Department of Applied Human Nutrition

The effect of a mixed meal formulated with *Chondrus crispus* seaweeds on satiety and food intake in young males

By

Tongtong Li

A Thesis

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Supervisor: Dr. Bohdan Luhovyy

Member(s) of Thesis Committee: Dr. Priya Kathirvel & Dr. Phillip Joy

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Abstract

Background & Objectives: Seaweeds, known as sea vegetables, are a rich source of nutrients, including minerals, polysaccharides, soluble and insoluble fibers, and trace elements. Seaweeds are traditionally consumed in Asian countries; however, their consumption in western countries, including Canada, is very limited. The major reasons for limited consumption include their unique taste and flavor, unfamiliarity with using seaweeds in traditional foods, unavailability of ready-to-use processed foods with seaweeds and lack of information regarding their health benefits. To enhance the development and consumption of seaweed products in Canada, there is a need to investigate the sensory acceptability of seaweed-containing foods and their link with appetite sensation in consumers. The objective was to assess the effect of *Chondrus crispus* seaweed on sensory characteristics, *ad libitum* food intake (FI) at 120min, subjective appetite (SA) and physical comfort over 120min in males (19-35y). *Methods*: Thirty male participants (19-35 y) with Body Mass Index (BMI) of 20.0-24.9kg/m^2 completed a randomized controlled, single-blinded cross-over. The treatments included the omelettes prepared with (S) or without (N) added dried *Chondrus crispus* (5g, 60ml) and energy-free water control (W). Visual Analogue Scales (VAS) were used to assess appetite and physical comfort throughout the study. *Results:* S resulted in a similar pleasantness, taste, texture, flavour, aftertaste and appearance compared to N ($P>0.05$). Both S and N led to reduced FI at 120min compared to W ($P<0.05$) while FI after S was 115 kcal less compared to N ($P=0.05$). The palatability of pizza at 120min was lower after S than W and N ($P<0.05$). Both S and N led to reduced subjective appetite over 2h compared to W ($P<0.0001$), and a similar physical comfort ($P>0.05$). *Conclusions:* In healthy male adults, adding 5g of *Chondrus crispus* to a meal results in acceptable sensory characteristics, high physical comfort, and SA reduction and FI suppression possibly due to increased mastication of the food with added seaweed, and a lower sensory acceptance of a subsequent meal.

Keywords: seaweed; red seaweed; *Chondrus crispus*, Irish moss; Hana Tsunomata; taste; pleasantness; meal; acceptance; perception; satiety; food intake; physical comfort

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List of Abbreviations

A
AA, average appetite
ANOVA: analysis of variance
Apo-A IV: apolipoprotein A-IV
AS: appetite sensation
AUC: area under the curve (t – total, i – incremental, ni – net incremental)
Available CHO: Available carbohydrate
B
BMI: body mass index
C
CCK: cholecystokinin
cFI: cumulative food intake
CNS: central nervous system
CVDs: cardiovascular diseases
D
DALYs: disability-adjusted life years
DHA: docosahexaenoic acid
DTE: desire to eat
E
EPA: eicosapentaenoic acid
F
FAO: Food and Agriculture Organization of the United Nations
FBG: fasting blood glucose
FFM: fat-free mass
FFNHP: functional foods and natural health products
FI: food intake
FM: fat mass
FM: fat mass
G
GLP-1: glucagon-like peptide-1
H
HDL-C: high-density lipoprotein-cholesterol
I
IBD: irritable bowel disease
IMTA: integrated multi-trophic aquaculture
L
LDL-C: low-density lipoprotein-cholesterol
M
MET: metabolic equivalents
MetS: metabolic syndrome
MTE: motivation to eat
N
N: Omelettes without *Chondrus crispus* seaweed
NAFLD: Nonalcoholic Fatty Liver Disease
NCDs: non-communicable diseases
NPY: neuropeptide Y
O
OXM: oxyntomodulin
P
PA: physical activity
PC: physical comfort
PFC: prospective food consumption
PUFA: polyunsaturated fatty acids
PYTPA: past year's total physical activity
PYY: peptide YY
R
RDA: recommended dietary allowances
S
S: Omelettes with *Chondrus crispus* seaweed
SA: subjective appetite
SD: standard deviation
SQ: satiety quotient
T
T2DM: type 2 diabetes mellitus
TBW: total body water
U
UIC: urinary iodine concentration
UL: tolerable upper level
V
VAS: visual analogue scale
W
W: water control
WHO: World Health Organization
wt: weight

