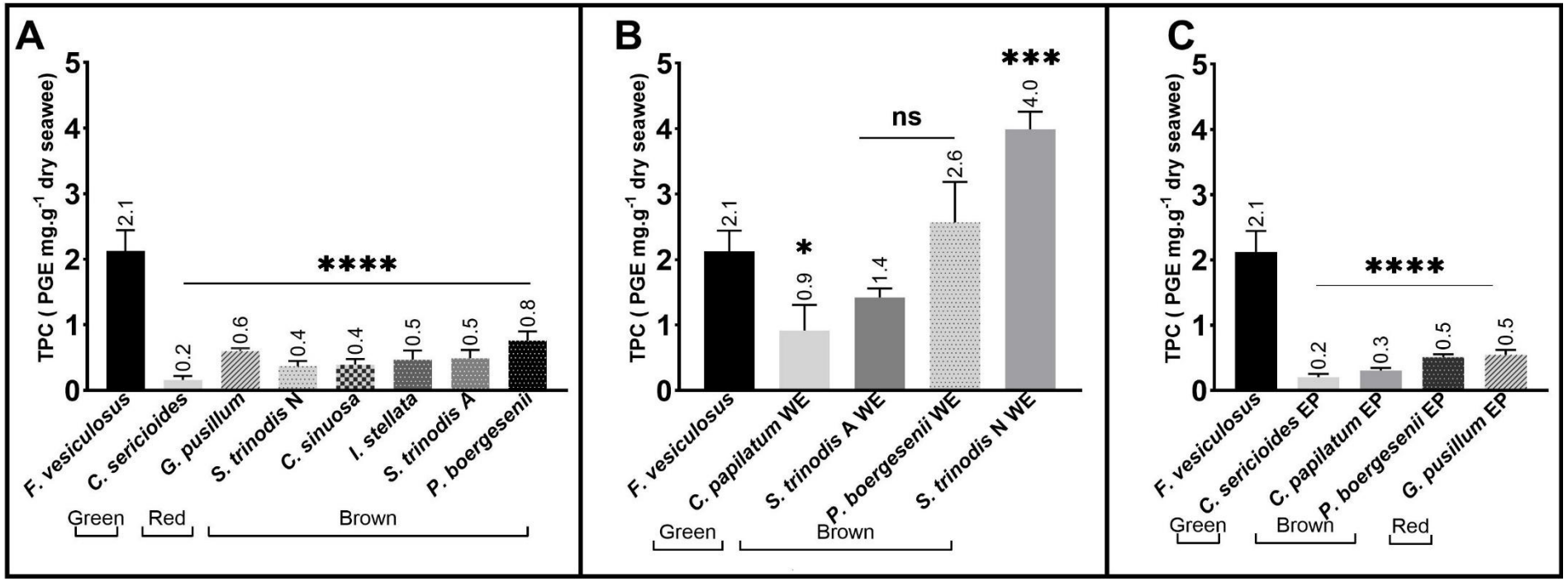


Figure 5. 11 Total polyphenol content (TPC) variations *in Fucus Vesiculosus* and different Kuwaiti seaweed extracts.

Figure 5. 11A shows TPC in *Fucus vesiculosus* and Kuwaiti seaweed homogenates. Figure 5. 11B shows TPC in *Fucus vesiculosus* homogenate and Kuwaiti seaweed deionised water extract (WE). Figure 5. 11C shows TPC in *Fucus Vesiculosus* homogenate and the Kuwaiti seaweed pellet remaining after ethanol extraction (EP). One-way ANOVA was performed with post-hoc Dunnett's test to evaluate the TPC differences between the homogenate from Fucus Vesiculosus and the Kuwaiti seaweed extract. Data are presented as mean ± standard deviation (SD) (n=3). *, **, *** and **** denote a significant difference at $p<0.05$, <0.006, < 0.0002 and <0.0001, respectively. PGE: phloroglucinol equivalent; *Fucus*: *Fucus vesiculosus*; *Clad*.: *Cladophora sericioides*; *Codi*: *Codium papillatum*; *Sir.* N: *Sirophysalis trinodis* collected in November 2020; *Sir.* A: *Sirophysalis trinodis* collected in April 2021; *Colp*: *Colpomenia Sinuosa*; *Iyng*: *Iyengaria stellata*; *Padi*: Padina *boergesenii*; *Geli*, *Gelidium Pusillum*; ns, non-significant.

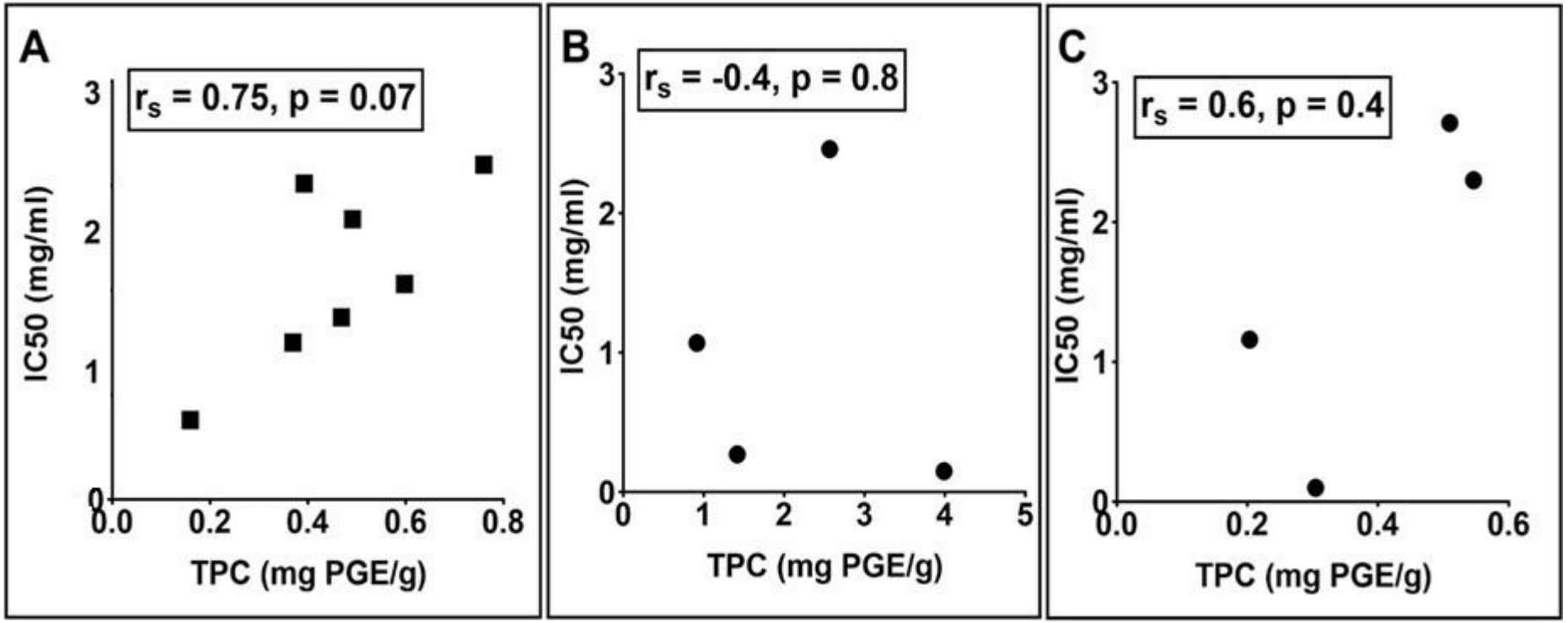


Figure 5. 12 Spearman Correlation coefficients between pancreatic lipase inhibition IC50 and total polyphenol content (TPC).

Figure 5. 12A shows no significant correlation between the TPC of Kuwaiti seaweed homogenates and its IC50 of pancreatic Lipase inhibition. Figure 5. 12B shows no correlation between pancreatic lipase inhibition IC50 and TPC of deionised water extract (WE) from Kuwaiti seaweed. Figure 5. 12C shows no correlation between pancreatic Lipase inhibition IC50 and the TPC of the pellet remaining after ethanol extraction (EP) from Kuwaiti seaweed. Spearman correlation analysis to evaluate the association between TPC and pancreatic lipase inhibition (IC 50). PGE: phloroglucinol equivalent.

5.5 Discussion

Polyphenols are well-established for their role in health and well-being, with potential mechanisms including antioxidant properties, acting as radical scavengers in lipid peroxidation chain reactions and chelators of metals. Specifically, polyphenols extracted from seaweeds have shown various potential health benefits through various biological properties. In this thesis, one of the biological properties of Kuwait seaweeds, through their ability to inhibit pancreatic lipase, was demonstrated (Chapter 2). The aim of this chapter was to determine TPC in Kuwaiti seaweeds and identify any association between pancreatic lipase inhibition and TPC in Kuwait seaweeds. In addition, this study also set out to evaluate any potential seasonal variation and identify the optimal approach for phenolic extraction. It was identified that the optimal approach for TPC in terms of both cost and efficacy was to employ food-grade solvents such as ethanol and WE (Cotas *et al.*, 2020). Additionally, it was observed that the TPC was higher in Kuwait seaweed when extracted in November, confirming seasonal variations. There was no significant correlation between TPC in Kuwaiti seaweed samples (homogenate, WE extract and ethanol pellet remaining after 100% or 50% EE) and its IC50 of pancreatic lipase inhibition.

5.5.1 TPC of Seaweed Homogenate

This study compared the TPC in green, brown, and red Kuwait seaweeds of the same species, revealing the highest TPC in brown seaweeds, followed by red and green seaweeds. Specifically, the seaweed homogenate from the brown seaweed *P. boergesenii* exhibited the highest TPC, followed by the red seaweed *G. Pusillum.* The data presented here demonstrate significant variation in TPC across samples of the same seaweed species. These findings indicate that seaweed's polyphenol content is inherently linked to the chemical composition of each individual seaweed. This may include linkages, functional groups and size despite sharing taxonomic classification (Duan *et al.*, 2023). These results support earlier work by Machu *et al.* (2015), who identified higher TPC in brown seaweed when compared to red and green seaweeds of the same species. One possible explanation for the high TPC in brown seaweed may be linked to the presence of phlorotannins found abundantly, particularly in brown seaweeds (Maheswari and Babu, 2022).

Phlorotannins, previously shown to be present in high concentrations in brown seaweeds, play a crucial role in the growth and development of brown algae, operating at both cellular and organismal levels (Gomez and Huovinen, 2020, p. 372). These findings help identify seaweeds with the highest TPC, potentially conferring enhanced biological properties compared to other seaweeds and polyphenol-containing foods. Moving forward, it is essential to identify which polyphenols are present in brown seaweed and understand the potential health benefits, opening avenues for pharmaceutical and clinical applications.

5.5.2 TPC Present in seaweed WE

This study revealed a significant difference in TPC across seaweed species present in WE. The observed variations in TPC suggest that there may be compositional and structural variations between seaweed species, leading to variations in polyphenol solubility (Fellah *et al.*, 2017; Ford *et al.*, 2019). The highest TPC was identified in the WE extract from the brown seaweed *S. trinodis* N*,* extracted in November, followed by *P. boergesenii*. These findings lend further support to the idea that brown seaweed contains a higher phenolic content than other seaweed species (Habeebullah and Jacobsen, 2013). In a similar manner, it was also observed that the highest level of TPC was found in brown seaweed extracts using WE and 70% acetone, compared to the same extracts from red and green seaweeds (Wang, Jónsdóttir and Ólafsdóttir, 2009).

Here, the TPC in the seaweed homogenate, WE, and the EP were compared. The TPC in the seaweeds was observed to be constantly higher in the WE compared to the other methods. These findings confirm the earlier work of López *et al.* (2011), who used the Folin-Ciocalteu assay to compare the TPC present in ethanol crude extracts with water, water/methanol in a 1/1 ratio and methanol alone in the brown seaweed *Stypocaulon scoparium.* The authors reported that the highest TPC was present following the aqueous extraction, confirming the data presented here (López *et al.*, 2011). Importantly, López *et al.* (2011) also confirmed that the TPC present in the aqueous solution also had the highest antioxidant activity.

The larger TPC in aqueous extracts is likely related to the structure and properties of the polyphenol compounds present in seaweed. Consequently, the utilisation of

solvents is likely to release a higher concentration of polyphenols from the seaweed and increase the solubility of polyphenolic compounds in solution (Berk, 2018, p. 289). In a recent study, Li *et al.* (2017) demonstrated that the phlorotannin content extracted from brown seaweed increased linearly in relation to the polarity of the solvent (the ratio of WE to ethanol). A potential explanation for this may be that the WE-soluble polyphenols derived from the Kuwaiti seaweed exhibit a significant degree of polarity (Wang, Jónsdóttir and Ólafsdóttir, 2009). These findings provide valuable insights into the optimal extraction methods concerning polyphenol content in Kuwait seaweeds.

5.5.3 TPC Present in seaweed EP

The extraction of polyphenols from plant materials has received considerable interest to maximise the polyphenol extraction yield (López *et al.*, 2011). Interestingly, the most common approach for extracting the TPC from whole seaweeds is using organic solvents and binary mixtures. However, the specific extraction method used may vary depending on the type of seaweed species (Sadeghi *et al.*, 2024). In addition to the yield of polyphenols extraction, both of which have been shown to be dependent upon the solvent characteristics. For example, aqueous acetone has been found to be optimal for high molecular weight flavonoids, while methanol may be more suitable for lower molecular weight polyphenols (Mojzer *et al.*, 2016). The present study measured the TPC in the EP. The highest TPC was present in the red seaweed *G. Pusillum*, followed by the brown seaweed *P. boergesenii*. The lowest TPC levels were observed in the green seaweeds. The efficacy of polyphenol extraction using ethanol and organic solvents may be attributed to their ability to precipitate proteins and extract less polar polyphenols (Habeebullah and Jacobsen, 2013; Wang *et al.*, 2012). These findings suggest that the TPC present in the EP may be composed of less polar (hydrophobic) polyphenols (Heffernan *et al.*, 2015). Thus, it is possible to assume that the tested Kuwaiti seaweeds contain low contents of these types of polyphenols. Moving forward, it is crucial to determine the types and concentrations of polyphenols in Kuwaiti seaweed.

5.5.4 Seasonal Variations in TPC from *S. trinodis*

The diversity of seaweeds is primarily influenced by geographical location, given different growing conditions, such as sunlight, temperature, salinity, and nutrient

availability (Park *et al.*, 2023). Seasonal variations, such as summer vs. winter, have also been shown to impact the polyphenol content in different seaweeds (Cotas *et al.*, 2020; Fellah *et al.*, 2017; Kumar, Sahoo and Levine, 2015; Stengel, Connan and Popper, 2011). This study identified a temporal effect in TPC variation in the WE from the brown seaweed *S. trinodis*, where a higher TPC was present in seaweed collected in November (Autumn) 2020 when compared with April (Spring) 2021. Consistent with our findings, Pandey *et al.* (2022) reported that the TPC was the highest in autumn compared to spring in the brown seaweeds *F. Vesiculosus* and *Pelvetia canaliculate* extracted from the coastline of Norway. Roleda *et al.* (2019) also observed similar findings to this study, reporting higher TPC in the brown seaweeds *Alaria esculenta* and *Saccharina latissimi* when extracted from the coastlines of Norway and France in winter compared to spring.

The temporal effect in TPC observed here is likely to be multifactorial, influenced by external seasonal climate changes (Kamal *et al.*, 2023). The Kuwait coastline is no different to other coastlines worldwide, experiencing varying seasonal changes. This is evidenced by the work of Devlin *et al.* (2019), who observed lower temperatures and higher salinity in November compared to April. Seasonal variations clearly have a significant impact on seaweed species, and different seaweed species are likely to respond differently to a myriad of abiotic and biotic fluctuations. Two of the key features identified previously by Devlin *et al.* (2019), specifically related to Kuwait seaweed, were temperature and salinity, which are likely to influence the seaweed's physical structure, chemical composition and TPC. For example, seasonal variations have been shown to influence the biochemical constituents of seaweeds, including amino acids, minerals, fatty acids, and sugars (Vinuganesh *et al.*, 2022). Furthermore, seaweeds have been shown to respond to different stressors by increasing their levels of antioxidant enzymes and non-enzymatic molecules such as polyphenols (Vinuganesh *et al.*, 2022). This is illustrated in a previous paper by Stengel, Connan and Popper (2011), who reported an inverse relationship between water temperature and polyphenol content. The same authors reported that the TPC extracted from *Ascophyllum nodosum* and *F. vesiculosus* from Ireland was reduced in the presence of lower salinity (Connan and Stengel, 2011). These data provide insight into conditions that may be optimal for maximising polyphenol yield. In addition to identifying the optimal timing for seaweed harvest, it is crucial to ensure that these seaweeds remain

safe for consumption throughout the year and that the extracted polyphenols retain their biological activities at varying times, aligning with the highest TPC levels.

5.5.5 TPC and Pancreatic Lipase Inhibition

Seaweed polyphenols have been reported to inhibit pancreatic lipase activity (Shannon, Conlon and Hayes, 2023). Therefore, this study examined the correlation between TPC in seaweed samples and their ability to inhibit pancreatic lipase activity. The seaweed samples used in this study included homogenates, WE and the EP.

The seaweed homogenate presented all bioactive compounds (Chater *et al.*, 2016). Whereas the WE and the EP have been reported to contain water-soluble and ethanol-insoluble compounds, respectively (Holdt and Kraan, 2011). This study revealed no correlation between TPC in Kuwaiti seaweed samples (homogenate, WE and EP) and their inhibitory effect on pancreatic lipase activity (chapter 2). A possible explanation for this might be that the seaweed and its extracts inhibit pancreatic lipase through a combination of multiple compounds that work together to create additive effects to each other. Consistent with our findings, Chater *et al.* (2016) reported that the inhibition of pancreatic lipase might be attributed to the combined effect of seaweed bioactive compounds (polyphenols and polysaccharides).

5.6 Conclusion

The purpose of this chapter was to extract and quantify the polyphenolic compounds in Kuwaiti seaweed. In this chapter, Kuwaiti seaweeds were demonstrated to contain high concentrations of polyphenolic compounds. Temporal variation in the TPC of the brown seaweed *S. trinodis* was also observed. Based on the TPC in the WE of *S. trinodis*, it was identified that Autumn, especially November, may be an optimal time to harvest seaweed to maximise polyphenol yield, which is likely to be influenced by the lower water temperatures and higher salinity. Further work research is required to measure TPC levels in Kuwait seaweed harvested throughout the year to confirm whether November is the optimal time point.