9-pt HS: 9-point hedonic scale

Chapter 1: Introduction

Seaweed, or macroalgae, or sea vegetables are forms of algae that grow in the sea. They are a food source for ocean life and range in various colors from red, green to brown. Seaweeds also come in various shapes; for instance, they may be shaped in the form of fingers, broad leaves, spheres, or resemble a fruit [1]. There are about 221 species of macroalgae: 125 Rhodophyta (Red seaweed), 64 Phaeophyta (Brown seaweed) and 32 Chlorophyta (Green seaweed) that have been cultivated and utilized throughout the world. Of those, approximately 66% are used in food. The rest are used wildly in the phycocolloid industry, traditional medicine, agriculture, animal feed and fertilizers [2,3].

Aquaculture is the science of cultivating aquatic animals, plants, and related organisms like fish, shellfish, seaweed, and microalgae, for human use and consumption; and is the fastest-growing food production sector in the world, with an annual increase of 5.8% between 2001 and 2016 [1]. Seaweed is a globally valued marine resource; it is estimated that the global seaweed industry has reached a production value of more than USD 6 billion annually. In 2005, the world's total seaweed production was about 15 million tonnes; by 2015, the total production was around 30 million tonnes [1]. According to the latest data from the Food and Agriculture Organization of the United Nations (FAO), in 2018, the world's total seaweed production was around 33 million tonnes [4]. Commercial harvesting occurs in approximately 35 countries throughout various climates and regions. Global seaweed production continues to grow with the largest producers of cultured species, including China, Indonesia, and the Philippines. In addition, the largest producers of wild-harvested species include Chile, China, and Norway [1,5]. Domestically, Canada occupies a special place among the world producers of seaweeds. There are numerous marine plants off the east coast of Canada, but those of economic significance include the rockweed (*Ascophyllum*), Irish moss (*Chondrus crispus*) and dulse (*Palmaria*) [6,7].

In many maritime countries, seaweeds are used as fertilizer for industrial applications and a food source, mainly in Asia. Notably, in China, Japan and Korea, seaweeds have long been used as a healthy food because of the highly diversified nutritious ingredients and have been considered as a compensation to the traditional food materials [8]. In Japan, seaweeds are served in approximately 21% of their meals, and their daily consumption could be as high as 5.3g/day [9–11]. Asian coastal countries consume the most significant proportion of seaweeds harvested yearly for human consumption [12]. As a sustainable natural resource, seaweeds represent an inexpensive valuable source of nutrients, including minerals, vitamins, soluble dietary fibers, and flavonoids, which are regarded as preventive agents against lifestyle-related diseases [13]. Seaweed products have the potential to be marketed as functional foods due to the presence of fucoxanthin, alginates, fucoidans and phlorotannins that may provide some benefits for body weight control [14]. Other seaweed ingredients, including carrageenan from *Chondrus crispus*, remain highly unexplored in their satiating properties [15,16]. Edible seaweeds, therefore, have received increasing interest in developing low-cost and highly nutritious diets for humans [17]. Besides the potential health benefits, edible seaweeds also provide several inter-sectorial benefits, including the provision of several critical ecosystem services; creating a more balanced and diverse local aquaculture industry; and participating in the dietary shift toward more decarbonized ocean-based sources of proteins [18].

However, the consumption and applications of seaweeds in Canada are very limited due to their unique taste and flavor, unfamiliarity as food ingredients, the widely perceived unavailability of ready-to-use seaweed-formulated foods and the lack of information regarding their health benefits [7]. Growing consumer demand for functional foods has led to a rise in the acceptance and use of traditional and novel whole foods and ingredients, and it is necessary to formulate a broad range of foods with seaweeds that have the potential to be introduced and accepted into the Canadian dietary pattern that might help to decrease the prevalence of metabolic syndrome and other non-communicable diseases by modulating certain physiological responses.

Chapter 2: Literature Review

2.1 Overview of Chronic Diseases in Canada

Chronic diseases, also known as non-communicable diseases or NCDs, are illnesses that persist a year or longer and necessitate continuing medical attention. They are treatable but not curable [19]. Common chronic conditions among Canadian adults are hypertension, osteoarthritis, anxiety disorders, osteoporosis, diabetes, asthma, chronic obstructive pulmonary disease, ischemic heart disease, cancer, and dementia. Of those, obesity, cardiovascular diseases, diabetes, and cancer are the most preventable diet and lifestyle-related diseases among Canadians [20]. According to the 2016 Canadian Community Health Survey, 44 percent of Canadians (aged 20 and above) suffer from at least one of ten common chronic diseases [20]. More than half of Canadian adults reported having one or more chronic illnesses in the 2018 Health Care in Canada (HCIC) survey [21].

2.1.1 Cardiovascular diseases

Cardiovascular diseases (CVDs) include atherosclerosis, coronary heart disease, stroke, heart failure, deep vein thrombosis, and peripheral arterial diseases are the leading cause of death globally. In 2019, an estimated 17.9 million people were reported to have died from CVDs, representing 32% of all global deaths. Of these deaths, 85% were due to heart attack and stroke [22]. Despite significant breakthroughs in therapy, disease management, and public health measures over the previous few decades, heart disease is the second leading cause of mortality in Canada; it is also the primary reason for hospitalization [23]. Approximately 2.4 million (or 1 in 12) Canadian adults (aged 20 and older) had ischemic heart disease in 2012–2013. Furthermore, in 2015, heart disease was the most significant cause of years of life lost owing to premature death in Canada and the second greatest cause of disability-adjusted life years (DALYs) lost [23]. Hypertension, dyslipidemia, and diabetes, all core components of the metabolic syndrome, are all key risk factors for developing CVDs. CVDs can also be influenced by genetic factors or lifestyle variables such as tobacco use, a sedentary lifestyle, or diet composition [22,24].

2.1.2 Cancer

Cancer is a broad term encompassing a wide range of diseases that can affect any body part. The other terminologies used for cancer include malignant tumors and neoplasms. Each year, the number of people diagnosed with cancer rises, and cancer remains a leading cause of global mortality, responsible for nearly 10 million deaths in 2020 [25,26]. Cancer is Canada's leading cause of mortality, accounting for 30% of all deaths [27]. In 2015, 2.1 million (7.1%) Canadians aged 12 years and older were diagnosed with cancer [28]. Cancer risk increases with age, with people aged 70 and up accounting for 43% of new cancer diagnoses and 62% of cancer deaths [28]. The yearly number of new cancer cases is expected to rise by 84 % and 74% in 2028–2032 in Canadian males and females, respectively [29]. Lung cancer is expected to be the most often diagnosed cancer in the general population in Canada, followed by breast cancer in women and prostate cancer in men. In 2022, lung cancer is anticipated to be the leading cause of cancer death, accounting for 24.3 percent of all cancer fatalities, ahead of colorectal, pancreatic, and breast cancers [30]. The incidence and death rates in the eastern provinces of Canada are expected to be higher than in the western provinces [30]. Early detection, diet and physical exercise, smoking and alcohol reduction, and other healthy behaviors can help prevent or control cancer. Primary preventive actions are aimed at preventing cancer before it develops, whereas secondary prevention is concerned with early detection and intervention [31].

2.1.3 Overweight/Obesity

Obesity, which is described as an abnormal buildup of body fat in the adipose tissue, energy imbalance, and lipogenesis, is a result of modern lifestyles that include high fat, sugar, and calorie intake, as well as insufficient exercise and physical activity [32]. According to World Health Organization (WHO), for adults, overweight is defined as a body mass index (BMI) greater or equal to 25 kg/m^2, and obesity is a BMI greater than or equal to 30 kg/m^2 [32]. Obesity has become one of the world's most serious public health problems. In 2016, more than 1.9 billion adults (accounting for around 39% of the world population) were overweight. Of these, over 650 million were obese (accounting for around 13% of the world population). It's expected to increase in the near future, especially among adults [32–34]. In 2018, the prevalence of overweight and obesity among Canadians aged 18 and above was 36.3% and 26.8%, respectively, bringing the total population with higher health risks to 63.1% due to excess weight [35]. Obesity is linked to several disorders, including dyslipidemia, hypertension, and hyperglycemia, all of which are components of metabolic syndrome (MetS) [36]. In addition, increased BMI is a leading cause of non-communicable diseases, such as cardiovascular disease, type II diabetes, musculoskeletal disorders (e.g. osteoarthritis), some cancers (e.g. including endometrial, breast, ovarian, prostate, liver, gallbladder, kidney, and colon) [32]. Moreover, obesity leads to a higher prevalence of Nonalcoholic Fatty Liver Disease (NAFLD), a chronic liver disease which is considered as the hepatic manifestation of MetS, than the general population [37].