This study also demonstrated that seaweed species and extraction methods significantly affect the TPC levels, with *P. boergesenii* and *G. Pusillum* exhibiting the highest TPC in their homogenates and crude extracts. The observed variance in TPC

across seaweed species suggests that each seaweed likely harbours species-specific polyphenolic compounds with distinctive structural features. These findings contribute to the existing literature on the associations between solvent selection for extraction and the selectivity of phenolic compounds. This study provides insight into the possibility that highly polar phenolic compounds may be present in Kuwaiti seaweeds, primarily since WE has proven to be an efficient polyphenol extractant. The data presented in this chapter demonstrate that Kuwaiti seaweed contains significant concentrations of phenolic content. Although further work is required, these findings may contribute to increasing the economic value of seaweeds, either as a dietary component or a natural bioactive ingredient, with potential benefits for improving human health through pancreatic lipase inhibition or antioxidant effects.

Chapter 6 Final Discussion and Suggested Future Works

Kuwait has been classified as a country with the highest obesity in the world (Samara, Andersen and Aro, 2019; WHO, 2020). The prevalence of obesity has been increased twofold from 1975 to 2016, with estimates reaching as high as 46 and 33% in females and males, respectively (Samara, Andersen and Aro, 2019; WHO, 2016). Consequently, the incidence of coronary heart diseases, diabetes, osteoarthritis and certain type of cancer are dramatically increased (WHO, 2014). There are many factors that has been associated with the alarming prevalence of obesity among Kuwaiti adults and children (i.e. economic, social, environmental as well as, genetic) (Musaiger *et al.*, 2012). Despite different ways for tackling this global health crises, there are serious side effects and limitations of current treatment options (Krentz, Fujioka and Hompesch, 2016). Alternative treatments have been intensively researched to overcome these side effects. Recently studies have focused on seaweeds (macroalgae) as alternative natural sources for modulating digestive enzymes activity and treating obesity (Chater *et al.*, 2015; Wan-Loy and Siew-Moi, 2016).

Seaweed contains novel and unique micro- and macronutrients compared with other terrestrial food sources that give its high nutritional and low caloric values. Macroalgae is a good source of dietary fibres, minerals, antioxidants and vitamins. Besides, seaweed is a good source of essentials amino acids, protein, polysaccharides, polyunsaturated fatty acids, polyphenols and carotenoids (Shannon and Abu-Ghannam, 2019). Seaweed biochemical composition and level and type of bioactive compounds are species- specific and also depends on external spatial and temporal factors (Cherry *et al.*, 2019). Geographic location and seasonal changes, such as sunlight exposure, temperature, and salinity levels, affect different seaweed chemical compositions (polysaccharides, phenols and other bioactive compounds) and therefore effects seaweed commercial value and utilization (Kamal *et al.*, 2023). Seaweeds must adapt to these challenging climates and seasonal changes, leading to structural modifications that can affect their functionality. Seaweed lives in a changing environment, which includes strong waves and rising and falling tides, so seaweed develops a thick cell wall to resist these fluctuations. In addition, some seaweeds exist in an environment where temperatures and UV rays change, so

seaweeds need to increase the rate of production of protective pigments and antioxidants to adapt to living in this environment (Konstantin *et al.*, 2023).

Various seaweeds from different regions have demonstrated similar potential in previous studies, including those from Japan (Bitou *et al.*, 1999), Tunisia (Ben Rebah *et al.*, 2008), Korea (Kim *et al.*, 2012; Eom *et al.*, 2013; Jung *et al.*, 2013), Malaysia (Balasubramaniam *et al.*, 2013), Scotland (Chater *et al.*, 2016; Austin *et al.*, 2018), China (Lu, Gu and Yu, 2024; Zhang *et al.*, 2021), Portugal (Catarino *et al.*, 2019) and Australie (Shannon, Conlon and Hayes, 2023). However, to the best of our knowledge, no studies have been conducted to date investigating the potential of Kuwaiti seaweed and its extracts as pancreatic lipase inhibitors

The aim of this research was to investigate the biological activities of Kuwaiti seaweeds and their aqueous and ethanolic extracts as a potential anti-obesity agent. To achieve this aim, there were four main objectives, each of which was separated into different chapters. Firstly, pancreatic lipase activity with Kuwaiti seaweeds and their extracts was determined using an in-vitro olive oil turbidity assay. Secondly, the seaweeds with the highest inhibitory effect on pancreatic lipase were used to determine their mode of inhibition using Micheales-Menten kinetics and Lineweaver-Burk plots. Then, these seaweeds were further evaluated for their ability to modulate fat digestion under conditions resembling physiological digestion using a synthetic model gut system. Finally, total phenolic content (TPC) in Kuwaiti seaweeds and their extracts was determined to see any correlation between TPC in these seaweeds and their inhibitory effect on pancreatic lipase activity.

This study emphasizes the significant ability of Kuwaiti seaweeds and their extracts to inhibit pancreatic lipase activity, providing the possibility for further investigations. Since the previous chapters have addressed the key results and discussed them in depth related to the literature, this chapter aimed to offer an overview of the research with a summary of each chapter’s results, as shown in Table 6. 1. In addition, this chapter discussed limitations encountered throughout the study and proposed possible future works.

Table 6. 1 Summary of key findings from each chapter

Seaweed	Tested Extracts	**Chapter 2** IC50 (mg/ml)	**Chapter 3** Mode of Inhibition	**Chapter 4** In-vitro Fat Digestion	**Chapter 5** TPC% (mg PGE/g dry seaweed)
Green	*C. sericioides* homogenate	0.38	Non-competitive inhibition	No significant changes	0.2 (± 0.06)
	C. sericioides EP	1.16	Mixed-type inhibition	No significant changes	0.2 (± 0.05)
	C. papillatum WE	1.07	Mixed-type inhibition	No significant changes	0.9 (± 0.3)
	C. papillatum EP	0.1	Mixed-type inhibition	No significant changes	0.3 (± 0.03)
Brown	*S. trinodis* N homogenate	0.75	Mixed-type inhibition	No significant changes	0.4 (± 0.08)
	S. trinodis N WE	0.15	Mixed-type inhibition	No significant changes	4.0 (± 0.2)
	S. trinodis A homogenate	1.34	Non-competitive inhibition	No significant changes	0.5 (± 0.12)
	S. trinodis A WE	0.27	Non-competitive inhibition	No significant changes	1.4 (± 0.1)
	C. Sinuosa homogenate	1.51	Mixed-type inhibition	No significant changes	0.4 (± 0.09)
	I. stellata homogenate	0.87	Mixed-type inhibition	No significant changes	0.5 (± 0.14)
	P. boergesenii homogenate	1.6	Mixed-type inhibition	No significant changes	0.8 (± 0.14)
	P. boergesenii WE	2.46	Mixed-type inhibition	No significant changes	2.6 (± 0.6)
	P. boergesenii EP	2.71	Mixed-type inhibition	No significant changes	0.5 (± 0.04)
Red	*G. Pusillum* homogenate	1.03	Mixed-type inhibition	No significant changes	0.6 (± 0.06)
	G. Pusillum EP	2.03	Mixed-type inhibition	No significant changes	0.5 (± 0.07)

IC50 : Half maximal inhibitory concentration; C. sericioides: Cladophora sericioides; C. papillatum: Codium papillatum; S. trinodis N: Sirophysalis trinodis collected in November 2020; S. trinodis A: Sirophysalis trinodis collected in April 2021; C. Sinuosa: Colpomenia Sinuosa; I. stellata: Iyengaria stellata; P. boergesenii i: Padina boergesenii; G. Pusillum; Gelidium Pusillu; EP: ethanol pellet remaning after ethanol extarct; WE: WE extract; PGE: phloroglucinol equivalent.

6.1 Thesis Overview

6.1.1 Anti-lipase Potential of Nine Kuwait Seaweeds and their Aqueous and Organic Extracts

Chapter 2 explained different seaweed species used and the extraction process and yields. Briefly, seven Kuwaiti seaweeds were collected from the Kuwaiti coastline between 2019 and 2022. The green seaweeds were *C. sericioides* and *C. papillatum*. The brown seaweeds were *S. trinodis* (collected in November 2020 and April 2021), *P. boergesenii*, *C. sinuosa* and *I. stellata*. The red seaweed was *G. Pusillum*. These seaweeds were used in Chapter 2 as homogenates, WE, EE and EP. Therefore 32 samples were investigated for their inhibitory effect on pancreatic lipase activity using an in-vitro olive oil turbidity assay. The seaweeds collected in 2019 and 2021 were subjected to absolute (100%) EE. Due to the lack of inhibitory effect, the seaweeds collected in 2022 were subjected to 50% EE to modify the extraction and inhibition effect. Then, the seaweed samples with the highest inhibitory effect on pancreatic lipase were further examined in the next chapters (Chapter 3, Chapter 4 and Chapter 5). In addition, the seasonal effect on brown seaweed *S. trinodis* collected in November 2020 and April 2023 was determined in each chapter.

Chapter 2 showed various levels of pancreatic lipase inhibition ranging from 6-99%. Significant inhibition of pancreatic lipase activity was observed with Kuwaiti seaweed homogenates, WE and EP. However, this study revealed that EE from Kuwaiti seaweed failed to induce any significant decrease in pancreatic lipase activity. Fifteen seaweed samples significantly inhibited pancreatic lipase were all seaweed homogenates, including *C. sericioides*, *S. trinodis* N, *S. trinodis* A, *P.* boergesenii, *C. sinuosa*, *I. stellata* and *G. Pusillum*, the WE, including *C. papillatum*, *S. trinodis* (collected in November 2020 and April 2021) and *P. boergesenii*, and EP, including *C. sericioides*, *C. papillatum*, *P. boergesenii* and *G. Pusillum*. In addition, The IC50 calculated from green, brown and red seaweed species and its extracts values were between 0.1 and 2.71 mg/ml (Table 6. 1). A possible explanation for the variances in inhibitory effect between each seaweed and its extract could be the unique phytochemical components found in each seaweed sample (Ferdouse *et al.*, 2018). The seaweed homogenate may contain a wide range of phytochemicals. The WE extracts may contain only water-

soluble components, such as polysaccharides and polyphenols (Austin *et al.*, 2018). Similarly, the EP may exclusively comprise ethanol-insoluble components, such as insoluble polysaccharides like alginate and fucoidan (Chater *et al.*, 2016). This chapter highlights the possibility of an additive effect between seaweed bioactive compounds to inhibit pancreatic lipase as the samples used either contain all the bioactive compounds as in used homogenates or contain a variety of bioactive compounds based on their solubility and extraction efficiency with used solvents. The findings of this chapter underscore the significant ability of Kuwaiti seaweed to inhibit pancreatic lipase activity, thus potentially providing natural bioactive compounds suitable for use in anti-obesity therapies.

6.1.2 Determination of Kinetics Parameters of Kuwait Seaweeds and their Aqueous and Organic Extracts for the Inhibition of Pancreatic Lipase

Chapter 3 determined the mode of inhibition of pancreatic lipase in 15 Kuwaiti seaweed samples, which were detailed previously, using Micheales-Menten kinetics and Lineweaver-Burk plots. According to the Kinetic parameters (Micheales-Menten kinetics and Lineweaver-Burk plots), the addition of 15 Kuwaiti seaweed samples in the reaction mixtures allowed the pancreatic lipase catalysed reaction to approach the Vmax at lower value than the Vmax of the control reaction, which did not contain seaweed. All Kuwaiti seaweeds and their aqueous and ethanolic pellets remaining after ethanol extraction exhibited a mixed-type inhibition of pancreatic lipase, except homogenate from green seaweed *C. sericioides* and homogenate and WE extract from brown seaweed *S. trinodis* A (collected in April), which exhibited a non-competitive inhibition (Table 6. 1). Seaweed homogenates contain a wide range of phytochemical compounds, with each seaweed species possessing its own distinct phytochemical profile (Leandro *et al.*, 2020).

In addition, this chapter examines the seasonal variations in the mode of inhibition of pancreatic lipase in homogenate and WE derived from brown seaweed *S. trinodis* collected in November 2020 and April 2021. The homogenate and WE derived from brown seaweed *S. trinodis* collected in November exhibited a mixed-type inhibition. However, the homogenate and WE derived from brown seaweed *S. trinodis* collected in April exhibited non-competitive inhibition. It has been shown that seasonal

variations, such as sunlight, temperature and salinity, have a major effect on seaweed physical and chemical properties (Ptak *et al.*, 2021; Yesilova, Balkis and Taskin, 2017). The Kuwait coastline has seasonal fluctuations similar to those around the world. Kuwait's coastal temperatures were decreased and salinities were increased in November compared to April (Devlin *et al.*, 2019). Therefore, the difference in the mode of inhibition between *S. trinodis* collected in November and *S. trinodis* collected in April is likely due to the seasonal variations, which impact the structure and function of seaweed, as detailed in 2.5.4.

It has previously been demonstrated that seaweed-derived bioactive components, including polysaccharides (Zhang *et al.*, 2021), polyphenols (Shannon, Conlon and Hayes, 2023) and carotenoids (Kurniawan *et al.*, 2023), efficiently inhibit pancreatic lipase activity. To date, there remains a scarcity of research definitively identifying the kinetic parameters of seaweeds. Caulerpenyne, a chemical derived from the Japanese green seaweed *Caulerpa taxifolia*, has been investigated as a competitive inhibitor of pancreatic lipase by the double reciprocal Lineweaver-Burk plot of reaction rate vs. substrate (triolein) concentrations (Bitou *et al.*, 1999). Dieckole, a polyphenolic chemical derived from the Korean brown seaweed *Eisenia bicyclis*, acts as a non-competitive inhibitor of pancreatic lipase using the double reciprocal Lineweaver-Burk plot of reaction rate vs. substrate (p-nitrophenyl butyrate (pNPB)) concentrations (Jung *et al.*, 2013). Finally, fucoidan, a sulfated polysaccharide derived from the Chinese brown seaweed *Laminaria japonica*, has been recognised as a mixed inhibitor using the double reciprocal Lineweaver-Burk plot of reaction rate vs. substrate (4-nitrophenyl laurate (pNPL)) concentrations (Lu, Gu and Yu, 2024).

However, several studies have examined the inhibitory effects of plant extracts on pancreatic lipase activity. A recent study by Khatchapuridze, Ploeger and Gulua (2023) revealed that Mukuzani dry red wine from Georgia showed a mixed-type inhibition through the use of the double reciprocal Lineweaver-Burk plot of reaction rate vs. olive oil concentrations, with a K_{ia} value lower than the K_{ib} value. It is noteworthy that the aqueous and ethyl-acetate extracts from the Mukuzani wine showed uncompetitive inhibition (Khatchapuridze, Ploeger and Gulua, 2023). The variation observed in the mode of inhibition may be attributed to active compounds in the dried wine and in the aqueous and ethyl-acetate extracts, likely due to the presence of diverse compounds

with varying affinities towards the free enzyme or the enzyme-substrate complex (Khatchapuridze, Ploeger and Gulua, 2023).

The findings presented in Chapter 3 suggest that the components within these seaweed homogenates have the potential to influence the inhibit properties of pancreatic lipase activity through distinct mechanisms, as evidenced by variance in kinetics data. This chapter provides insight into the regulation and inhibition of pancreatic lipase by Kuwaiti seaweed, emphasizing natural bioactive compounds suitable for managing weight and metabolic health. Therefore, this can add an economical value to Kuwaiti seaweed.

6.1.3 The Effect of Kuwaiti Seaweed and their Aqueous and Organic Extracts on in-vitro Fat Digestion Using a Model Gut

Chapter 4 investigated the effect of 15 Kuwaiti seaweed samples, which were detailed previously, on fat digestion using an in-vitro model gut that replicates digestion in the upper GIT. In-vitro digestion models provide a simplified and controlled system to reliably replicate the intricate dynamics of fat digestion. The primary advantage of using a model gut system lies in its reproducibility and ability to mitigate common challenges often encountered in human studies, such as high cost, time-intensive procedures, and ethical considerations (Mulet-Cabero *et al.*, 2020). Therefore, the objective of this research was to examine the effect of the Kuwaiti seaweed samples and their extracts, identified from Chapter 2, on fat digestion within a model gut.

In this study, free glycerol, released from fat substrate (olive oil), served as a marker of fat digestion. This chapter demonstrates that the model gut accurately simulates fat digestion in both the gastric and intestinal phases. There was a negligible quantity of free glycerol in the gastric phase (0-60 minutes). However, the content of released free glycerol increased during the intestinal phase (61-180 minutes). These findings are consistent with the physiological process of fat digestion and absorption in the GIT (Chater *et al.*, 2016; Omer and Chiodi, 2024; Xu *et al.*, 2021).

The primary finding of Chapter 4 was that seaweed homogenates, WE and EP did not inhibit fat digestion, as shown in Table 6. 1, despite the earlier findings outlined in Chapter 2. This lack of significant reduction may, in part, be attributed to the complexity of the model gut compared to the olive oil assay used in Chapter 2. The model gut

simulates mechanical and biomedical conditions similar to in-vivo digestion, including diverse enzymes, variable pH levels, bile salts and continual stirring. It is possible that, individually or collectively, these factors may have impaired the efficacy of Kuwait seaweeds and their extracts in inhibiting pancreatic lipase activity. Previous studies indicate that polyphenol can enter the lower GIT by forming complexes with poor digestion, when combined with different dietary fibres (Saura-Calixto and Díaz-Rubio, 2007). If this is the case in the Kuwait seaweed samples, this may confer advantages for lower GIT health. For example, the fermentation of dietary fibres in the lower GIT may reduce the risk of colon pathophysiology and enhance overall colon health (Ding *et al.*, 2020). The gel formation or precipitation utilising Kuwait seaweeds in this study may elucidate the absence of fat digestion inhibition. Although not demonstrated in the present study, further research is necessary to study the gel-forming properties and structural characteristics of Kuwait seaweeds. Moreover, investigating Kuwait seaweeds in diverse pH conditions is crucial to determine whether changes in solution pH influence the noted absence of fat digestion inhibition observed in this work. These investigations may reveal new therapeutic properties of Kuwait seaweeds, similar to those observed with Gaviscon and alginates.