2.1.4 Diabetes

Diabetes is a metabolic disorder that is characterized by chronic hyperglycemia resulting from disturbances in insulin secretion or tissue resistance to its action [38]. Uncontrolled diabetes will result in consistently high blood glucose levels, leading to severe complications such as blindness, kidney failure, heart attacks, stroke, nerve damage and lower limb amputation [38]. There are three main types of diabetes: type I, type II and gestational diabetes [38]. Type I diabetes, common in children and youth, is an autoimmune disease in which the pancreas' insulin-producing cells are destroyed by the immune system, causing the body to rely on an external source of insulin for life. Type II diabetes, the most common diabetes among Canadian adults [39], is a metabolic disorder that happens when the pancreas fails to produce enough insulin or when the body fails to use it appropriately (resistant to insulin). Gestational diabetes develops during pregnancy and usually disappears after delivery. However, gestational diabetes increases the risk of developing type 2 diabetes in the future [38].

Among Canadian adults, 6.1% have pre-diabetes, 7.0% have high blood glucose and 1.7% live with undiagnosed high blood glucose [40]. From 2013 to 2014, it was reported that about 3.0 million were living with diagnosed diabetes. In other words, 1 in 10 adults was diabetic [38]. With an average annual increase of 3.3% in prevalence from 2000 to 2016, 3.4 million Canadians, representing 8.1% of the population, were living with diabetes in 2017 - 2018 [41]. According to the national data, an average of 549 Canadians are diagnosed with diabetes every day [40]. In 2019, diabetes was the ninth leading cause of death globally, with an estimated 1.5 million deaths directly caused by diabetes [42].

Although some type II diabetes risk factors such as age, family history, gender, and ethnicity are non-modifiable [40], certain risk factors can be managed by making healthy lifestyle choices such as eating healthily, exercising regularly, and achieving/maintaining a healthy weight. Medication can also help prevent the development of type II diabetes in people with pre-diabetes [43]. Furthermore, social determinants of health such as food insecurity, social support, and built environment can influence individual behavior and must be addressed at a societal level [39].

2.2 Current Trends in Incorporating Functional Foods in Diets

Hippocrates, the father of modern medicine, said, "Let food be thy medicine, thy medicine shall be thy food." Foods are well known for providing nutrients for nourishment and additional health benefits in maintaining biological functions and health [44]. The growing economic and social expenses associated with diet-related disorders such as cancer, diabetes, and cardiovascular disease have fueled growing concerns about diet's impact on health [45]. Consumers are increasingly looking for food ingredients beyond basic nutritional benefits to optimize overall health and prevent chronic illness. Growing consumer interest, combined with a greater understanding of food-health relationships, rising healthcare expenses, and an aging population, has driven the insatiable market for functional foods and natural health products (FFNHP) [46]. The concept of "functional foods" first appeared in Japan in the 1980s, characterizing enriched food ingredients that bring health benefits to the body. United States, Canada, and European countries, eventually adopted the concept, indicating a clear trend toward innovative food marketing [47]. Although the term "functional foods" is not regulated in Canada and is defined in various ways by different organizations, in general, functional foods describe foods being part of the usual diet, being in a food form and containing a bioactive that provides a health benefit. Functional foods can be categorized in various ways and may or may not include conventional foods [48].

In 2015, the global market for functional foods was valued at USD 129.39 billion, with rising consumer awareness of their health and correct diet serving as a significant determinant element driving the sector forward in the following years with an average annual growth rate of 8.5% [47,49]. Japan, the United States, and Europe are the world's leading markets [47]. In Canada, sales from FFNHP companies were $21.5 billion in 2007, with $3.7 billion coming from FFNHP activities. Between 2004 and 2007, revenue in the functional foods industry increased by 28% [48]. Currently, the most common functional foods on the market are yogurt (digestive health), cereals (cardiac health), margarine/butter (cholesterol metabolism), and energy/protein bars and drink (hunger-reduction) [50]. According to a 2012 Ipsos-Reid report, 98% of respondents purchased functional food or beverage the previous year. Cereals (e.g., whole grain, higher fiber, added omega-3s or omega-6s), healthy snack foods, yoghurt with additional probiotics, and juices (with added nutrients and antioxidants) were the most popular functional food products [46].