6.1.4 Differences in Total Polyphenol Content of Kuwait Seaweed Based on Seaweed Species and Seasonal Variation.

Chapter 5 quantified TPC in 15 Kuwaiti seaweed samples, which were detailed previously. In addition, *F. vesiculosus*, which is well established for its abundant polyphenolic content (Catarino *et al.*, 2019), were used to evaluate tested Kuwaiti seaweed TPC. In this chapter, variances in TPC were observed across tested seaweed samples (Table 6. 1). The variation in TPC across seaweed species indicates that each seaweed likely harbours species-specific polyphenolic compounds with distinctive structural features (Fellah *et al.*, 2017). Several seaweeds have been shown to contain different polyphenolic compounds, including flavonoids, bromophenols, phenolic terpenoids, mycosporine-like amino acids, and non-typical phenolic compounds (Cotas *et al.*, 2020; Zhao *et al.*, 2023). This study also revealed that seaweed species and extraction methods significantly affect the TPC levels. The highest TPC was observed in the WE from the brown seaweed *S. trinodis* N, collected in November, followed by *P. boergesenii*. These findings lend further support to the idea that brown

seaweed contains a higher phenolic content than other seaweed (Habeebullah and Jacobsen, 2013). In addition, the larger TPC in aqueous extracts (WE) is likely related to the structure and properties of the polyphenol compounds present in seaweed. A potential explanation for this may be that the WE-soluble polyphenols derived from the Kuwaiti seaweed exhibit a significant degree of polarity (Li *et al.*, 2017).

Temporal variation in the TPC of the brown seaweed *S. trinodis* was also observed. Based on the TPC in the WE of *S. trinodis*, it was identified that Autumn, especially November, may be an optimal time to harvest seaweed to maximise polyphenol yield. In accordance with our findings, Pandey *et al.* (2022) reported that the TPC was the highest in autumn compared to spring in the brown seaweeds *F. Vesiculosus* and *Pelvetia canaliculate* extracted from the coastline of Norway. Roleda *et al.* (2019) also observed similar findings, reporting higher TPC in the brown seaweeds *Alaria esculenta* and *Saccharina latissimi* when harvested from the coastlines of Norway and France in winter compared to spring. These results provide insight into the optimal timing for seaweed harvest with the highest maximum TPC levels.

This study revealed no correlation between TPC in 15 Kuwaiti seaweed samples (homogenate, WE and EP) and its IC50 of pancreatic lipase inhibition. A possible explanation for this might be that the seaweed and its extracts inhibit pancreatic lipase through a combination of multiple compounds that work together to create additive effects to each other. Consistent with our findings, Chater *et al.* (2016) reported that the inhibition of pancreatic lipase might be attributed to the additive effect of seaweed bioactive compounds (polyphenols and polysaccharides).

6.2 Limitation

The research has encountered some limitations during this study. Regarding external conditions, COVID-19 and its unavoidable lost time, interruptions and struggles to provide Kuwaiti seaweeds were among the biggest obstacles. Regarding samples, some seaweed was not ground completely to powder, which might reduce the efficiency of extracting more active compounds, thus reducing their effect on enzyme inhibition. Kuwaiti seaweeds *Ulva intestinalis* and *Colpomenia sinuosa*, which were both collected from the Salmiya coastline in February 2020 and March 2021, respectively, were excluded from the middle of this study because of their limited quantity and abruptly vanished from the laboratory, respectively. This all happened before 2022 when Kuwait University provided me with more seaweed.

6.3 Suggested Future Work

Further research with advanced analytical techniques must elucidate which bioactive components are responsible for pancreatic lipase inhibition. High-Performance Liquid Chromatography (HPLC) will be used to separate the bioactive components. In addition, Mass Spectrometry (MS) will be used to determine the structure of these bioactive components.

Furthermore, with the same seaweeds, research could be performed using different solvent combinations and different extraction methods to improve bioactive extraction. In addition, the extraction conditions such as temperature and pH, could be changed to optimize the extraction. Other Kuwaiti seaweed species may also be investigated in order to enhance the commercial value of seaweeds as either a food supplement or a natural bioactive. Moreover, the influence of seaweed obtained from different seasons on pancreatic lipase can be conducted to discover an optimal time to harvest seaweed.

Further in-vitro digestion research could be tried with olive oil that is free from fatty acids, to clarify the possibility of seaweed bioactive compounds interacting with free fatty acids and hence preventing them from inhibiting pancreatic lipase. Further investigation can be done to indicate any interference by seaweed components in the glycerol assay.

To hit two targets with one arrow, lab work with the same seaweed samples, extracts and concentrations can be done to test their inhibitory impact on other digestive enzymes such as α-amylase and α-glucosidase.

References

Abdelaziz, H. M. M., Hamdi, M., Mahfouz, E. M. E., Zidan, S. G. E.-d. I., Hatata, A. and Elfadl, H. G. A. (2025) 'Epicardial Fat Volume Assessment via Cardiac Computed Tomography: A Novel Indicator in Heart Failure with Preserved Ejection Fraction', *Egyptian Journal of Radiology and Nuclear Medicine,* 56(1), pp. 10-20.

Abidov, M., Ramazanov, Z., Seifulla, R. and Grachev, S. (2010) 'The Effects of Xanthigen™ in the Weight Management of Obese Premenopausal Women with Non-alcoholic Fatty Liver Disease and Normal Liver Fat', *Diabetes, Obesity & Metabolism*, 12(1), pp. 72-81, Available at: Oxford, UK: Blackwell Publishing Ltd. DOI: 10.1111/j.1463-1326.2009.01132.x.

Afonso, C., Matos, J., Guarda, I., Gomes-Bispo, A., Gomes, R., Cardoso, C., Gueifão, S., Delgado, I., Coelho, I., Castanheira, I. and Bandarra, N. M. (2021) 'Bioactive and Nutritional Potential of Alaria esculenta and Saccharina latissima', *Journal of Applied Phycology,* 33(1), pp. 501-513.

Ahmed, M. (2022) 'Functional, Diagnostic and Therapeutic Aspects of Bile', *Clinical and Experimental Gastroenterology,* 15, pp. 105-120.

Ajayan, K. V., Shamna, P., Haritha, D. S. and Harilal, C. C. (2021) 'Fatty Acid Profile and Metal Composition of Seaweeds from Three Locations of the

Continental Shelf of Kerala Coast, India', *Regional Studies in Marine Science*, 45, pp. 1-9, Available at: Elsevier B.V. DOI: 10.1016/j.rsma.2021.101864.

AL Nohair, S. (2014) 'Obesity in Gulf Countries', *International Journal of Health Sciences,* 8(1), pp. 79-83.

Alabdulghani, E., Elsammak, A. and Sarawi, M. (2013) 'Environmental Assessment of Kuwait Bay: An Integrated Approach', *Journal of Coastal Conservation*, 17(3), pp. 445-462, Available at: Dordrecht: Springer. DOI: 10.1007/s11852-013-0242-7.

Aladilah, H., Alsharrah, T. K., Albader, D., Ebel, R., Küpper, F. C. and Kumari, P. (2021) 'Assessment of Arabian Gulf Seaweeds from Kuwait as Sources of Nutritionally Important Polyunsaturated Fatty Acids (PUFAs)', *Foods,* 10(10), pp. 2442-2458.

Alara, O. R., Abdurahman, N. H. and Ukaegbu, C. I. (2021) 'Extraction of Phenolic Compounds: A Review', *Current Research in Food Science,* 4, pp. 200-214.

Alfaris, N. (2021) 'Management of Obesity in Saudi Arabia During the Era of COVID-19: A Clash of Two Pandemics', *Obesity (Silver Spring, Md.)*, 29(1), pp. 18-18, Available at: United States: Blackwell Publishing Ltd. DOI: 10.1002/oby.23055.

Alkandari, A., Alarouj, M., Elkum, N., Sharma, P., Devarajan, S., Abu-Farha, M., Al-Mulla, F., Tuomilehto, J. and Bennakhi, A. (2020) 'Adult Diabetes and Prediabetes

Prevalence in Kuwait: Data from the Cross-Sectional Kuwait Diabetes Epidemiology Program', *Journal of Clinical Medicine,* 9(11), pp. 3420.

Allaert, F.-A., Demais, H. and Collén, P. N. (2018) 'A randomized controlled double-blind clinical trial comparing versus placebo the effect of an edible algal extract (Ulva Lactuca) on the component of depression in healthy volunteers with anhedonia', *BMC psychiatry,* 18, pp. 1-10.

Almarri, F., Al Sabah, S., Al Haddad, E. and Vaz, J. D. (2017) 'A Call for More Research from the Arabian Gulf', *Obesity Surgery*, 27(8), pp. 2034-2043, Available at: New York: Springer US. DOI: 10.1007/s11695-017-2588-7.

Almutairi, N., Abahussain, A. and Elbattay, A. (2014) 'Spatial and temporal Characterizations of Water Quality in Kuwait Bay', *Marine Pollution Bulletin,* 83(1), pp. 127-131.

Alsabah, A., Al Sabah, S., Al-Sabah, S., Al-Serri, A., Al Haddad, E. and Renno, W. M. (2017) 'Investigating Factors Involved in Post Laparoscopic Sleeve Gastrectomy (LSG) Neuropathy', *Obesity Surgery,* 27, pp. 1271-1276.

AlSarawi, H. A., Jha, A. N., AlSarawi, M. A. and Lyons, B. P. (2015) 'Historic and Contemporary Contamination in the Marine Environment of Kuwait: an Overview', *Marine Pollution Bulletin,* 100(2), pp. 621-628.

Althumiri, N. A., BinDhim, N. F., Aldabaeab, A. E., AlMousa, N., Aljabbary, R. A. and Alumran, A. (2024) 'Comparing Lifestyle and Behavior of Post-Bariatric Surgery and Participants with Obesity: A Community-Based Cross-Sectional Study', *Diabetes, Metabolic Syndrome and Obesity,* 17, pp. 31-44.

AlYamani, F. Y., Alkandari, M., Polikarpov, I. and Grintsov, V. (2019) *Field Guide Oforder Amphipoda Malacostraca, Crustacea) of Kuwait.* first edn. Kuwait Kuwait Institute for Scientific Research

AlYamani, F. Y., Polikarpov, I., AlGhunaim, A. and Mikhaylova, T. (2014) *Field Guide of Marine Macroalgae (Chlorophyta, Rhodophyta, Phaeophyceae) of Kuwait.* First Edition edn. Kuwait: Kuwait Institute for Scientific Research.

Angrisani, L., Santonicola, A., Iovino, P., Vitiello, A., Zundel, N., Buchwald, H. and Scopinaro, N. (2017) 'Bariatric Surgery and Endoluminal Procedures: IFSO Worldwide Survey 2014', *Obesity Surgery,* 27, pp. 2279-2289.

Astorga-España, M. S., Mansilla, A., Ojeda, J., Marambio, J., Rosenfeld, S., Mendez, F., Rodriguez, J. P. and Ocaranza, P. (2017) 'Nutritional Properties of Dishes Prepared with Sub-Antarctic Macroalgae—An Opportunity for Healthy Eating', *Journal of Applied Phycology,* 29, pp. 2399-2406.

Austin, C., Stewart, D., Allwood, J. W. and McDougall, G. J. (2018) 'Extracts from the Edible Seaweed,: Ascophyllum nodosum, Inhibit Lipase Activity In-vitro: Contributions of Phenolic and Polysaccharide Components', *Food & Function*, 9(1), pp. 502-510, Available at: Cambridge: Royal Soc Chemistry. DOI: 10.1039/c7fo01690e.

Balasubramaniam, V., Mustar, S., Mustafa Khalid, N., Abd Rashed, A., Mohd Noh, M. F., Wilcox, M. D., Chater, P. I., Brownlee, I. A. and Pearson, J. P. (2013) 'Inhibitory Activities of Three Malaysian Edible Seaweeds on Lipase and α-amylase', *Journal of Applied Phycology*, 25(5), pp. 1405-1412, Available at: Dordrecht: Springer Netherlands. DOI: 10.1007/s10811-012-9964-4.

Becker, K., Hartmann, A., Ganzera, M., Fuchs, D. and Gostner, J. M. (2016) 'Immunomodulatory Effects of the Mycosporine-Like Amino Acids Shinorine and Porphyra-334', *Marine Drugs,* 14(6), pp. 119-131.

Belattmania, Z., Engelen, A., Pereira, H., Serrão, E. A., Barakate, M., Elatouani, S., Zrid, R., Bentiss, F., Chahboun, N. and Reani, A. (2016) 'Potential uses of the brown seaweed Cystoseira humilis biomass: 2-Fatty acid composition, antioxidant and antibacterial activities', *Journal of Materials and Environmental Science,* 7(6), pp. 2074-2081.

Ben Rebah, F., Smaoui, S., Frikha, F., Gargouri, Y. and Miled, N. (2008) 'Inhibitory Effects of Tunisian Marine Algal Extracts on Digestive Lipases', *Applied Biochemistry and Biotechnology*, 151(1), pp. 71-79, Available at: New York: Humana Press Inc. DOI: 10.1007/s12010-008-8167-6.

Benslima, A., Sellimi, S., Hamdi, M., Nasri, R., Jridi, M., Cot, D., Li, S., Nasri, M. and Zouari, N. (2021) 'Brown Seaweed Cystoseira Schiffneri as a Promising Source of Sulfated Fucans: Seasonal Variability of Structural, Chemical, and Antioxidant Properties', *Food Science & Nutrition,* 9(3), pp. 1551-1563.

Berg, J. M., Tymoczko, J. L., Gatto, G. J. and Stryer, L. (2019) *Biochemistry.* Ninth edn.: W. H. Freeman and Company, p. 1171.

Berk, Z. (2018) 'Chapter 11 Extraction', in Berk, Z. (ed.) *Food Process Engineering and Technology (Third Edition)*: Academic Press, pp. 289-310.

Beysseriat, M., Decker, E. A. and McClements, D. J. (2006) 'Preliminary Study of the Influence of Dietary Fiber on the Properties of Oil-in-water Emulsions Passing through an In-vitro Human Digestion Model', *Food Hydrocolloids,* 20(6), pp. 800-809.

Bhuyar, P., Sundararaju, S., Rahim, M. H. A., Unpaprom, Y., Maniam, G. P. and Govindan, N. (2021) 'Antioxidative Study of Polysaccharides Extracted from Red (Kappaphycus alvarezii), Green (Kappaphycus striatus) and Brown (Padina gymnospora) Marine Macroalgae/Seaweed', *SN Applied Sciences,* 3(4), pp. 485-494.

Białecka-Florjańczyk, E., Fabiszewska, A. U., Krzyczkowska, J. and Kuryłowicz , A. (2018) 'Synthetic and Natural Lipase Inhibitors', *Mini-Reviews in Medicinal Chemistry,* 18(8), pp. 672-683.

Biancacci, C., McDougall, G. J., Allwood, J. W., Day, J. G., Davidson, K. and Stanley, M. S. (2021) 'LC-MSn Profiling Reveals Seasonal Variation in the Composition of Osmundea pinnatifida (Hudson) Stackhouse', *Journal of Applied Phycology,* 33(4), pp. 2443-2458.

Bisswanger, H. (2014) 'Enzyme Assays', *Perspectives in Science,* 1(1-6), pp. 41-55.

Bitou, N., Ninomiya, M., Tsujita, T. and Okuda, H. (1999) 'Screening of Lipase Inhibitors from Marine Algae', *Lipids,* 34(5), pp. 441-445.

Bray, G. A., Kim, K. K. and Wilding, J. H. (2017) 'Obesity:A Chronic Relapsing Progressive Disease Process. A Position Statement of the World Obesity Federation', *Obesity Reviews,* 18(7), pp. 715-723.

Buelga, C. S.-., Paramas, A. M. G.-. and Manzano, S. G.-. (2023) 'Phenolic Acids and Derivatives: Description, Sources, Properties, and Applications', in *Natural Secondary Metabolites: From Nature, Through Science, to Industry*. pp. 37-72 [Online]. Version. Available at: https://doi.org/10.1007/978-3-031-18587-8_3.

Burki, T. K. (2016) 'Country in Focus: Gulf Region States Face Major Health Challenges from Obesity and Diabetes', *The lancet Diabetes & Endocrinology*, 4(9), pp. 737-738, Available at: England: Elsevier Ltd. DOI: 10.1016/S2213-8587(16)30189-9.

Caldwell, G. W., Yan, Z., Lang, W. and Masucci, J. A. (2012) 'The IC50 Concept Revisited', *Current Topics in Medicinal Chemistry,* 12(11), pp. 1282-1290.

Catarino, M. D., Silva, A. M. S., Mateus, N. and Cardoso, S. M. (2019) 'Optimization of Phlorotannins Extraction from Fucus vesiculosus and Evaluation of their Potential to Prevent Metabolic Disorders', *Marine Drugs*, 17(3), pp. 162, Available at: Switzerland: MDPI AG. DOI: 10.3390/md17030162.

Chater, P. I., Wilcox, M., Cherry, P., Herford, A., Mustar, S., Wheater, H., Brownlee, I., Seal, C. and Pearson, J. (2016) 'Inhibitory Activity of Extracts of Hebridean Brown Seaweeds on Lipase Activity', *Journal of Applied Phycology*, 28(2), pp. 1303-1313, Available at: Dordrecht: Springer Netherlands. DOI: 10.1007/s10811-015-0619-0.

Chater, P. I., Wilcox, M. D., Houghton, D. and Pearson, J. P. (2015) 'The Role of Seaweed Bioactives in the Control of Digestion: Implications for Obesity Treatments', *Food & Function*, 6(11), pp. 3420-3427, Available at: CAMBRIDGE: ROYAL SOC CHEMISTRY. DOI: 10.1039/c5fo00293a.

Chatzidaki, M. D., Mateos-Diaz, E., Leal-Calderon, F., Xenakis, A. and Carrière, F. (2016) 'Water-in-Oil Microemulsions Emulsions as Carriers of Hydroxytyrosol: an Gastrointestinal Lipolysis Study Using the pHstat Technique', *Food & Function,* 7(5), pp. 2258-2269.

Cherry, P., O'Hara, C., Magee, P. J., McSorley, E. M. and Allsopp, P. J. (2019) 'Risks and Benefits of Consuming Edible Seaweeds', *Nutrition Reviews*, 77(5), pp. 307-329, Available at: United States: Oxford University Press. DOI: 10.1093/nutrit/nuy066.

Connan, S. and Stengel, D. B. (2011) 'Impacts of Ambient Salinity and Copper on Brown Algae: 2. Interactive Effects on Phenolic Pool and Assessment of Metal Binding Capacity of Phlorotannin', *Aquatic Toxicology,* 104(1), pp. 1-13.

Copeland, R. A. (2013) *Evaluation of Enzyme Inhibitors in Drug Discovery: A Guide for Medicinal Chemists and Pharmacologists.* Second edn.: John Wiley & Sons, p. 538.

Copeland, R. A. (2023) *Enzymes: a Practical Introduction to Structure, Mechanism, and Data Analysis.* Third edn.: John Wiley & Sons.

Corstens, M. N., Berton-Carabin, C. C., Elichiry-Ortiz, P. T., Hol, K., Troost, F. J., Masclee, A. A. and Schroën, K. (2017) 'Emulsion-Alginate Beads Designed to Control In-Vitro Intestinal Lipolysis: Towards Appetite Control', *Journal of Functional Foods,* 34, pp. 319-328.

Cory, H., Passarelli, S., Szeto, J., Tamez, M. and Mattei, J. (2018) 'The Role of Polyphenols in Human Health and Food Systems: A Mini-Review', *Frontiers in Nutrition,* 5(87), pp. 1-9.

Cotas, J., Leandro, A., Monteiro, P., Pacheco, D., Figueirinha, A., Gonçalves, A. M. M., da Silva, G. J. and Pereira, L. (2020) 'Seaweed Phenolics: From Extraction to Applications', *Marine Drugs*, 18(8), pp. 384-431*, NLM*. DOI: 10.3390/md18080384 (Accessed Jul 24).

Cvitanovic, A. B., Durgo, K., Hudek, A., Družina, V. B. and Komes, D. (2018) 'Overview of Polyphenols and

Their Properties ', in *Polyphenols: Properties, Recovery, and Applications*. pp. 3-44 [Online]. Version. Available at: https://app.knovel.com/hotlink/toc/id:kpPPRA0006/polyphenols-properties/polyphenols-properties.

da Costa, E., Melo, T., Reis, M., Domingues, P., Calado, R., Abreu, M. H. and Domingues, M. R. (2021) 'Polar Lipids Composition, Antioxidant and Anti-Inflammatory Activities of the Atlantic Red Seaweed Grateloupia Turuturu', *Marine Drugs,* 19(8), pp. 414-438.

Dang, T. T., Van Vuong, Q., Schreider, M. J., Bowyer, M. C., Van Altena, I. A. and Scarlett, C. J. (2017) 'Optimisation of Ultrasound-Assisted Extraction Conditions for Phenolic Content and Antioxidant Activities of the Alga Hormosira banksii Using Response Surface Methodology', *Journal of Applied Phycology,* 29(6), pp. 3161-3173.

Das, I. J. and Bal, T. (2024) 'Exploring Carrageenan: From Seaweed to Biomedicine—A Comprehensive Review', *International Journal of Biological Macromolecules,* 268, pp. 1-31.

DDI, D. D. I. (2006): Kuwait Available at: https://www.dasmaninstitute.org/ 2024).

Devlin, M. J., Breckels, M., Graves, C. A., Barry, J., Capuzzo, E., Huerta, F. P., Al Ajmi, F., Al-Hussain, M. M., LeQuesne, W. J. and Lyons, B. P. (2019) 'Seasonal and Temporal Drivers Influencing Phytoplankton Community in Kuwait Marine Waters: Documenting a Changing Landscape in the Gulf', *Frontiers in Marine Science,* 6, pp. 141-163.

Devlin, M. J., Massoud, M. S., Hamid, S. A., Al-Zaidan, A., Al-Sarawi, H., Al-Enezi, M., Al-Ghofran, L., Smith, A. J., Barry, J., Stentiford, G. D., Morris, S., da Silva, E. T. and Lyons, B. P. (2015) 'Changes in the Water Quality Conditions of Kuwait's Marine Waters: Long-term Impacts of Nutrient Enrichment', *Marine Pollution Bulletin*, 100(2), pp. 607-620, Available at: Oxfod: Elsevier Ltd. DOI: 10.1016/j.marpolbul.2015.10.022.

Devlin, M. J., Quesne, W. J. F. L. and Lyons, B. P. (2015) 'The Marine Environment of Kuwait—Emerging Issues in a Rapidly Changing Environment', *Marine Pollution Bulletin*, 100(2), pp. 593-596, Available at: Oxford: Elsevier Ltd. DOI: 10.1016/j.marpolbul.2015.11.046.

Din, N. A. S., Mohd Alayudin, A. S., Sofian-Seng, N.-S., Rahman, H. A., Mohd Razali, N. S., Lim, S. J. and Wan Mustapha, W. A. (2022) 'Brown Algae as Functional Food Source of Fucoxanthin: A Review', *Foods,* 11(15), pp. 1-35.

Ding, S., Xu, S., Fang, J. and Jiang, H. (2020) 'The Protective Effect of Polyphenols for Colorectal Cancer', *Frontiers in Immunology,* 11, pp. 1-9.

Draget, K. I., Skjåk-Bræk, G. and Stokke, B. T. (2006) 'Similarities and Differences Between Alginic Acid Gels and Ionically Crosslinked Alginate Gels', *Food Hydrocolloids,* 20(2-3), pp. 170-175.

Duan, X., Subbiah, V., Xie, C., Agar, O. T., Barrow, C. J., Dunshea, F. R. and Suleria, H. A. R. (2023) 'Evaluation of the Antioxidant Potential of Brown Seaweeds Extracted by Different Solvents and Characterization of their Phenolic Compounds by LC–ESI-QTOF–MS/MS', *Journal of Food Science,* 88(9), pp. 3737-3757.

Duijsens, D., Verkempinck, S. H. E. and Grauwet, T. (2024) 'Impact of the Stepwise Implementation of INFOGEST Semi-Dynamic Conditions on In-vitro Starch and Protein Digestion: A Case Study on Lentil Cotyledon Cells', *Food Research International,* 197(1), pp. 1-12.

Duta-Bratu, C.-G., Nitulescu, G. M., Mihai, D. P. and Olaru, O. T. (2023) 'Resveratrol and Other Natural Oligomeric Stilbenoid Compounds and Their Therapeutic Applications', *Plants*, 12(16). DOI: 10.3390/plants12162935.

Edmonds, N. J., AlZaidan, A. S., AlSabah, A. A., Quesne, W. J. F. L., Devlin, M. J., Davison, P. I. and Lyons, B. P. (2021) 'Kuwait's Marine Biodiversity: Qualitative Assessment of Indicator Habitats and Species', *Marine Pollution Bulletin*, 163, pp. 111915-111930, Available at: England: Elsevier Ltd. DOI: 10.1016/j.marpolbul.2020.111915.

El Khoury, D., Goff, H. D., Berengut, S., Kubant, R. and Anderson, G. H. (2014) 'Effect of Sodium Alginate Addition to Chocolate Milk on Glycemia, Insulin, Appetite and Food Intake in Healthy Adult Men', *European Journal of Clinical Nutrition*, 68(5), pp. 613-618, Available at: London: Nature Publishing Group. DOI: 10.1038/ejcn.2014.53.

Elkum, N., Al-Arouj, M., Sharifi, M., Shaltout, A. and Bennakhi, A. (2016) 'Prevalence of Childhood Obesity in the State of Kuwait: Prevalence of Childhood Obesity in Arabs', *Pediatric Obesity*, 11(6), pp. 30-34. DOI: 10.1111/ijpo.12090.

Emad, A. M., Rasheed, D. M., El-Kased, R. F. and El-Kersh, D. M. (2022) 'Antioxidant, Antimicrobial Activities and Characterization of Polyphenol-Enriched Extract of Egyptian Celery (Apium graveolens L., Apiaceae) Aerial Parts via UPLC/ESI/TOF-MS', *Molecules (Basel, Switzerland),* 27(3), pp. 698-717.

Eom, S.-H., Lee, M.-S., Lee, E.-W., Kim, Y.-M. and Kim, T. H. (2013) 'Pancreatic Lipase Inhibitory Activity of Phlorotannins Isolated from Eisenia bicyclis', *Phytotherapy Research*, 27(1), pp. 148-151, Available at: Hoboken: Blackwell Publishing Ltd. DOI: 10.1002/ptr.4694.

Fard, S. G., Shamsabadi, F. T., Emadi, M., Meng, G. Y., Muhammad, K. and Mohamed, S. (2011) 'Ethanolic extract of Eucheuma cottonii promotes in vivo hair growth and wound healing', *Journal of Animal and Veterinary Advances,* 10(5), pp. 601-605.

Fathiraja, P., Gopalrajan, S., Karunanithi, M., Nagarajan, M., Obaiah, M. C., Durairaj, S. and Neethirajan, N. (2022) 'Response Surface Methodology Model to Optimize Concentration of Agar, Alginate and Carrageenan for the Improved Properties of Biopolymer Film', *Polymer Bulletin,* 79(8), pp. 6211-6237.

Fellah, F., Louaileche, H., Dehbi-Zebboudj, A. and Touati, N. (2017) 'Seasonal Variations in the Phenolic Compound Content and Antioxidant Activities of Three Selected Species of Seaweeds from Tiskerth Islet, Bejaia, Algeria', *Journal of Materials and Environmental Sciences,* 8(2), pp. 4451-4456.

Fenton, S., Burrows, T. L., Collins, C. E., Rayward, A. T., Murawski, B. and Duncan, M. J. (2021) 'Efficacy of a Multi-Component m-Health Diet, Physical Activity, and Sleep Intervention on Dietary Intake in Adults with Overweight and Obesity: A Randomised Controlled Trial', *Nutrients,* 13(7), pp. 2468-2485.

Ferdouse, F., Holdt, S. L., Smith, R., Murua, P. and Yang, Z. (2018) *The Global Status of Seaweed Production, Trade and Utilization.* Rome: Food and Agriculture Organization of the United Nations.

Field, C. J. and Robinson, L. (2019) 'Dietary Fats', *Advances in Nutrition,* 10(4), pp. 722-724.

Fluhr, J. W., Breternitz, M., Kowatzki, D., Bauer, A., Bossert, J., Elsner, P. and Hipler, U. C. (2010) 'Silver-loaded seaweed-based cellulosic fiber improves epidermal skin physiology in atopic dermatitis: Safety assessment, mode of action and controlled, randomized single-blinded exploratory in vivo study', *Experimental Dermatology,* 19(8), pp. 9-15.

Ford, L., Theodoridou, K., Sheldrake, G. N. and Walsh, P. J. (2019) 'A Critical Review of Analytical Methods Used for the Chemical Characterisation and Quantification of Phlorotannin Compounds in Brown Seaweeds', *Phytochemical Analysis*, 30(6), pp. 587-599, Available at: England: Wiley Subscription Services, Inc. DOI: 10.1002/pca.2851 (Accessed September 2023).

Fujiki, H., Sueoka, E., Watanabe, T. and Suganuma, M. (2015) 'Synergistic Enhancement of Anticancer Effects on Numerous Human Cancer Cell Lines Treated with the Combination of EGCG, Other Green Tea Catechins, and Anticancer Compounds', *Journal of Cancer Research and Clinical Oncology,* 141(9), pp. 1511-1522.

Ganesan, A. R., Tiwari, U. and Rajauria, G. (2019) 'Seaweed Nutraceuticals and their Therapeutic Role in Disease Prevention', *Food Science and Human Wellness,* 8(3), pp. 252-263.

Gaskell, C., Lutimba, S., Bendriss, G. and Aleem, E. (2024) 'Obesity, Physical Activity, and Cancer Incidence in Two Geographically Distinct Populations

The Gulf Cooperation Council Countries and the United Kingdom-A Systematic Review and Meta-Analysis', *Cancers,* 16(24), pp. 4205.

Getachew, A. T., Jacobsen, C. and Holdt, S. L. (2020) 'Emerging Technologies for the Extraction of Marine Phenolics: Opportunities and Challenges', *Marine Drugs*, 18(8), pp. 389-411. DOI: 10.3390/md18080389 (Accessed Jul 27).

Gheda, S., Naby, M. A., Mohamed, T., Pereira, L. and Khamis, A. (2021) 'Antidiabetic and Antioxidant Activity of Phlorotannins Extracted from the Brown Seaweed Cystoseira Compressa in Streptozotocin-Induced Diabetic Rats', *Environmental Science and Pollution Research,* 28, pp. 22886-22901.

Gholamhoseinian, A., Shahouzehi, B. and Sharifi-Far, F. (2010) 'Inhibitory Effect of Some Plant Extracts on Pancreatic Lipase', *International Journal of Pharmacology,* 6(1), pp. 18-24.

Glisan, S. L., Grove, K. A., Yennawar, N. H. and Lambert, J. D. (2017) 'Inhibition of Pancreatic Lipase by Black Tea Theaflavins: Comparative Enzymology and in Silico Modeling Studies', *Food Chemistry,* 216, pp. 296-300.

Gomez, I. and Huovinen, P. (2020) 'Brown Algal Phlorotannins: An Overview of Their Functional Roles', in *Antarctic Seaweeds*. pp. 365-388 [Online]. Version.

Gorniak, I., Bartoszewski, R. and Kroliczewski, J. (2019) 'Comprehensive Review of Antimicrobial Activities of Plant Flavonoids', *Phytochemistry Reviews,* 18, pp. 241-272.

Guglielmi, V., Bettini, S., Sbraccia, P., Busetto, L., Pellegrini, M., Yumuk, V., Colao, A. M., El Ghoch, M. and Muscogiuri, G. (2023) 'Beyond Weight Loss: Added Benefits Could Guide the Choice of Anti-obesity Medications', *Current Obesity Reports,* 12(2), pp. 127-146.

Guimarães, A. C., Meireles, L. M., Lemos, M. F., Guimarães, M. C. C., Endringer, D. C., Fronza, M. and Scherer, R. (2019) 'Antibacterial Activity of Terpenes and Terpenoids Present in Essential Oils', *Molecules,* 24(13), pp. 2471-2483.

Gulliford, M. C. F., Charlton, J. M., Prevost, T. P., Booth, H. P., Fildes, A. P., Ashworth, M. D. M., Littlejohns, P. M. D., Reddy, M. F., Khan, O. F. and Rudisill, C. P. (2016)

'Costs and Outcomes of Increasing Access to Bariatric Surgery: Cohort Study and Cost-Effectiveness Analysis Using Electronic Health Records', *Value in Health*, 20(1), pp. 85-92, Available at: NEW YORK: Elsevier Inc. DOI: 10.1016/j.jval.2016.08.734.

Gunnarson, G. L., Frøyen, J. K., Sandbu, R., Thomsen, J. B. and Hjelmesæth, J. (2015) 'Plastic Surgery after Bariatric Surgery', *Tidsskrift for Den Norske Legeforening*, 135(11), pp. 1044-1049, Available at: Norway. DOI: 10.4045/tidsskr.14.0814.

GÜR, İ. and Polat, S. (2023) 'Seasonal Changes in Proximate and Bioactive Compounds of Brown and Red Seaweeds from İskenderun Bay, the North-Eastern Mediterranean Sea', *Journal of Marine Sciences and Fisheries,* 6(1), pp. 33-43.

Habeebullah, S. F. K., Alagarsamy, S., Sattari, Z., Al-Haddad, S., Fakhraldeen, S., Al-Ghunaim, A. and Al-Yamani, F. Y. (2020) 'Enzyme-assisted Extraction of Bioactive Compounds from Brown Seaweeds and Characterization', *Journal of Applied Phycology*, 32(1), pp. 615-629, Available at: Dordrecht: Springer Nature B.V. DOI: 10.1007/s10811-019-01906-6.

Habeebullah, S. F. K. and Jacobsen, C. (2013) 'Phenolic Compounds and Antioxidant Activities of Selected Species of Seaweeds from Danish Coast', *Food Chemistry*, 138(2-3), pp. 1670-1681, Available at: Oxford: Elsevier Ltd. DOI: 10.1016/j.foodchem.2012.10.078.

Habeebullah, S. F. K., Surendraraj, A., Al-Ghunaim, A. and Al-Yamani, F. (2019) 'Chemical Profile and Antioxidant Activities of 26 Selected Species of Seaweeds from Kuwait Coast', *Journal of Applied Phycology*, 31(4), pp. 2653-2668, Available at: Dordrecht: Springer Netherlands. DOI: 10.1007/s10811-019-1739-8.

Halimi, H., De Caro, J., Carrière, F. and De Caro, A. (2005) 'Closed and Open Conformations of the Lid Domain Induce Different Patterns of Human Pancreatic Lipase Antigenicity and Immunogenicity', *Biochimica et Biophysica Acta- Proteins and Proteomics,* 1753(2), pp. 247-256.

Hall, A. C., Fairclough, A. C., Mahadevan, K. and Paxman, J. R. (2012) 'Ascophyllum nodosum Enriched Bread Reduces Subsequent Energy Intake with No Effect on Post-prandial Glucose and Cholesterol in Healthy, Overweight Males. A Pilot Study', *Appetite*, 58(1), pp. 379-386, Available at: London: Elsevier Ltd. DOI: 10.1016/j.appet.2011.11.002.

Han, H., Yilmaz, H. and Gulcin, I. (2018) 'Antioxidant Activity of fFaxseed (Linum usitatissimum L.) Shell and Analysis of its Polyphenol Contents by LC-MS/MS', 12(4), pp. 397-402.

Handing, G., Pathak, U. I., Balasubramanian, A., Liu, A. and Mayer, W. A. (2022) 'Case–Orlistat-Induced Calcium Oxalate Crystalluria', *Canadian Urological Association Journal,* 16(10), pp. 371-378.

Harman, C., Bekkby, T., Calabrese, S., Trannum, H., Oug, E., Hagen, A. G., Green, N., Kaste, Ø. and Frigstad, H. (2019) 'The Environmental Status of Norwegian Coastal Waters', *World Seas: An Environmental Evaluation*: Elsevier, pp. 69-84.

Hartmann, A., Gostner, J., Becker, K. and Ganzera, M. 'A Novel HILIC-Method for The Analysis of Mycosporine-like Amino Acids and Their Biological Relevance on Anti-Inflammatory Targets and Collagenase'. *Planta Medica*, 2015.

Hata, Y., Nakajima, K., Uchida, J.-i., Hidaka, H. and Nakano, T. (2001) 'Clinical effects of brown seaweed, Undaria pinnatifida (wakame), on blood pressure in hypertensive subjects', *Journal of Clinical Biochemistry and Nutrition,* 30, pp. 43-53.

Hebbar, P., Nizam, R., Melhem, M., Alkayal, F., Elkum, N., John, S. E., Tuomilehto, J., Alsmadi, O. and Thanaraj, T. A. (2018) 'Genome-wide Association Study Identifies Novel Recessive Genetic Variants for High TGs in an Arab Population', *Journal of Lipid Research*, 59(10), pp. 1951-1966, Available at: ROCKVILLE: Elsevier Inc. DOI: 10.1194/jlr.P080218.

Heck, A. M., Yanovski, J. A. and Calis, K. A. (2000) 'Orlistat, a New Lipase Inhibitor for the Management of Obesity', *Pharmacotherapy,* 20(3), pp. 270-279.