Certain factors influence the trend of incorporating functional foods into diets. Researchers have identified five determinants that influence consumers' acceptance of functional foods: product characteristics, socio-demographic characteristics, psychological characteristics, behavioral characteristics, and physical characteristics [51]. People tend to purchase functional foods perceived as healthier, less processed, familiar food vehicles and natural enrichments. For instance, they prefer margarine and yogurt over chewing gum, ice cream, and chocolate [52]. Fiber-enriched cereals were found to be more accepted than calcium-enriched juice because of the less healthy combination of juice with calcium [53]. In addition, consumers have higher purchase intentions toward those functional foods with reasonable prices and accurate and objective health information about the efficacy of functional properties or attributes [51]. Regarding socio-demographic characteristics, such as age and gender, a study indicated that older people are typically the primary consumers of functional foods, and female consumers are more likely to consume functional foods than males [51]. Consumers with particular physical characteristics (e.g., diet-related health problems, subjective health complaints, higher body mass index, or poor subjective health) are more likely to incorporate functional foods to improve their health status [51].

In summary, given the competitive nature of the functional foods market and consumers' complicated process of accepting novel foods, understanding the determinants that influence consumer acceptance and their relationships is critical to a successful product launch and developing marketing strategies for the novel functional foods industry.

2.3 Regulation of Satiety/Appetite and Food Intake

Food intake regulation via modification of gastrointestinal reactions to ingested foods is becoming an increasingly important aspect of therapeutic strategies to combat the obesity epidemic. Seaweed has recently received much attention for its potential role in energy regulation by inhibiting energy intake and increasing feelings of satiety [54]. Short- and long-term food intake is regulated by a complex set of physiological mechanisms involving a wide range of molecules interacting through various biochemical circuits [54]. The concept of appetite includes at least two separate components: satiation and satiety. Satiation refers to processes that promote meal termination and thus limit meal size. Satiation could cause a variety of postprandial sensations, such as pleasure, distress, fullness, nausea, or bloating. Satiety refers to postprandial events that affect the interval to the next meal and thus regulate meal frequency [55]. In other words, satiety is a state of suppressing appetite, while satiation moves into a deeper state of satiety. Appetite regulation is largely controlled by an interaction of peripheral signals (hormones, nutrients, neuronal signals) with the central nervous system (CNS), in which the hypothalamus plays a pivotal role [56]. Appetite sensations may also be altered by psychological, social and environmental factors [57,58]. Peripheral appetite regulation mechanisms include the stomach's motor functions, such as rate of emptying and accommodation, which transmit satiation signals to the brain. More specifically, the hypothalamus expresses several important receptors that control appetite, including cannabinoid (CB)3 and neuropeptide Y (NPY) receptors. Many peripherally released long-term and short-term peptides and hormones, including cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), oxyntomodulin (OXM), peptide YY (PYY), apolipoprotein A-IV (Apo-A IV), ghrelin, insulin (a pancreatic hormone) and leptin offer feedback from the arrival of nutrients in various parts GI tract, where they are released to exert satiating effects or regulate metabolism via incretin actions [59]. Ultimately, these peripheral factors provide input to the highly organized hypothalamic circuitry and vagal complex of nuclei to determine the cessation of energy intake during meal ingestion and the return of appetite and hunger after fasting [55,56,60]. The two prominent families of gastrointestinal hormones are appetite stimulators and satiety stimulators. The formal one includes ghrelin, a 28 amino acid peptide that promotes meal initiation by increasing appetite and hunger feelings. Satiety stimulators, such as the gut hormones glucagon-like peptide-1 (GLP-1), PYY3-36 cleaved from PYY1-36, cholecystokinin (CCK) and leptin that signal the brain to decrease hunger and promote meal cessation. In addition, to these GI hormones, insulin, a pancreatic hormone, as well as insulin-regulated glucose, play a significant role in human metabolism and eating behaviour [54,58]

Appetite is also affected by various factors other than physiologic demands. Food provides strong visual, smell, and sensory signals that can override satiety and stimulate feeding [61]. Food taste affects satiation and satiety through a regulatory mechanism, including the mouth cavity, gut, and brain. Taste, as a significant component of flavor, influences the food choices of customers [57]. People prefer to eat sweet and salty foods while consuming fewer bitter or sour ones. The taste and smell of food can have a significant impact on eating behaviors. Sensory-specific satiety directly impacts the sensory characteristics of foods and can help people stop and gradually reduce further eating after having a specific meal [57].