Hees, D. H. V., Olsen, Y. S., Wernberg, T., Alstyne, K. L. V. and Kendrick, G. A. (2017) 'Phenolic Concentrations of Brown Seaweeds and Relationships to Nearshore Environmental Gradients in Western Australia', *Marine Biology*, 164(4), pp. 1-13, Available at: Berlin/Heidelberg: Springer Berlin Heidelberg. DOI: 10.1007/s00227-017-3115-z.

Heffernan, N., Smyth, T. J., Soler-Villa, A., Fitzgerald, R. J. and Brunton, N. P. (2015) 'Phenolic Content and Antioxidant Activity of Fractions Obtained from Selected Irish Macroalgae Species (Laminaria digitata, Fucus serratus, Gracilaria gracilis and Codium fragile)', *Journal of Applied Phycology,* 27(1), pp. 519-530.

Heo, S.-J., Park, E.-J., Lee, K.-W. and Jeon, Y.-J. (2005) 'Antioxidant Activities of Enzymatic Extracts from Brown Seaweeds', *Bioresource Technology*, 96(14), pp. 1613-1623, Available at: Oxford: Elsevier Ltd. DOI: 10.1016/j.biortech.2004.07.013.

Heo, S. J., Lee, G. W., Song, C. B. and Jeon, Y. J. (2003) 'Antioxidant Activity of Enzymatic Extracts from Brown Seaweeds', *Algae (Korean Phycological Society),* 18(1), pp. 71-81.

Heslehurst, N., Vieira, R., Hayes, L., Crowe, L., Jones, D., Robalino, S., Slack, E. and Rankin, J. (2017) 'Maternal Body Mass Index and Post-term Birth: A Systematic Review and Meta-analysis', *Obesity Reviews,* 18(3), pp. 293-308.

Hitoe, S. and Shimoda, H. (2017) 'Seaweed Fucoxanthin Supplementation Improves Obesity Parameters in Mildly Obese Japanese Subjects', *Functional Foods in Health and Disease*, 7(4), pp. 246-262.

Hoad, C. L., Marciani, L., de Celis Alonso, B., Traynor, C., Gowland, P. A., Rayment, P., Spiller, R. C., Mela, D. J. and Peters, H. P. (2004) 'In-vivo Imaging of Intragastric Gelation and Its Effect on Satiety in Humans', *The Journal of Nutrition,* 134(9), pp. 2293-2300.

Hoffmann, S. L., Kohlstedt, M., Jungmann, L., Hutter, M., Poblete-Castro, I., Becker, J. and Wittmann, C. (2021) 'Cascaded Valorization of Brown Seaweed to Produce l-lysine and Value-Added Products Using Corynebacterium Glutamicum Streamlined by Systems Metabolic Engineering', *Metabolic Engineering,* 67, pp. 293-307.

Holdt, S. L. and Kraan, S. (2011) 'Bioactive Compounds in Seaweed: Functional Food Applications and Legislation', *Journal of Applied Phycology*, 23(3), pp. 543-597, Available at: Dordrecht: Springer Netherlands. DOI: 10.1007/s10811-010-9632-5.

Houghton, D., Wilcox, M. D., Brownlee, I. A., Chater, P., Seal, C. J. and Pearson, J. P. (2014) 'Method for Quantifying Alginate and Determining Release from a Food Vehicle in Gastrointestinal Gigesta', *Food Chemistry*, 151, pp. 352-357, Available at: Oxford: Elsevier Ltd. DOI: 10.1016/j.foodchem.2013.11.070.

Hu, B., Cui, F., Yin, F., Zeng, X., Sun, Y. and Li, Y. (2015) 'Caffeoylquinic Acids Competitively Inhibit Pancreatic Lipase through Binding to the Catalytic Triad', *International Journal of Biological Macromolecules,* 80, pp. 529-535.

Hua, F., Shi, L. and Zhou, P. (2022) 'Phenols and Terpenoids: Natural Products as Inhibitors of NLRP3 Inflammasome in Cardiovascular Diseases', *Inflammopharmacology,* 30(1), pp. 137-147.

Hwang, P.-A., Phan, N. N., Lu, W.-J., Hieu, B. T. N. and Lin, Y.-C. (2016) 'Low-molecular-weight Fucoidan and High-stability Fucoxanthin from Brown Seaweed Exert Prebiotics and Anti-inflammatory Activities in Caco-2 Cells', *Food & nutrition research,* 60(1), pp. 1-9.

Ihua, M. W., Guihéneuf, F., Mohammed, H., Margassery, L. M., Jackson, S. A., Stengel, D. B., Clarke, D. J. and Dobson, A. D. W. (2019) 'Microbial Population Changes in Decaying Ascophyllum nodosum Result in Macroalgal- Polysaccharide-Degrading Bacteria with Potential Applicability in Enzyme-Assisted Extraction Technologies', *Marine Drugs,* 17(4), pp. 200-220.

Iso, H. and Kubota, Y. (2007) 'Nutrition and Disease in the Japan Collaborative Cohort Study for Evaluation of Cancer (JACC)', *Asian Pacific Journal of Cancer Prevention,* 8(Suppl), pp. 35-80.

Jacobtorweihen, J., Hartmann, A., Hofer, S. and Spiegler, V. (2024) 'Antibacterial Activities of the Algal Bromophenol Methylrhodomelol Against Pseudomonas aeruginosa', *Planta Medica,* 90(6), pp. 469-481.

Janarthanan, M. and Kumar, M. S. (2018) 'The Properties of Bioactive Substances Obtained from Seaweeds and their Applications in Textile Industries', *Journal of Industrial Textiles*, 48(1), pp. 361-401, Available at: London, England: SAGE Publications. DOI: 10.1177/1528083717692596.

Jang, W., Kim, M. and Cho, J. (2022) 'Antioxidant, Anti-Inflammatory, Anti-Menopausal, and Anti-Cancer Effects of Lignans and Their Metabolites', *International Journal of Molecular Sciences,* 23(24), pp. 1422-1441.

Javan, A. J., Javan, M. J. and Tehrani, Z. A. (2013) 'Theoretical Investigation on Antioxidant Activity of Bromophenols from the Marine Red Alga Rhodomela confervoides: H-Atom vs Electron Transfer Mechanism', *Journal of Agricultural and Food Chemistry,* 61(7), pp. 1534-1541.

Jensen, M. G., Kristensen, M. and Astrup, A. (2012) 'Effect of Alginate Supplementation on Weight Loss in Obese Subjects Completing a 12-wk Energy-restricted diet: A Randomized Controlled Trial', *The American Journal of Clinical Nutrition*, 96(1), pp. 5-13, Available at: Oxford: Oxford Univ Press. DOI: 10.3945/ajcn.111.025312.

Jensen, M. G., Kristensen, M., Belza, A., Knudsen, J. C. and Astrup, A. (2012) 'Acute Effect of Alginate-Based Preload on Satiety Feelings, Energy Intake, and Gastric Emptying Rate in Healthy Subjects', *Obesity (Silver Spring, Md.)*, 20(9), pp. 1851-1858, Available at: Oxford, UK: Blackwell Publishing Ltd. DOI: 10.1038/oby.2011.232.

Jerbi, A., Amal, D., Abdelfatteh, E. and and Kammoun, M. (2017) 'Essential Oil Composition and Biological Activities of Eucalyptus globulus Leaves Extracts from Tunisia', *Journal of Essential Oil Bearing Plants,* 20(2), pp. 438-448.

Jin, B.-R., Kim, H.-J., Sim, S.-A., Lee, M. and An, H.-J. (2021) 'Anti-Obesity Drug Orlistat Alleviates Western-Diet-Driven Colitis-Associated Colon Cancer via Inhibition of STAT3 and NF-κB-Mediated Signaling', *Cells,* 10(8), pp. 2060.

Jin, M., Shen, M.-h., Jin, M.-h., Jin, A.-h., Yin, X.-z. and Quan, J.-s. (2018) 'Hypoglycemic property of soy isoflavones from hypocotyl in Goto-Kakizaki diabetic rats', *Journal of Clinical Biochemistry and Nutrition,* 62(2), pp. 148-154.

Jofre, J., Celis-Plá, P. S. M., Figueroa, F. L. and Navarro, N. P. (2020) 'Seasonal Variation of Mycosporine-Like Amino Acids in Three Subantarctic Red Seaweeds', *Marine Drugs,* 18(2), pp. 75-92.

Jung, S.-A., Kim, K.-B.-W.-R., Kim, D.-H., Cho, J.-Y., Kim, T.-W. and Ahn, D.-H. (2013) 'Lipase Inhibitory Mode of Dieckol Isolated from Eisenia bicyclis Ethanol Extract', *Microbiology and Biotechnology Letters,* 41(1).

Kamal, M., Abdel-Raouf, N., Alwutayd, K., AbdElgawad, H., Abdelhameed, M. S., Hammouda, O. and Elsayed, K. N. (2023) 'Seasonal Changes in the Biochemical Composition of Dominant Macroalgal Species Along the Egyptian Red Sea Shore', *Biology,* 12(3), pp. 411-432.

Kang, J.-I., Kim, M.-K., Lee, J.-H., Jeon, Y.-J., Hwang, E.-K., Koh, Y.-S., Hyun, J.-W., Kwon, S.-Y., Yoo, E.-S. and Kang, H.-K. (2017) 'Undariopsis Peterseniana Promotes Hair Growth by the Activation of Wnt/β-Catenin and ERK Pathways', *Marine Drugs,* 15(5), pp. 130-146.

Kardum, N. and Glibetic, M. (2018) 'Polyphenols and their Interactions with other Dietary Compounds: Implications for Human Health', *Advances in Food and Nutrition Research*: Elsevier, pp. 103-144.

Katimbwa, D. A., Oh, J., Jang, C. H. and Lim, J. (2022) 'Orlistat, a Competitive Lipase Inhibitor Used as an Antiobesity Remedy, Enhances Inflammatory Reactions in the Intestine', *Applied Biological Chemistry,* 65(1), pp. 47-57.

Kessel, A. and Ben-Tal, N. (2018) *Introduction to Proteins Structure, Function and Motion* Second edn. US: Taylor & Francis Group, p. 891.

Khalil, A. B., Beshyah, S. A., Abdella, N., Afandi, B., Al-Arouj, M. M., Al-Awadi, F., Benbarka, M., Nakhi, A. B., Fiad, T. M. and Al Futaisi, A. (2018) 'Diabesity in the Arabian Gulf: Challenges and Opportunities', *Oman Medical Journal,* 33(4), pp. 273-282.

Khan, M., Goto, R., Kobayashi, K., Suzumura, S., Nagata, Y., Sonoda, T., Sakauchi, F., Washio, M. and Mori, M. (2004) 'Dietary habits and cancer mortality among middle aged and older Japanese living in hokkaido, Japan by cancer sites and sex', *Asian Pacific Journal of Cancer Prevention,* 5(1), pp. 58-65.

Khatchapuridze, Z., Ploeger, A. and Gulua, L. (2023) 'Kinetic Behaviour of Pancreatic Lipase Inhibition by Wine', *Food Research,* 7(3), pp. 157-164.

Kidgell, J. T., Magnusson, M., de Nys, R. and Glasson, C. R. (2019) 'Ulvan: A systematic Review of Extraction, Composition and Function', *Algal Research,* 39, pp. 1-20.

Kim, A. T. and Park, Y. (2023) 'Trifuhalol A, a Phlorotannin from the Brown Algae Agarum Cribrosum, Reduces Adipogenesis of Human Primary Adipocytes through Wnt/β-Catenin and AMPK-Dependent Pathways', *Current Research in Food Science,* 7, pp. 1-8.

Kim, J. K., Kraemer, G. and Yarish, C. (2019) 'Evaluation of the Metal Content of Farm Grown Gracilaria tikvahiae and Saccharina latissima from Long Island Sound and New York Estuaries', *Algal Research (Amsterdam),* 40, pp. 1-9.

Kim, K. B. W. R., Jung, J. Y., Cho, J. Y. and Ahn, D. H. (2012) 'Lipase Inhibitory Activity of Ethyl Acetate Fraction from Ecklonia cava Extracts', *Biotechnology and Bioprocess Engineering*, 17(4), pp. 739-745, Available at: Heidelberg: The Korean Society for Biotechnology and Bioengineering. DOI: 10.1007/s12257-011-0671-9.

Kishida, R., Yamagishi, K., Muraki, I., Sata, M., Tamakoshi, A., Iso, H. and Group, J. S. (2020) 'Frequency of seaweed intake and its association with cardiovascular disease mortality: The JACC Study', *Journal of Atherosclerosis and Thrombosis,* 27(12), pp. 1340-1347.

Konstantin, B., Anastasia, P., Nikolay, I. and Daria, P. (2023) 'Seasonal Variations in the Chemical Composition of Arctic Brown Macroalgae', *Algal Research,* 72, pp. 103112.

Koseki, K., Yoshimura, R., Ido, K., Katsuura, K., Bito, T. and Watanabe, F. (2023) 'Determination of Vitamin B12 and Folate Compounds in Commercially Available Edible Seaweed Products', *Frontiers in Bioscience-Elite,* 15(2), pp. 10-18.

Kosmalski, M., Deska, K., Bąk, B., Różycka-Kosmalska, M. and Pietras, T. 'Pharmacological Support for the Treatment of Obesity—Present and Future'. *Healthcare*: MDPI, 433-365.

Krentz, A. J., Fujioka, K. and Hompesch, M. (2016) 'Evolution of Pharmacological Obesity Treatments: Focus on Adverse Side-effect Profiles', *Diabetes, Obesity & Metabolism*, 18(6), pp. 558-570, Available at: Oxford, UK: Blackwell Publishing Ltd. DOI: 10.1111/dom.12657.

Kumar, K. S., Ganesan, K. and Rao, P. V. S. (2015) 'Seasonal Variation in Nutritional Composition of Kappaphycus alvarezii (Doty) Doty—an Edible Seaweed', *Journal of Food Science and Technology*, 52(5), pp. 2751-2760. DOI: 10.1007/s13197-014-1372-0 (Accessed 2015/05/01).

Kumar, S., Sahoo, D. and Levine, I. (2015) 'Assessment of Nutritional Value in a Brown Seaweed Sargassum wightii and Their Seasonal Variations', *Algal Research*, 9, pp. 117-125, Available at: Amsterdam: Elsevier B.V. DOI: 10.1016/j.algal.2015.02.024.

Kurniawan, R., Nurkolis, F., Taslim, N. A., Subali, D., Surya, R., Gunawan, W. B., Alisaputra, D., Mayulu, N., Salindeho, N. and Kim, B. (2023) 'Carotenoids Composition of Green Algae Caulerpa Racemosa and their Antidiabetic, Anti-obesity, Antioxidant, and Anti-inflammatory Properties', *Molecules,* 28(7), pp. 1-15.

Kushner, R. F. (2014) 'Weight Loss Strategies for Treatment of Obesity', *Progress in Cardiovascular Diseases*, 56(4), pp. 465-472, Available at: PHILADELPHIA: Elsevier Inc. DOI: 10.1016/j.pcad.2013.09.005.

Kushner, R. F. (2018) 'Weight Loss Strategies for Treatment of Obesity: Lifestyle Management and Pharmacotherapy', *Progress in Cardiovascular Diseases*, 61(2), pp. 246-252, Available at: Elsevier DOI: https://doi.org/10.1016/j.pcad.2018.06.001 (Accessed 10/2023).

Kwon, Y.-J., Kwon, G. E., Lee, H. S., Choi, M. H. and Lee, J.-W. (2022) 'The Effect of Orlistat on Sterol Metabolism in Obese Patients', *Frontiers in Endocrinology,* 13, pp. 1-10.

Lange, K. W., Hauser, J., Nakamura, Y. and Kanaya, S. (2015) 'Dietary Seaweeds and Obesity', *Food Science and Human Wellness*, 4(3), pp. 87-96, Available at: Elsevier B.V. DOI: 10.1016/j.fshw.2015.08.001.

Leandro, A., Pacheco, D., Cotas, J., Marques, J. C., Pereira, L. and Gonçalves, A. M. M. (2020) 'Seaweed's Bioactive Candidate Compounds to Food Industry and Global Food Security', *Life*, 10(8), pp. 140-177, Available at: Basel: MDPI AG. DOI: 10.3390/life10080140.

Lee, S.-H. and Jeon, Y.-J. (2015) 'Efficacy and safety of a dieckol-rich extract (AG-dieckol) of brown algae, Ecklonia cava, in pre-diabetic individuals: A double-blind, randomized, placebo-controlled clinical trial', *Food & Function,* 6(3), pp. 853-858.

Lee, Y.-J., Park, J.-H., Park, S.-A., Joo, N.-R., Lee, B. H., Lee, K. B. and Oh, S.-M. (2020) 'Dieckol or Phlorofucofuroeckol Extracted from Ecklonia Cava Suppresses Lipopolysaccharide-Mediated Human Breast Cancer Cell Migration and Invasion', *Journal of Applied Phycology,* 32, pp. 631-640.

Lee, Y. H., Yoon, S.-J., Kim, A., Seo, H. and Ko, S. (2016) 'Health Performance and Challenges in Korea: A Review of the Global Burden of Disease Study 2013', *Journal of Korean Medical Science,* 31(2), pp. 114-121.

Leelapornpisid, P., Mungmai, L., Sirithunyalug, B., Jiranusornkul, S. and Peerapornpisal, Y. (2014) 'A novel moisturizer extracted from freshwater macroalga [Rhizoclonium hieroglyphicum (C. Agardh) Kützing] for skin care cosmetic', *Chiang Mai Journal of Science,* 41(5.2), pp. 1195-1207.

Li, Y., Fu, X., Duan, D., Liu, X., Xu, J. and Gao, X. (2017) 'Extraction and Identification of Phlorotannins from the Brown Alga, Sargassum fusiforme (Harvey) Setchell', *Marine Drugs*, 15(2), pp. 49-49, Available at: BASEL: MDPI AG. DOI: 10.3390/md15020049.

Lim, S. Y., Steiner, J. M. and Cridge, H. (2022) 'Lipases: It's Not Just Pancreatic Lipase!', *American Journal of Veterinary Research,* 83(8), pp. 1-8.

Liu, J., Lin, S., Wang, Z., Wang, C., Wang, E., Zhang, Y. and Liu, J. (2011) 'Supercritical Fluid Extraction of Flavonoids from Maydis Stigma and Its Nitrite-Scavenging Ability', *Food and Bioproducts Processing,* 89(4), pp. 333-339.

Liu, Z., Ni, W., Huang, Y., Gurzadyan, G. and Chen, X. (2024) 'Experimental Evidence of the S x State and Fluorescence Emission from the Intramolecular Charge Transfer States in Fucoxanthin', *Physical Chemistry Chemical Physics,* 26(48), pp. 29879-29886.

López, A., Rico, M., Rivero, A. and Suárez de Tangil, M. (2011) 'The Effects of Solvents on the Phenolic Contents and Antioxidant Activity of Stypocaulon scoparium Algae Extracts', *Food Chemistry*, 125(3), pp. 1104-1109, Available at: Oxford: Elsevier Ltd. DOI: 10.1016/j.foodchem.2010.09.101.

Lu, C., Gu, Q. and Yu, X. (2024) 'Effect of In-vitro Simulated Digestion on the Physicochemical Properties and Pancreatic Lipase Inhibitory Activity of Fucoidan', *Journal of the Science of Food and Agriculture,* 104(7), pp. 4157-4164.

Lucas, K. H. and Kaplan-Machlis, B. (2001) 'Orlistat-A Novel Weight Loss Therapy', *Annals of Pharmacotherapy,* 35(3), pp. 314-328.

Luo, J., Jiang, B., Li, C., Jia, X. and Shi, D. (2019) 'CYC27 Synthetic Derivative of Bromophenol from Red Alga Rhodomela confervoides: Anti-Diabetic Effects of Sensitizing Insulin Signaling Pathways and Modulating RNA Splicing-Associated RBPs', *Marine Drugs,* 17(1), pp. 49.

Machu, L., Misurcova, L., Ambrozova, J. V., Orsavova, J., Mlcek, J., Sochor, J. and Jurikova, T. (2015) 'Phenolic Content and Antioxidant Capacity in Algal Food

Products', *Molecules (Basel, Switzerland)*, 20(1), pp. 1118-1133, Available at: Basel: MDPI AG. DOI: 10.3390/molecules20011118.

Maheswari, V. and Babu, P. A. S. (2022) 'Phlorotannin and its Derivatives, a Potential Antiviral Molecule from Brown Seaweeds, an Overview', *Russian Journal of Marine Biology,* 48(5), pp. 309-324.

Martinez-Gonzalez, A. I., Alvarez-Parrilla, E., Díaz-Sánchez, Á. G., Rosa, L. d. l., Núñez-Gastélum, J. A., Vazquez-Flores, A. A. and Gonzalez-Aguilar, G. A. (2017) 'In-vitro Inhibition of Pancreatic Lipase by Polyphenols: a Kinetic, Fluorescence Spectroscopy and Molecular Docking Study', *Food Technology and Biotechnology,* 55(4), pp. 519-530.

Matsumoto, M., Hosokawa, M., Matsukawa, N., Hagio, M., Shinoki, A., Nishimukai, M., Miyashita, K., Yajima, T. and Hara, H. (2010) 'Suppressive Effects of the Marine Carotenoids, Fucoxanthin and Fucoxanthinol on Triglyceride Absorption in Lymph Duct-cannulated Rats', *European Journal of Nutrition,* 49(4), pp. 243-249.

May, M., Schindler, C. and Engeli, S. (2020) 'Modern Pharmacological Treatment of Obese Patients', *Therapeutic Advances in Endocrinology and Metabolism,* 11, pp. 1-19.

McDougall, G. J., Kulkarni, N. N. and Stewart, D. (2009) 'Berry Polyphenols Inhibit Pancreatic Lipase Activity In-vitro', *Food Chemistry,* 115(1), pp. 193-199.

Mohamed, S., Hashim, S. N. and Rahman, H. A. (2012) 'Seaweeds: A Sustainable Functional Food for Complementary and Alternative Therapy', *Trends in Food Science & Technology*, 23(2), pp. 83-96, Available at: LONDON: Elsevier Ltd. DOI: 10.1016/j.tifs.2011.09.001.

Mohammed, M. S., Sendra, S., Lloret, J. and Bosch, I. (2018) 'Systems and WBANs for Controlling Obesity', *Journal of Healthcare Engineering*, 2018, pp. 1-21, Available at: LONDON: Hindawi. DOI: 10.1155/2018/1564748.

Mohandas, G. G. and Kumaraswamy, M. (2018) 'Antioxidant Activities of Terpenoids from Thuidium Tamariscellum (C. Muell.) Bosch. and Sande-Lac. a Moss', *Pharmacognosy Journal,* 10(4).

Mojzer, E. B., Hrnčič, M. K., Škerget, M., Knez, Ž. and Bren, U. (2016) 'Polyphenols: Extraction Methods, Antioxidative Action, Bioavailability and Anticarcinogenic Effects', *Molecules,* 21(7), pp. 901-939.

Montero, L., Del Pilar Sánchez-Camargo, A., Ibáñez, E. and Gilbert-López, B. (2018) 'Phenolic Compounds from Edible Algae: Bioactivity and Health Benefits', *Current Medicinal Chemistry,* 25(37), pp. 4808-4826.

Montonen, J., Knekt, P., Härkänen, T., Järvinen, R., Heliövaara, M., Aromaa, A. and Reunanen, A. (2005) 'Dietary patterns and the incidence of type 2 diabetes', *American Journal of Epidemiology,* 161(3), pp. 219-227.

Morimoto, A., Ohno, Y., Tatsumi, Y., Mizuno, S. and Watanabe, S. (2012) 'Effects of healthy dietary pattern and other lifestyle factors on incidence of diabetes in a rural Japanese population', *Asia Pacific Journal of Clinical Nutrition,* 21(4), pp. 601-608.

Mulet-Cabero, A.-I., Egger, L., Portmann, R., Ménard, O., Marze, S., Minekus, M., Le Feunteun, S., Sarkar, A., Grundy, M. M.-L. and Carrière, F. (2020) 'A Standardised Semi-dynamic In-vitro Digestion Method Suitable for Food–an International Consensus', *Food & Function,* 11(2), pp. 1702-1720.

Murai, U., Yamagishi, K., Kishida, R. and Iso, H. (2021) 'Impact of Seaweed Intake on Health', *European Journal of Clinical Nutrition,* 75(6), pp. 877-889.

Murai, U., Yamagishi, K., Sata, M., Kokubo, Y., Saito, I., Yatsuya, H., Ishihara, J., Inoue, M., Sawada, N. and Iso, H. (2019) 'Seaweed Intake and Risk of Cardiovascular Disease: the Japan Public Health Center–based Prospective (JPHC) Study', *The American Journal of Clinical Nutrition,* 110(6), pp. 1449-1455.

Murray, M., Dordevic, A. L., Cox, K., Scholey, A., Ryan, L. and Bonham, M. P. (2021) 'Twelve Weeks' Treatment with a Polyphenol-Rich Seaweed Extract Increased HDL Cholesterol with No Change in Other Biomarkers of Chronic Disease Risk in Overweight Adults: A Placebo-Controlled Randomized Trial', *The Journal of Nutritional Biochemistry,* 96, pp. 1-13.

Musaiger, A. O., Al-Mannai, M., Al-Haifi, A. R., Nabag, F., Elati, J., Abahussain, N., Tayyem, R., Jalambo, M., Benhamad, M. and Al-Mufty, B. (2016) 'Prevalence of Overweight and Obesity among Adolescents in Eight Arab Countries: Comparison between Two International Standards (ARABEAT-2)', *Nutrición Hospitalaria*, 33(5), pp. 1062-1065, Available at: MADRID: AULA MEDICA EDICIONES. DOI: 10.20960/nh.567.

Musaiger, A. O., Al-Mannai, M., Tayyem, R., Al-Lalla, O., Ali, E. Y. H., Kalam, F., Benhamed, M. M., Saghir, S., Halahleh, I., Djoudi, Z. and Chirane, M. (2012) 'Prevalence of Overweight and Obesity among Adolescents in Seven Arab Countries: A Cross-Cultural Study', *Journal of Obesity*, 2012, pp. 1-5, Available at: United States: Hindawi Limiteds. DOI: 10.1155/2012/981390.

Myers, C. A. and Broyles, S. T. (2020) 'Fast Food Patronage and Obesity Prevalence During the COVID-19 Pandemic: An Alternative Explanation', *Obesity (Silver Spring, Md.)*, 28(10), pp. 1796-1797, Available at: United States: Blackwell Publishing Ltd. DOI: 10.1002/oby.22993.

Nagahawatta, D., Liyanage, N., Jayawardena, T. U., Yang, F., Jayawardena, H., Kurera, M., Wang, F., Fu, X., Jeon, Y.-J. and Nagahawatta, D. (2023) 'Functions and Values of Sulfated Polysaccharides from Seaweed', *Algae,* 38(4), pp. 217-240.

Nanri, A., Kimura, Y., Matsushita, Y., Ohta, M., Sato, M., Mishima, N., Sasaki, S. and Mizoue, T. (2010) 'Dietary patterns and depressive symptoms among Japanese men and women', *European Journal of Clinical Nutrition,* 64(8), pp. 832-839.

Naumann, S., Schweiggert-Weisz, U., Eglmeier, J., Haller, D. and Eisner, P. (2019) 'In-vitro Interactions of Dietary Fibre Enriched Food Ingredients with Primary and Secondary Bile Acids', *Nutrients,* 11(6), pp. 1424-1442.

Nelson, S. M., Gao, Y.-T., Nogueira, L. M., Shen, M.-C., Wang, B., Rashid, A., Hsing, A. W. and Koshiol, J. (2017) 'Diet and biliary tract cancer risk in Shanghai, China', *Plos One,* 12(3), pp. 1-10.

Niemi, C., Mortensen, A. M., Rautenberger, R., Matsson, S., Gorzsás, A. and Gentili, F. G. (2023) 'Rapid and Accurate Determination of Protein Content in North Atlantic Seaweed by NIR and FTIR Spectroscopies', *Food Chemistry,* 404, pp. 1-10.

Nishida, Y., Kumagai, Y., Michiba, S., Yasui, H. and Kishimura, H. (2020) 'Efficient Extraction and Antioxidant Capacity of Mycosporine-like Amino Acids from Red Alga Dulse palmaria palmata in Japan', *Marine Drugs,* 18(10), pp. 502.

Obluchinskaya, E. D., Pozharitskaya, O. N. and Shikov, A. N. (2022) 'In-vitro Anti-inflammatory Activities of Fucoidans from Five Species of Brown Seaweeds', *Marine Drugs,* 20(10), pp. 606.

Olsson, J., Toth, G. B. and Albers, E. (2020) 'Biochemical Composition of Red, Green and Brown Seaweeds on the Swedish West Coast', *Journal of Applied Phycology,* 32(5), pp. 3305-3317.

Omer, E. and Chiodi, C. (2024) 'Fat Digestion and Absorption: Normal Physiology and Pathophysiology of Malabsorption, Including Diagnostic Testing', *Nutrition in Clinical Practice,* 39, pp. 6-16.

Orfanoudaki, M., Hartmann, A., Alilou, M., Gelbrich, T., Planchenault, P., Derbré, S., Schinkovitz, A., Richomme, P., Hensel, A. and Ganzera, M. (2020) 'Absolute Configuration of Mycosporine-like Amino Acids, their Wound Healing Properties and In-vitro Anti-aging Effects', *Marine Drugs,* 18(1), pp. 35.

Otero, P., López-Martínez, M. I. and García-Risco, M. R. (2019) 'Application of Pressurized Liquid Extraction (PLE) to Obtain Bioactive Fatty Acids and Phenols from Laminaria ochroleuca Collected in Galicia (NW Spain)', *Journal of Pharmaceutical and Biomedical Analysis,* 164, pp. 86-92.

Panche, A. N., Diwan, A. D. and Chandra, S. R. (2016) 'Flavonoids: an Overview', *Journal of Nutritional Science* 5, pp. e47-e47, Available at: Cambridge, UK: Cambridge University Press. DOI: 10.1017/jns.2016.41.

Pandey, D., Hansen, H. H., Dhakal, R., Aryal, N., Rai, S. P., Sapkota, R., Nielsen, M. O., Novoa-Garrido, M. and Khanal, P. (2022) 'Interspecies and Seasonal Variations in Macroalgae from the Nordic Region: Chemical Composition and Impacts on Rumen Fermentation and Microbiome Assembly', *Journal of Cleaner Production,* 363, pp. 1-18.

Park, E., Yu, H., Lim, J.-H., Choi, J. H., Park, K.-J. and Lee, J. (2023) 'Seaweed Metabolomics: A Review on its Nutrients, Bioactive Compounds and Changes in Climate Change', *Food Research International,* 163, pp. 1-17.

Park, H., Lee, K. W. and Shin, D. (2022) 'Association of Seaweed Consumption with Metabolic Syndrome and Its Components: Findings from the Korean Genome and Epidemiology Study', *Foods,* 11(11), pp. 1635-1646.

Pelkman, C. L., Navia, J. L., Miller, A. E. and Pohle, R. J. (2007) 'Novel calcium-gelled, Alginate-pectin Beverage Reduced Energy Intake in Nondieting Overweight and Obese Women: Interactions with Dietary Restraint Status', *The American Journal of Clinical Nutrition*, 86(6), pp. 1595-1602, Available at: Bethesda, MD: American Society for Clinical Nutrition. DOI: 10.1093/ajcn/86.5.1595.

Pereira, L. (2018) 'Biological and therapeutic properties of the seaweed polysaccharides', *International Biology Review,* 2(2), pp. 1-50.

Pereira, T., Horta, A., Barroso, S., Mendes, S. and Gil, M. M. (2021) 'Study of the Seasonal Variations of the Fatty Acid Profiles of Selected Macroalgae', *Molecules,* 26(19), pp. 5807-5822.

Pérez-Lloréns, J. L., Critchley, A., Cornish, M. and Mouritsen, O. (2023) 'Saved by Seaweeds (II): Traditional Knowledge, Home Remedies, Medicine, Surgery, and Pharmacopoeia', *Journal of Applied Phycology,* 35(5), pp. 2049-2068.

Picot-Allain, M. C. N., Amiri-Rigi, A., Abdoun-Ouallouche, K., Aberkane, L., Djefal-Kerrar, A., Mahomoodally, M. F. and Emmambux, M. N. (2022) 'Assessing the Bioactivity, Cytotoxicity, and Rheological Properties of Pectin Recovered from Citrus Peels', *Food Bioscience,* 46, pp. 1-9.

Pietta, P.-G. (2000) 'Flavonoids as Antioxidants', *Journal of Natural Products,* 63(7), pp. 1035-1042.

Pillon, N. J., Loos, R. J., Marshall, S. M. and Zierath, J. R. (2021) 'Metabolic Consequences of Obesity and Type 2 Diabetes: Balancing Genes and Environment for Personalized Care', *Cell,* 184(6), pp. 1530-1544.

Pimentel, F. B., Alves, R. C., Rodrigues, F. and PP Oliveira, M. B. (2017) 'Macroalgae-derived ingredients for cosmetic industry—An update', *Cosmetics,* 5(1), pp. 2-19.

Pozharitskaya, O. N., Obluchinskaya, E. D. and Shikov, A. N. (2020) 'Mechanisms of Bioactivities of Fucoidan from the Brown Seaweed Fucus vesiculosus L. of the Barents Sea', *Marine drugs,* 18(5), pp. 275.

Pradhan, B., Bhuyan, P. P., Patra, S., Nayak, R., Behera, P. K., Behera, C., Behera, A. K., Ki, J.-S. and Jena, M. (2022) 'Beneficial Effects of

Seaweeds and Seaweed-derived Bioactive Compounds: Current Evidence and Future Prospective', *Biocatalysis and Agricultural Biotechnology,* 39, pp. 1-10

Prior, R. L., Wu, X., Gu, L., Hager, T. J., Hager, A. and Howard, L. R. (2008) 'Whole Berries versus Berry Anthocyanins: Interactions with Dietary Fat Levels in the C57BL/6J Mouse Model of Obesity', *Journal of Agricultural and Food Chemistry,* 56(3), pp. 647-653.

Ptak, S. H., Hjuler, A. L., Ditlevsen, S. I., Fretté, X., Errico, M. and Christensen, K. V. (2021) 'The Effect of Seasonality and Geographic Location on Sulphated Polysaccharides from Brown Algae', *Aquaculture Research,* 52(12), pp. 6235-6243.

Qiu, Y., Jiang, H., Fu, L., Ci, F. and Mao, X. (2021) 'Porphyran and oligo-porphyran originating from red algae Porphyra: Preparation, biological activities, and potential applications', *Food Chemistry,* 349, pp. 1-12.

Radünz, M., Camargo, T. M., Raphaelli, C. d. O., Radünz, A. L., Gandra, E. Á. and Zavareze, E. d. R. (2024) 'Chemical Composition, Atimicrobial and Antioxidant Activities of Broccoli, Kale, and Cauliflower Extracts', *Plant Foods for Human Nutrition,* 79(3), pp. 700-706.

Rajapakse, N. and Kim, S.-K. (2011) 'Nutritional and Digestive Health Benefits of Seaweed', *Advances in Food and Nutrition Research,* 64, pp. 17-28.

Rana, A., Samtiya, M., Dhewa, T., Mishra, V. and Aluko, R. E. (2022) 'Health Benefits of Polyphenols: A Cconcise Review', *Journal of Food Biochemistry*, 46(10), pp. 14264-14288, Available at: United States: Wiley Subscription Services, Inc. DOI: 10.1111/jfbc.14264.

Rangel, J. C. S.-., Benavides, J., Heredia, J. B., Cisneros-Zevallos, L. and Jacobo-Velázquez, D. A. (2013) 'The Folin-Ciocalteu Assay Revisited: Improvement of its Specificity for Total Phenolic Content Determination', *Analytical Methods*, 5(21), pp. 5990-5999, Available at: Cambridge: Royal Soc Chemistry. DOI: 10.1039/c3ay41125g.

Razem, M., Ding, Y., Morozova, K., Mazzetto, F. and Scampicchio, M. (2022) 'Analysis of Phenolic Compounds in Food by Coulometric Array Detector: A Review', *Sensors,* 22(19), pp. 1-18.

Reinisalo, M., Kårlund, A., Koskela, A., Kaarniranta, K. and Karjalainen, R. O. (2015) 'Polyphenol Stilbenes: Molecular Mechanisms of Defence Against Oxidative Stress and Aging-related Diseases', *Oxidative Medicine and Cellular Longevity,* 2015, pp. 1-25.

Reis, P., Holmberg, K., Watzke, H., Leser, M. E. and Miller, R. (2009) 'Lipases at Interfaces: A Review', *Advances in Colloid and Interface Science,* 147-148, pp. 237-250.

Ring, B., Wrighton, S. A. and Mohutsky, M. (2014) 'Reversible Mechanisms of Enzyme Inhibition and Resulting Clinical Significance', in Nagar, S., Argikar, U.A. and Tweedie, D.J. (eds.) *Enzyme Kinetics in Drug Metabolism: Fundamentals and Applications*. Totowa, NJ: Humana Press, pp. 37-56.