2.4 Seaweeds Classification

Seaweeds are also called macroalgae that are visible to the naked eye and include a vast and diverse group of relatively complex, multicellular algal species, with sizes ranging from a few centimeters to specimens that can reach 100 m in length [62]. Approximately 10,500 species of this group have been described [63]. The word 'algae' is used to classify a large group of heterogenous organisms that do not have a specific taxonomic status [64]. Over 30,000 species of algae have been discovered and classified in a variety of colors, shapes, and sizes [65]. Despite seaweeds, like plants, take on many distinct shapes and structures, they have four basic parts consisting of a holdfast, stipe, blade and float. The structure as a whole is called the thallus [66]. Although seaweeds are also autotrophic and photosynthetic beings, they are different from terrestrial plants. Unlike land plants that nourish themselves by the root system, seaweeds adsorb nutrients throughout their whole surface by passive diffusion and active transport; they also do not present conductive tissues. They also lack roots, though some present rhizoids or basal discs that allow them to adhere to rocks as a method of restraint but not to nourish themselves [65].

Seaweeds can be classified into three broad groups based on pigmentation, carbohydrate storage chemicals, and cell wall structure: brown macroalgae (Phaeophyta), red macroalgae (Rhodophyta), and green macroalgae (Chlorophyta) [64]. Brown seaweeds, such as *Saccharina japonica* (Japanese *kombu*), *Undaria pinnatifid* (Japanese *wakame*) and *Sargassum fusiforme* (Japanese *hiziki*), are usually large and range from the giant kelp that is often 20 m long to smaller species that are 30–60 cm long. Red seaweeds, such as *Porphyra* spp., *Kappaphycus alvarezii* and *Gracilaria* spp., are usually smaller, generally ranging from a few centimeters to about a meter in length. However, red seaweeds are not always red. Some of them are purple or brownish-red. Green seaweeds (*Enteromorpha clathrate, Monostroma nitidum Caulerpa* spp.*)* are also small, with a similar size range to the red seaweeds [1,7].

2.5 Seaweed Aquaculture

Seaweeds have represented the largest group of marine and coastal aquaculture organisms since 2004 and were the first group of organisms to pass the 50 percent farmed/wild harvest in 1971. Since then, farmed freshwater fish production reached this milestone in 1986, farmed mollusc production in 1994, farmed diadromous fish production in 1997 and farmed crustacean production in 2010 [67]. Recently, the FAO released The State of World Fisheries and Aquaculture, which provides an abundance of information on both fisheries and aquaculture globally. According to this report, seaweeds represent 51.3% of the total production of marine and coastal aquaculture, followed by molluscs (27.4%), finfish (11.6%), and crustaceans (9.1%) and other aquatic animals (0.6%) in 2018 [68]. Compared with 2000 (10.6 million tonnes), the world seaweed production in 2018 has more than tripled, up to32.4 million tonnes in 2018 [68]. In 2018, farmed seaweeds and wild-collected seaweed represented 97.1% and 2.9% of total seaweed production, which amounted to 31.5 million tonnes and 0.9 million tonnes, respectively [68]. The rapid growth rates in seaweed mariculture occurred within the periods of 2000 to 2005 (increased by 40%), 2005 to 2010 (increased by 36%) and 2010 to 2015 (increased by 53.4%) [68]. From 2000 to 2015, the world's seaweed production nearly tripled from 10.6 million tonnes in 2000 to 31.1 million tonnes in 2015. The slowdown in growth rates in recent years seems to be caused mainly by the slowing growth in the farming of tropical species in southeast Asia, while the farming of temperate and cold-water species is still rising [18,68].

World seaweeds mariculture has taken place dominantly within the east and southeast Asian countries (China 51.7%, Indonesia 28.8%, Republic of Korea 5.3%, Philippines 4.6%, Democratic People's Republic of Korea 1.7%, Japan 1.2%, Malaysia 0.5%) with a share of 99.5% world total production in 2018. Zanzibar (United Republic of Tanzania) and Chile produced minimal amounts (0.3 and 0.1%, respectively) of the world's seaweed aquaculture production, while other producers of the world only shared 0.1% of seaweed production [68]. The global seaweed industry is relatively underdeveloped, fragmented and regionalized outside of Asia. However, there has been significant progress on seaweed farming in North America, Europe and South America, mainly through the development of integrated multi-trophic aquaculture (IMTA), which promotes ecosystem services [69].

Eight genera of seaweeds: *Saccharina japoni*ca (known as kombu or *Laminaria japonica*; 35.3%), the carrageenophytes *Eucheuma* (29.0%), the agarophytes *Gracilaria* (10.7%), *Porphyra* and *Pyropia* (known as nori; 8.9%), *Undaria pinnatifida* (known as wakame; 7.2%), the carrageenophytes *Kappaphycus* (4.9%) and *Sargassum* (0.8%), were the major organisms produced in the global mariculture and provided 96.8% (31.3 million tonnes) of the world seaweed aquaculture production in

2018. Of those, two genera of seaweeds, the brown seaweed *Saccharina* and the red seaweed *Eucheuma*, were the two most-produced organisms in mariculture in the world. Some seaweed species, such as *Undaria pinnatifida* and *Porphyra* (produced in East and Southeast Asia), are directly used as human food [68].

2.6 Seaweed Resources in Canada

Canada is the second-largest country in the world, with the longest coastlines of any country, measuring 243,042 km on three oceans (Pacific, Arctic and Atlantic) [70]. Canadian coastal waters support valuable biological resources, including seaweeds. Many species of seaweeds occur along Canadian coasts: about 175 are reported in the Arctic, 350 in the Atlantic, and nearly 500 in the Pacific. Canada ranked first as the world's red seaweed *Chondrus crispus* producer approximately from 1950 to 1970 [6]. Domestically, seaweed aquaculture is relatively small but is a growing sector in Canada. There were approximately 600 seaweed species found in British Columbia (BC). The cold, clean water and a steady supply of nutrients of the Pacific Northwest coast support the world's greatest diversity of kelp species. There are 20 kinds of kelp alone; of these, the giant kelp (*Macrocystis integrifolia*) and bull kelp (*Nereocystis luetkeana*) are the dominant kelp species in BC [71]. These two species are also known as "floating", or "canopy" kelps due to much of their stipe and blades floating at the water surface that form a dense canopy [72]. Historically, kelps have been harvested mainly for their alginates used in various industrial activities, such as food processing, fertilizer and construction. Kelp harvest in BC has been attempted since the mid-20th century. However, several industrial larger-scale harvest attempts have failed, and all harvesting now is small-scale [72]. Kelp production in BC increased 43% between 2014 and 2016. The production in 2016 was almost double the production from the early 2000s [72]. Although most red seaweeds are less abundant than brown seaweeds, they still support traditional harvests in BC, especially in northern BC among first nation communities. On the Pacific Coast of Canada, indigenous people have been harvesting and using *Porphyra* species (red seaweed, *nori*) for a long time. The main species eaten is *Porphyra abbottae* [73].

On the Atlantic Coast of Canada, the biodiversity richness of seaweeds is exceptional, given that seaweeds in this region have to survive drastic temperature changes. Nevertheless, a few species have led to the development of three notable fisheries: Irish moss (*Chondrus crispus*), rockweed (*Ascophyllum* *nodosum*) and dulse (*Palmaria palmata*) harvesting [6]. The red seaweed *Chondrus crispus* is widely distributed in the northern Atlantic. Large beds of *Chondrus crispus* were identified in the Maritimes as early as 1830. However, harvesting in Prince Edward Island (PEI) and Nova Scotia (NS) remained minimal until World War II [6]. The harvest of *Chondrus crispus* was primarily for producing gels and Acadian desserts back then. However, since the early 1990s, *Chondrus crispus* harvesting has become more and more a complementary fishery. *Ascophyllum nodosum,* representing the highest biomass landings since 1987, is the dominant species of the Maritimes. Harvesting for commercial purposes in southwestern NS began in 1959. Harvesting and consumption of dulse in coastal communities along the Bay of Fundy have a long tradition; most dulse originates from Grand Manan Island [6]. Moreover, in NS, Brier Island has the highest species richness of a limited area of eastern Canada, with 152 species and varieties of seaweeds in 2017. These species included 62 Rhodophyta, 44 Chlorophyta and 44 Phaeophyceae. Of these species, over 35 were new to Digby Neck, 12 were new for the Bay of Fundy, one was new for NS, and three were new records for Canada [74].