Rioux, L.-E., Turgeon, S. L. and Beaulieu, M. (2009) 'Effect of Season on the Composition of Bioactive Polysaccharides from the Brown Seaweed Saccharina longicruris', *Phytochemistry,* 70(8), pp. 1069-1075.

Robinson, P. K. (2015) 'Enzymes: Principles and Biotechnological Applications', *Essays in Biochemistry,* 59, pp. 1-41.

Roleda, M. Y., Marfaing, H., Desnica, N., Jónsdóttir, R., Skjermo, J., Rebours, C. and Nitschke, U. (2019) 'Variations in Polyphenol and Heavy Metal Contents of Wild-harvested and Cultivated Seaweed Bulk Biomass: Health Risk Assessment and Implication for Food Applications', *Food Control,* 95, pp. 121-134.

Sadeghi, A., Rajabiyan, A., Nabizade, N., Meygolinezhad, N. and Ahmady, A. Z. (2024) 'Seaweed-derived Phenolic Compounds as Diverse Bioactive Molecules: A Review on Identification, Application, Extraction and Purification Strategies', *International Journal of Biological Macromolecules,* 266, pp. 1-23.

Sahu, A. K. and Mishra, A. K. (2022) 'Photophysical Behavior of Plant Flavonols Galangin, Kaempferol, Quercetin, and Myricetin in Homogeneous Media and The DMPC Model Membrane: Unveiling the Influence of the B-ring hydroxylation of Flavonols', *The Journal of Physical Chemistry B,* 126(15), pp. 2863-2875.

Sakai, C., Abe, S., Kouzuki, M., Shimohiro, H., Ota, Y., Sakinada, H., Takeuchi, T., Okura, T., Kasagi, T. and Hanaki, K. (2019) 'A randomized Placebo-controlled Trial of an Oral Preparation of High Molecular Weight Fucoidan in Patients with Type 2 diabetes with Evaluation of Taste Sensitivity', *Yonago Acta Medica,* 62(1), pp. 14-23.

Salgado, L. T., Tomazetto, R., Cinelli, L. P., Farina, M. and Amado Filho, G. M. (2007) 'The Influence of Brown Algae Alginates on Phenolic Compounds Capability of Ultraviolet Radiation Absorption In-vitro', *Brazilian Journal of Oceanography,* 55, pp. 145-154.

Samara, A., Andersen, P. T. and Aro, A. R. (2019) 'Health Promotion and Obesity in the Arab Gulf States: Challenges and Good Practices', *Journal of Obesity*, 2019, pp. 1-6, Available at: United States: Hindawi. DOI: 10.1155/2019/4756260.

Sánchez-Camargo, A. d. P., Montero, L., Stiger-Pouvreau, V., Tanniou, A., Cifuentes, A., Herrero, M. and Ibáñez, E. (2016) 'Considerations on the Use of Enzyme-assisted Extraction in Combination with Pressurized Liquids to Recover Bioactive Compounds from Algae', *Food Chemistry*, 192, pp. 67-74, Available at: Oxford: Elsevier Ltd. DOI: 10.1016/j.foodchem.2015.06.098.

Santos, S. A. O., Felix, R., Pais, A. C. S., Rocha, S. M. and Silvestre, A. J. D. (2019) 'The Quest for Phenolic Compounds from Macroalgae: A Review of Extraction and Identification Methodologies', *Biomolecules*, 9(12), pp. 847-903, Available at: Switzerland: MDPI. DOI: 10.3390/biom9120847 (Accessed September 2023).

Sapatinha, M., Oliveira, A., Costa, S., Pedro, S., Gonçalves, A., Mendes, R., Bandarra, N. and Pires, C. (2022) 'Red and Brown Seaweeds Extracts: A Source of Biologically Active Compounds', *Food Chemistry,* 393, pp. 1-10.

Saraswati, S., Giantina, G., Giriwono, P. E., Faridah, D. N., Iskandriati, D. and Andarwulan, N. (2020) 'Water and Lipid-Soluble Component Profile of Sargassum cristaefolium from Different Coastal Areas in Indonesia with Potential for Developing Functional Ingredient', *Journal of Oleo Science,* 69(11), pp. 1517-1528.

Saura-Calixto, F. and Díaz-Rubio, M. E. (2007) 'Polyphenols Associated with Dietary Fibre in Wine: A Wine Polyphenols Gap?', *Food Research International,* 40(5), pp. 613-619.

Savira, A., Amin, M. and Alamsjah, M. 'The Effect of Different Type of Solvents on the Antioxidant Activity of Fucoxanthin Extract from Brown Seaweed Sargassum Duplicatum'. *IOP Conference Series: Earth and Environmental Science*: IOP Publishing, 1-7.

Seyler, M., Barry, C., Loisel, A., Moro, M. R., Lachal, J. and Lefèvre, H. (2025) 'Anxiety Disorders and Major Depressive Disorders in 299 Adolescents Hospitalized for Assessment of Common Obesity: A Retrospective Cohort Study', *Archives de pédiatrie : Organe Officiel de la Société française de pédiatrie*.

Shannon, E. and Abu-Ghannam, N. (2019) 'Seaweeds as Nutraceuticals for Health and Nutrition', *Phycologia (Oxford)*, 58(5), pp. 563-577, Available at: Abingdon: Taylor & Francis. DOI: 10.1080/00318884.2019.1640533.

Shannon, E., Conlon, M. and Hayes, M. (2023) 'In-vitro Enzyme Inhibitory Effects of Green and Brown Australian Seaweeds and Potential Impact on Metabolic Syndrome', *Journal of Applied Phycology,* 35(2), pp. 893-910.

Shephard, R. J. (2019) 'On Determining How Much Obesity is Costing Society', *The Health & Fitness Journal of Canada,* 12(1), pp. 80-116.

Shimazu, T., Kuriyama, S., Hozawa, A., Ohmori, K., Sato, Y., Nakaya, N., Nishino, Y., Tsubono, Y. and Tsuji, I. (2007) 'Dietary patterns and cardiovascular disease mortality in Japan: a prospective cohort study', *International journal of epidemiology,* 36(3), pp. 600-609.

Shishikura, Y., Khokhar, S. and Murray, B. S. (2006) 'Effects of Tea Polyphenols on Emulsification of Olive Oil in a Small Intestine Model System', *Journal of Agricultural and Food Chemistry,* 54(5), pp. 1906-1913.

Silva, V., Igrejas, G., Falco, V., Santos, T. P., Torres, C., Oliveira, A. M., Pereira, J. E., Amaral, J. S. and Poeta, P. (2018) 'Chemical Composition, Antioxidant and Antimicrobial Activity of Phenolic Compounds Extracted from Wine Industry by-Products', *Food Control,* 92, pp. 516-522.

Simonnet, A., Chetboun, M., Poissy, J., Raverdy, V., Noulette, J., Duhamel, A., Labreuche, J., Mathieu, D., Pattou, F. and Jourdain, M. (2020) 'High Prevalence of Obesity in Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) Requiring Invasive Mechanical Ventilation', *Obesity*, 28(7), pp. 1195-1199, Available at: Hoboken: John Wiley and Sons Inc. DOI: 10.1002/oby.23006.

Singh, M., Kaur, M., Vyas, B. and Silakari, O. (2018) 'Design, Synthesis and Biological Evaluation of 2-Phenyl-4 H-chromen-4-one Derivatives as Polyfunctional Compounds Against Alzheimer's Disease', *Medicinal Chemistry Research,* 27, pp. 520-530.

Sjöström, L., Rissanen, A., Andersen, T., Boldrin, M., Golay, A., Koppeschaar, H. P. and Krempf, M. (1998) 'Randomised Placebo-controlled Trial of Orlistat for Weight Loss and Prevention of Weight Regain in Obese Patients', *The Lancet,* 352(9123), pp. 167-172.

Sosnowska, D., Podsędek, A. and Kucharska, A. Z. (2022) 'Proanthocyanidins as the Main Pancreatic Lipase Inhibitors in Chokeberry Fruits', *Food & Function,* 13(10), pp. 5616-5625.

Stengel, D. B., Connan, S. and Popper, Z. A. (2011) 'Algal Chemodiversity and Bioactivity: Sources of Natural Variability and Implications for Commercial Application', *Biotechnology Advances*, 29(5), pp. 483-501, Available at: Oxford: Elsevier Inc. DOI: 10.1016/j.biotechadv.2011.05.016.

Tabassum, M. R., Xia, A. and Murphy, J. D. (2016) 'Seasonal Variation of Chemical Composition and Biomethane Production from the Brown Seaweed Ascophyllum nodosum', *Bioresource Technology,* 216, pp. 219-226.

Tai-fang, G., Cai-yun, F. and Wen, C. (2007) 'Physiochemical properties of alginate in tissue engineering research and its clinical application', *ZHONGGUO ZUZHI GONGCHENG YANJIU YU LINCHUANG KANGFU,* 11(18), pp. 3613.

Tanniou, A., Vandanjon, L., Incera, M., Serrano Leon, E., Husa, V., Le Grand, J., Nicolas, J. L., Poupart, N., Kervarec, N., Engelen, A., Walsh, R., Guerard, F., Bourgougnon, N. and Stiger-Pouvreau, V. (2014) 'Assessment of the Spatial Variability of Phenolic Contents and Associated Bioactivities in the Invasive Alga Sargassum Muticum Sampled Along its European Range from Norway to Portugal', *Journal of Applied Phycology,* 26(2), pp. 1215-1230.

Teas, J., Baldeón, M. E., Chiriboga, D. E., Davis, J. R., Sarriés, A. J. and Braverman, L. E. (2009a) 'Could dietary seaweed reverse the metabolic syndrome?', *Asia Pacific Journal of Clinical Nutrition,* 18(2), pp. 145-154.

Teas, J., Hurley, T. G., Hebert, J. R., Franke, A. A., Sepkovic, D. W. and Kurzer, M. S. (2009b) 'Dietary seaweed modifies estrogen and phytoestrogen metabolism in healthy postmenopausal women', *The Journal of nutrition,* 139(5), pp. 939-944.

Teas, J. and Irhimeh, M. (2012) 'Dietary algae and HIV/AIDS: proof of concept clinical data', *Journal of Applied Phycology,* 24, pp. 575-582.

Terasaki, M., Kawagoe, C., Ito, A., Kumon, H., Narayan, B., Hosokawa, M. and Miyashita, K. (2017) 'Spatial and Seasonal Variations in the Biofunctional Lipid Substances (fucoxanthin and fucosterol) of the Laboratory-grown Edible Japanese Seaweed (Sargassum horneri Turner) Cultured in the Open Sea', *Saudi Journal of Biological Sciences*, 24(7), pp. 1475-1482, Available at: Amsterdam: Elsevier B.V. DOI: 10.1016/j.sjbs.2016.01.009.

Tilbeurgh, H. v., Bezzine, S., Cambillau, C., Verger, R. and Carrière, F. (1999) 'Colipase: Structure and Interaction with Pancreatic Lipase', *Biochimica et Biophysica Acta,* 1441(2-3), pp. 173-84.

Trigo, J. P., Palmnäs-Bédard, M., Juanola, M. V.-L. and Undeland, I. (2023) 'Effects of Whole Seaweed Consumption on Humans: Current Evidence from Randomized-controlled Intervention Trials, Knowledge Gaps, and Limitations', *Frontiers in Nutrition,* 10, pp. 1-17.

Van Gaal, L., Mertens, I., Ballaux, D. and Verkade, H. J. (2004) 'Modern, New Pharmacotherapy for Obesity. A Gastrointestinal Approach', *Best Practice and Research: Clinical Gastroenterology,* 18(6 SPEC.ISS.), pp. 1049-1072.

Venkatesan, J., Lowe, B., Anil, S., Manivasagan, P., Kheraif, A. A. A., Kang, K.-H. and Kim, S.-K. (2015) 'Seaweed Polysaccharides and their Potential Biomedical Applications', *Starch* 67(5-6), pp. 381-390, Available at: Weinheim: Blackwell Publishing Ltd. DOI: 10.1002/star.201400127.

Verwei, M., Minekus, M., Zeijdner, E., Schilderink, R. and Havenaar, R. (2016) 'Evaluation of Two Dynamic In-vitro Models Simulating Fasted and Fed State Conditions in the Upper Gastrointestinal Tract (TIM-1 and tiny-TIM) for Investigating the Bioaccessibility of Pharmaceutical Compounds from Oral Dosage Forms', *International Journal of Pharmaceutics,* 498(1-2), pp. 178-186.

Victor Calero, Patrícia M. Rodrigues, Tiago Dias, Alar Ainla, Adriana Vilaça, Lorenzo Pastrana, Miguel Xavier and Catarina Gonçalves (2024) 'A Miniaturised Semi-dynamic In-vitro Model of Human Digestion ', *Scientific Reports,* 14(11923), pp. 1-11.

Vinuganesh, A., Kumar, A., Korany, S. M., Alsherif, E. A., Selim, S., Prakash, S., Beemster, G. T. and AbdElgawad, H. (2022) 'Seasonal Changes in the Biochemical Constituents of Green Seaweed Chaetomorpha antennina from Covelong, India', *Biomolecules,* 12(10), pp. 1475-1491.

Vo, C.-V. T., Luu, N. V. H., Nguyen, T. T. H., Nguyen, T. T., Ho, B. Q., Nguyen, T. H., Tran, T.-D. and Nguyen, Q.-T. (2022) 'Screening for Pancreatic Lipase Inhibitors: Evaluating Assay Conditions Using p-Nitrophenyl Palmitate as Substrate', *All life (Online),* 15(1), pp. 13-22.

Wadden, T. A., Webb, V. L., Moran, C. H. and Bailer, B. A. (2012) 'Lifestyle Modification for Obesity: New Developments in Diet, Physical Activity, and Behavior Therapy', *Circulation*, 125(9), pp. 1157-1170, Available at: Philadelphia: Lippincott Williams & Wilkins. DOI: 10.1161/CIRCULATIONAHA.111.039453.

Wagenblast, A. L., Laessoe, L. and Printzlau, A. (2014) 'Self-reported Problems and Wishes for Plastic Surgery after Bariatric Surgery', *Journal of Plastic Surgery and Hand Surgery*, 48(2), pp. 115-121, Available at: ABINGDON: Informa Healthcare. DOI: 10.3109/2000656X.2013.822384.

Wan-Loy, C. and Siew-Moi, P. (2016) 'Marine Algae as a Potential Source for Anti-obesity Agents', *Marine Drugs*, 14(12), pp. 222-241, Available at: Basel: MDPI. DOI: 10.3390/md14120222.

Wang, J., Ye, X., Su, Z., Zou, P., Pang, J. and Chen, J.-C. (2021) 'ACE-Inhibitory Peptides from Laminaria Japonica and their Potential Anti-Hypertensive Mechanism', *CyTA-Journal of Food,* 19(1), pp. 333-340.

Wang, J., Zheng, J., Huang, C., Zhao, J., Lin, J., Zhou, X., Naman, C. B., Wang, N., Gerwick, W. H. and Wang, Q. (2018) 'Eckmaxol, a phlorotannin extracted from Ecklonia maxima, produces anti-β-amyloid oligomer neuroprotective effects possibly via directly acting on glycogen synthase kinase 3β', *ACS chemical neuroscience,* 9(6), pp. 1349-1356.

Wang, S., Wang, L.-J., Jiang, B., Wu, N., Li, X., Liu, S., Luo, J. and Shi, D. (2015) 'Anti-angiogenic Properties of bddpm, A Bromophenol from Marine Red Alga Rhodomela Confervoides, with Multi Receptor Tyrosine Kinase Inhibition Effects', *International Journal of Molecular Sciences,* 16(6), pp. 13548-13560.

Wang, T., Jónsdóttir, R. and Ólafsdóttir, G. (2009) 'Total Phenolic Compounds, Radical Scavenging and Metal Chelation of Extracts from Icelandic Seaweeds', *Food Chemistry*, 116(1), pp. 240-248. DOI: https://doi.org/10.1016/j.foodchem.2009.02.041 (Accessed 2009/09/01/).

Wang, T., Jónsdóttir, R. s., Liu, H., Gu, L., Kristinsson, H. G., Raghavan, S. and Ólafsdóttir, G. n. (2012) 'Antioxidant Capacities of Phlorotannins Extracted from the Brown Algae Fucus vesiculosus', *Journal of agricultural and food chemistry,* 60(23), pp. 5874-5883.

Weiderpass, E., Botteri, E., Longenecker, J. C., Alkandari, A., Al-Wotayan, R., Al Duwairi, Q. and Tuomilehto, J. (2019) 'The Prevalence of Overweight and Obesity in an Adult Kuwaiti Population in 2014', *Frontiers in Endocrinology,* 10, pp. 449-458.

Wells, M. L., Potin, P., Craigie, J. S., Raven, J. A., Merchant, S. S., Helliwell, K. E., Smith, A. G., Camire, M. E. and Brawley, S. H. (2017) 'Algae as Nutritional and Functional Food Sources: Revisiting our Understanding', *Journal of Applied Phycology,* 29, pp. 949-982.

Wen, L., Tan, S., Zeng, L., Wang, Z., Ke, X., Zhang, Z., Tang, H., Guo, H. and Xia, E. (2020) 'Ultrasound-Assisted Extraction and In-Vitro Simulated Digestion of Porphyra Haitanensis Proteins Exhibiting Antioxidative and α-Glucosidase Inhibitory Activity', *Journal of Food Measurement and Characterization,* 14, pp. 3291-3298.

WHO, W. H. O. (2014) *Country Cooperation Strategy for WHO and Kuwait for 2012–2016*: World Health Organization.

WHO, W. H. O. (2016) *Global Health Observatory (GHO) Data: Overweight and Obesity*: World Health Organization. Available at: https://www.who.int/gho/ncd/risk_factors/overweight_obesity/obesity_adults/en/ 2020).

WHO, W. H. O. (2020) *Obesity and Overweight*: World Health Organization. Available at: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight 2021).

WHO, W. H. O. (2022) *Prevalence of Obesity among Adults, BMI & Greater Equal*: World Health Organization. Available at: https://www.who.int/data/gho/data/indicators/indicator-details/GHO/prevalence-of-obesity-among-adults-bmi--30-(crude-estimate)-(-) 2024).

Wilcox, M. D., Brownlee, I. A., Richardson, J. C., Dettmar, P. W. and Pearson, J. P. (2014) 'The Modulation of Pancreatic Lipase Activity by Alginates', *Food Chemistry*, 146(100), pp. 479-484, Available at: Oxford: Elsevier Ltd. DOI: 10.1016/j.foodchem.2013.09.075.

Wilding, J. P., Batterham, R. L., Calanna, S., Davies, M., Van Gaal, L. F., Lingvay, I., McGowan, B. M., Rosenstock, J., Tran, M. T. and Wadden, T. A. (2021) 'Once-Weekly Semaglutide in Adults with Overweight or Obesity', *New England Journal of Medicine,* 384(11), pp. 989-1002.

Wu, G., Zhuang, D., Chew, K. W., Ling, T. C., Khoo, K. S., Van Quyen, D., Feng, S. and Show, P. L. (2022a) 'Current Status and Future Trends in Removal, Control, and Mitigation of Algae Food Safety Risks for Human Consumption', *Molecules,* 27(19), pp. 6633-6652.

Wu, M., Cai, J., Fang, Z., Li, S., Huang, Z., Tang, Z., Luo, Q. and Chen, H. (2022b) 'The Composition and Anti-aging Activities of Polyphenol Extract from Phyllanthus Emblica L. Fruit', *Nutrients,* 14(4), pp. 857-875.

Wu, X., He, W., Yao, L., Zhang, H., Liu, Z., Wang, W., Ye, Y. and Cao, J. (2013) 'Characterization of Binding Interactions of (−)-Epigallocatechin-3-gallate from Green Tea and Lipase', *Journal of Agricultural and Food Chemistry,* 61(37), pp. 8829-8835.

Xiao, X. and Lowe, M. E. (2015) 'The β5-loop and Lid Domain Contribute to the Substrate Specificity of Pancreatic Lipase-Related Protein 2 (PNLIPRP2)', *Journal of Biological Chemistry,* 290(48), pp. 28847-28856.

Xiccato, G. (2010) 'Fat Digestion', *Nutrition of the Rabbit*: CABI Wallingford UK, pp. 56-65.

Xu, E., Chen, C., Fu, J., Zhu, L., Shu, J., Jin, M., Wang, Y. and Zong, X. (2021) 'Dietary Fatty Acids in Gut Health: Absorption, Metabolism and Function', *Animal Nutrition,* 7(4), pp. 1337-1344.

Yang, J., Liu, L. and Han, S. (2017) 'Preparation of octyl-grafted alginate-amide gel particle and its application in Pickering emulsion', *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* 529, pp. 320-327.

Yang, M., Zhang, Z., He, Y., Li, C., Wang, J. and Ma, X. (2021) 'Study on the Structure Characterization and Moisturizing Effect of Tremella Polysaccharide Fermented from GCMCC5. 39', *Food Science and Human Wellness,* 10(4), pp. 471-479.

Yang, Y. J., Nam, S.-J., Kong, G. and Kim, M. K. (2010) 'A case–control study on seaweed consumption and the risk of breast cancer', *British Journal of Nutrition,* 103(9), pp. 1345-1353.

Yao, Z., Qi, W., Zhang, H., Zhang, Z., Liu, L., Shao, Y., Zeng, H., Yin, J., Pan, H. and Guo, X. (2023) 'Down-Regulated GAS6 Impairs Synovial Macrophage Efferocytosis and Promotes Obesity-Associated Osteoarthritis', *Elife,* 12, pp. 1-19.

Yesilova, K., Balkis, N. and Taskin, E. (2017) 'Seasonal Investigation of the Protein, Carbohydrate and Lipid Contens of Dominant Macroalgae on the Western Coast of the Black Sea', *Fresenius Environmental Bulletin,* 26(1), pp. 46-55.

Yoshinaga, K. and Mitamura, R. (2019) 'Effects of Undaria pinnatifida (Wakame) on Postprandial Glycemia and Insulin Levels in Humans: a Randomized Crossover Trial', *Plant Foods for Human Nutrition,* 74(4), pp. 461-467.

Yuan, Y., Zhang, J., Fan, J., Clark, J., Shen, P., Li, Y. and Zhang, C. (2018) 'Microwave Assisted Extraction of Phenolic Compounds from Four Economic Brown Macroalgae Species and Evaluation of their Antioxidant Activities and Inhibitory Effects on α-amylase, α-glucosidase, Aancreatic Lipase and Tyrosinase', *Food Research International*, 113, pp. 288-297, Available at: Amsterdam: Elsevier Ltd. DOI: 10.1016/j.foodres.2018.07.021.

Zahrah, Z., Amin, M. N. G. and Alamsjah, M. A. 'The Effect of Fucoxanthin as Coloring Agent on the Quality of Shrimp Paste'. *IOP Conference Series: Earth and Environmental Science*: IOP Publishing, 1-11.

Zargarzadeh, M., Amaral, A. J., Custódio, C. A. and Mano, J. F. (2020) 'Biomedical applications of laminarin', *Carbohydrate Polymers,* 232, pp. 1-8.

Zhang, Q., Zhang, J., Shen, J., Silva, A., Dennis, D. A. and Barrow, C. J. (2006) 'A Simple 96-Well Microplate Method for Estimation of Total Polyphenol Content in Seaweeds', *Journal of Applied Phycology,* 18(3), pp. 445-450.

Zhang, T., Wu, S., Ai, C., Wen, C., Liu, Z., Wang, L., Jiang, L., Shen, P., Zhang, G. and Song, S. (2021) 'Galactofucan from Laminaria japonica Is Not Degraded by the Human Digestive System but Inhibits Pancreatic Lipase and Modifies the Intestinal Microbiota', *International Journal of Biological Macromolecules,* 166, pp. 611-620.

Zhang, Y., Cai, P., Cheng, G. and Zhang, Y. (2022) 'A Brief Review of Phenolic Compounds Identified from Plants: Their Extraction, Analysis, and Biological Activity', *Natural Product Communications,* 17(1), pp. 1-17.

Zhao, D. (2021) 'Epidemiological Features of Cardiovascular Disease in Asia', *Jorurnals of American College of Cardiology,* 1(1), pp. 1-13.

Zhao, L., Zhao, X., Xu, Y., Liu, X., Zhang, J. and He, Z. (2021) 'Simultaneous Determination of 49 Amino Acids, B Vitamins, Flavonoids, and Phenolic Acids in Commonly Consumed Vegetables by Ultra-Performance Liquid Chromatography–Tandem Mass Spectrometry', *Food Chemistry,* 344, pp. 128712-128722.

Zhao, P., Yang, X., Gan, J., Renes, I., Abrahamse, E., Bartke, N., Wei, W. and Wang, X. (2024) 'In Vitro Lipid Digestion of Milk Formula with Different Lipid Droplets: A Study on the Gastric Digestion Emulsion Structure and Lipid Release Pattern', *Journal of Agricultural and Food Chemistry,* 72(44), pp. 24736-24748.

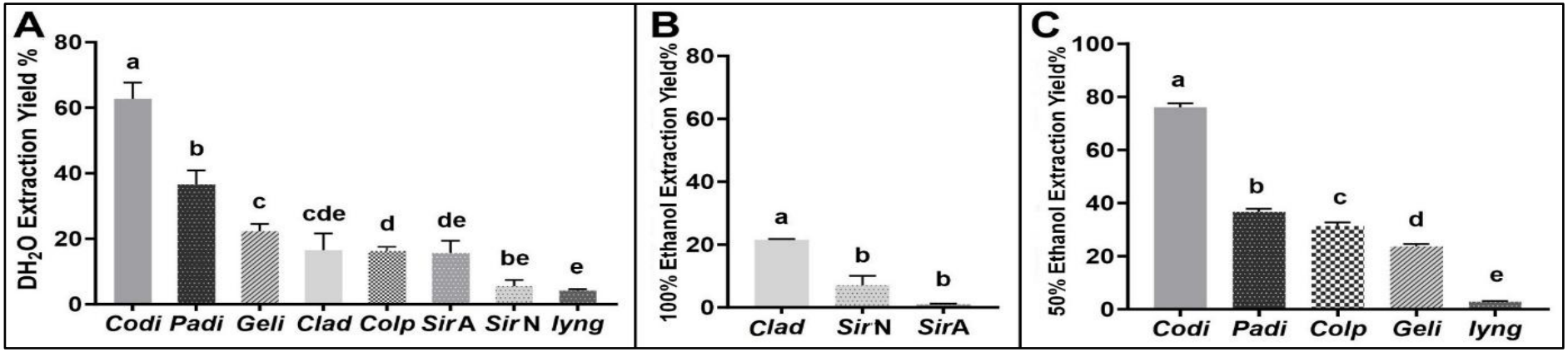
Zhao, W., Subbiah, V., Xie, C., Yang, Z., Shi, L., Barrow, C., Dunshea, F. and Suleria, H. A. (2023) 'Bioaccessibility and Bioavailability of Phenolic Compounds in Seaweed', *Food Reviews International,* 39(8), pp. 5729-5760.

Zhu, G., Fang, Q., Zhu, F., Huang, D. and Yang, C. (2021) 'Structure and Function of Pancreatic Lipase-Related Protein 2 and Its Relationship with Pathological States', *Frontiers in Genetics,* 12, pp. 1-7.

Zhu, X., Healy, L. E., Sevindik, O., Sun, D.-W., Selli, S., Kelebek, H. and Tiwari, B. K. (2022) 'Impacts of Novel Blanching Treatments Combined with Commercial Drying Methods on the Physicochemical Properties of Irish Brown Seaweed Alaria Esculenta', *Food Chemistry,* 369, pp. 1-9.

Appendices

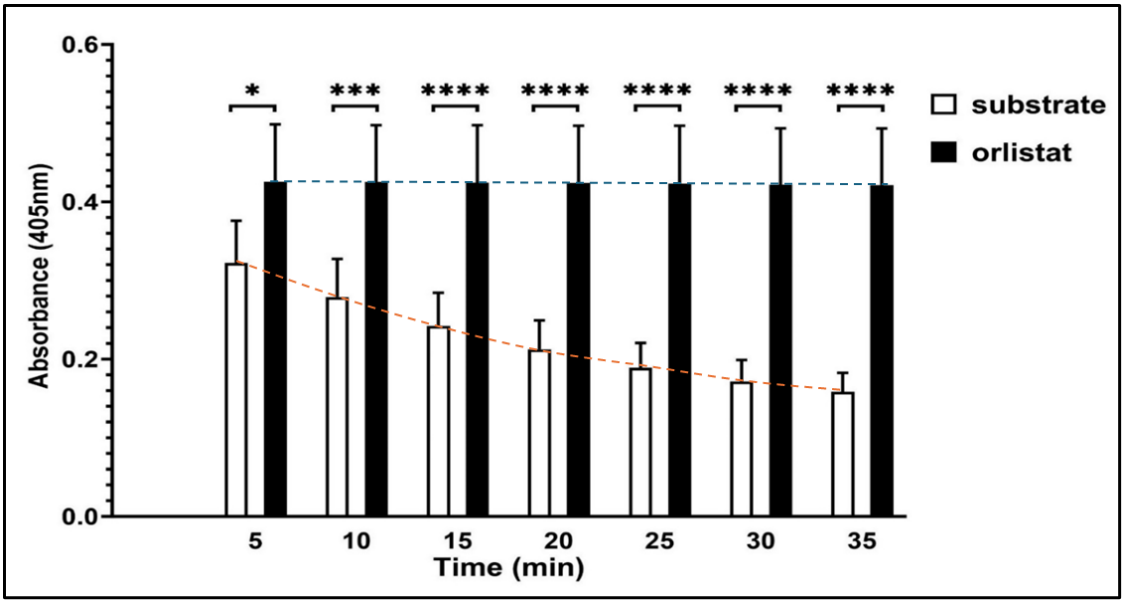
Appendix 1



Appendix 1Comparing the extraction yields from Kuwaiti seaweed.

Appendix 1A shows the significant differences in WE extraction yields from all seaweed species. Appendix 1B shows the significant differences in 100% EEion yields. Appendix 1C shows the significant differences in 50% extraction yields. A one-way ANOVA was performed with post-hoc Tukey's test to evaluate the significant differences between extraction yields and seaweed species. Data is presented as mean (± SD) (n=3). Values with different letters show statistically significant differences ($P < 0.05$). Clad, Cladophora sericioides; Geli, Gelidium Pusillum; Sir. N, Sirophysalis trinodis collected in November; Colp, Colpomenia Sinuosa, lyng, lyngaria stellata; Sir. A, Sirophysalis trinodis collected in April and Padi, Padina boergesenii.

Appendix 2



Changes in absorbance reflect changes in turbidity over a 35-minute duration. Diminished turbidly signifies the hydrolysis of triacylglycerides and was employed as an indicator of pancreatic lipase activity. A two-way ANOVA was conducted with a post-hoc Bonferroni's analysis, to determine the variation in absorbance levels every 5 minutes for 35 minutes between the olive oil substrate control and Orlistat as a positive control inhibitor. The Correlation between absorbance values measured at 405 nm and time is shown in the dotted line. Data are presented as mean ± standard deviation (n=6). *, *** and **** denote significance at P<0.05, p<0.0005 and <0.0001, respectively.

Appendix 3

KISR
Kuwait Institute for Scientific Research

TO WHOM IT MAY CONCERN

Based on the request of Mrs. Hebah Alshammari as she is a PhD student to collect algae samples, I have been collected algae samples from Kuwait institute for scientific research KISR – Marina- Raas Alard- Salmeya, for her to complete the required analytical studies essential for her to complete PhD study.

This certificate is issued upon his/her request with no obligation to KISR.

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State of Kuwait

Appendix 4

قسم العلوم البيولوجية
Department of Biological Sciences
كلية العلوم
Faculty of Science
جامعة الكويت
Kuwait University

7

TO WHOME IT MAY CONCERN

Based on the request of Mrs Heba Alshammari, a PhD student at Newcastle University-UK, I have collected and prepared these dried algal samples from the local marine environment of Kuwait for her to complete the required analytic studies of her PhD studies at Newcastle University.

This certificate is issued upon her request with no obligations to Kuwait University.

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MATERIAL SAFETY DATA SHEET — 15 Sections

SECTION 1 — CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

Product Identifier: [dry algae with silica gel beads -scientific samples]			[WHMIS Classification] Not classified
Product Use: Strictly Scientific research			
Sender Name: Dhia Al-Bader, Kuwait University Kuwait		Recipient Name: Hebah Alshammari-PhD student , Newcastle Univ. – UK	
Street Address: Kuwait University, faculty of science, Department of Biological Sciences, P.O. Box (5969)		Street Address: Newcastle University Newcastle upon Tyne, NE1 7RU - UK	
City: Kuwait	Province-	City: Newcastle upon Tyne	Province
Postal Code: Safat 13060	Emergency Telephone: 0096599882516	Postal Code	Emergency Telephone
Date MSDS Prepared: 28/04/2021	MSDS Prepared By: Dhia Al-Bader		Phone Number: +44-7988997648

SECTION 2 — COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous Ingredients *(specific)*	%	CAS Number	LD 50 of Ingredient *(specify species and route*	LC 50 of Ingredient *(specify species)*
None				

SECTION 3 — HAZARDS IDENTIFICATION

[Biological Hazard] Route of Entry: ☐ Skin Contact ☐ Skin Absorption ☐ Eye Contact ☐ Inhalation ☐ Ingestion

THE CONTENT IS NON-INFECTIOUS/NON-HAZARDOUS/NOT AN ETILOGIC AGENT/NOT FOR HUMAN CONSUMPTION. CONSISTS OF DRY MARINE ALGAL SAMPLES WITH SILICA GEL BEADS TO KEEP CONTENT DRY.

WHMIS Symbols]

Potential Health Effects

No effect on health

SECTION 4 — FIRST AID MEASURES

Skin Contact: Wash with water and household detergent.

Eye Contact: Wash with water

Inhalation:

Ingestion:

SECTION 5 — FIRE FIGHTING MEASURES

Flammable ☐ Yes X No	If yes, under which conditions?	
Means of Extinction		
Flashpoint (° C) and Method	Upper Flammable Limit (% by volume)	Lower Flammable Limit (% by volume)
Autoignition Temperature (°C)	Explosion Data — Sensitivity to Impact	Explosion Data — Sensitivity to Static Discharge
Hazardous Combustion Products		
[NFPA]		

SECTION 6 — ACCIDENTAL RELEASE MEASURES

Leak and Spill Procedures

wash with household detergent

SECTION 7 — HANDLING AND STORAGE

Handling Procedures and Equipment

Do not fold

Storage Requirements

SECTION 8 — EXPOSURE CONTROL / PERSONAL PROTECTION

Exposure Limits ☐ ACGIH TLV ☐ OSHA PEL ☐ Other *(specify)*

Specific Engineering Controls *(such as ventilation, enclosed process)*

Personal Protective Equipment X Gloves ☐ Respirator X Eye ☐ Footwear X Clothing ☐ Other

If checked, please specify type

When handling (unpacking) wear latex gloves, laboratory coat (TO AVOID CONTAMINATING THE SAMPLE).

SECTION 9 — PHYSICAL AND CHEMICAL PROPERTIES

Physical State **dry**	Odour and Appearance **None**	Odor – None
Specific Gravity -	Vapour Density (air = 1) -	Vapour Pressure (mmHg) -
Evaporation Rate -	Boiling Point (° C)	Freezing Point (° C)
pH	Coefficient of Water/Oil Distribution -	[Solubility in Water]

SECTION 10 — STABILITY AND REACTIVITY

Chemical Stability X Yes ☐ No	If no, under which conditions?
Incompatibility with Other Substances ☐ Yes X No	If yes, which ones?
Reactivity, and under what conditions?	
Hazardous Decomposition Products	

SECTION 11 — TOXICOLOGICAL INFORMATION

Effects of Acute Exposure

None

Effects of chronic exposure

None

Irritancy of Product

Skin sensitization	No	Respiratory sensitization	No
Carcinogenicity-IARC	No	Carcinogenicity - ACGIH	No
Reproductive toxicity	No	Teratogenicity	No
Embrotoxicity	No	Mutagenicity	No

Name of synergistic products/effects **have no effect**

SECTION 12 — ECOLOGICAL INFORMATION

[Aquatic Toxicity] **It is non toxic, non infectious and non hazardous to humans or animals**

SECTION 13 — DISPOSAL CONSIDERATIONS

Waste Disposal **dispose normally.**

SECTION 14 — TRANSPORT INFORMATION

KEEP at room temperature .

SECTION 15 — OTHER INFORMATION

Special Shipping Information

1. **The samples are non hazardous, non infectious and represent no risk to health.**
2. **The above information is correct to the best of our knowledge.**

Completed by Sender:
Name of sender: Dr. Dhia Al-Bader
Kuwait University

Signature: .....

Date: ...JUNE 28, 2022.....