2.7 Seaweed Cultivation and Harvest

There are two basic types of seaweed farms: small-scale cultivation and large-scale cultivation. Small-scale seaweed farms produce red algae in small patches of intertidal sand flats, primarily in the tropics. Larger-scale farms, mainly in the temperate zones, where rope lines seeded with (usually) brown algae spores in a hatchery are applied [75]. Asia has a long history of large-scale cultivation of seaweeds globally, while it is a recent commercial activity in North America and other parts of the world. Although there are also pilot-scale and pre-commercial farming projects for selected brown and red seaweed in Europe, Latin America and North America, the primary production of the seaweeds outside Asia is still natural stock harvesting [76]. Seaweed aquaculture is relatively small but is a growing sector in Canada. In Nova Scotia, the largest land-based seaweed cultivation operation in the world has been developed resulting in a high productivity [77]. However, traditional methods of harvesting wild seaweed are, by its nature, unreliable. The work is labour-intensive and costly. With the increasing global efforts to develop seaweed farms, the countries' production and commercialization strategies differ. For instance, there is a higher demand for seaweeds as direct food products in Asia, whereas, in Western countries, seaweeds' application is in the polysaccharide industry [78]. Experts observed that the increasing demand for seaweeds as food products could only be adequately met by cultivation [79].

Generally, seawater without contamination is the primary environmental requirement for seaweed cultivation. Other environmental conditions such as temperature, solar radiation, salinity, pH and nutrient availability also influence seaweed growth. In other words, areas with adequate light and nutrients, salinity and temperatures suitable for seaweed growth are required for seaweed cultivation [76]. However, different species do require different environmental conditions. Moreover, seaweed cultivation also depends on the productivity and adaptability of a species in the aquatic ecosystem [80]. Seaweed cultivation can be performed offshore, onshore and even in aquaculture integrated systems. All cultivation methods may be divided into two main groups: extensive and intensive. Extensively cultivated seaweeds are grown in natural water areas using only naturally available light, heat, water motion energy, and nutrients. Extensive cultivation is usually used for gathering large crops of seaweeds with definite properties, such as a high content of polysaccharides (for the production of alginates, agar, carrageenan) or good taste (softness, taste, and smell). Sometimes seaweeds are cultivated extensively for the production of medicines or dietary supplements [80]. On the other hand, intensive cultivation includes the cultivation of one or several seaweed species in tanks using natural or artificial light, nutrients and phytohormones; in small natural water such as ponds, lakes, and lagoons, using organic and inorganic fertilizers and applying agronomic techniques such as weeding, reducing epiphyte growth, regulating light, and water motion [80].

To date, commercial seaweed cultivation has primarily occurred in areas with adequate sunlight, moderate temperatures, ready water sources, and low-cost nutrients. Open ponds raceway (OPR) systems are the most prevalent commercial-scale seaweed cultivation methods. These are considered the most cost-effective and relatively low-technique, which offer good potential for a viable and economically sustainable operation [81]. However, the OPR system has significant drawbacks and vulnerabilities from a geographic and climatic perspective and is not ideal for a Canadian context. Since cultivation can only operate for four to six months annually, this is not economically viable. In order to overcome the climatic challenge, several alternatives (controlled environmental algae growth technologies) have been developed, including photo-bio-reactor (PBR) systems [81]. These systems allow seaweed cultivation under phototrophic/autotrophic conditions, flat plate and membrane systems, plastic/glass tube systems, and fermenters that take advantage of algae's unique capability to grow in heterotrophic conditions in the absence of light and rely on carbon sources for the energy used in growth [81]. Other seaweed cultivation systems use mixotrophic (both autotrophic and heterotrophic) conditions to achieve growth objectives [81].

Wild harvesting represents only 2.9% of total world production; however, it supports local consumption and a sizable phycocolloid industry in several parts of the world [68]. Traditionally, two modalities are followed: harvesting seaweeds directly from their natural growing sites and gathering them from beach cast, especially following storms [82]. Harvesting from the natural environment is usually either total, by removing the complete plants, or partial, by cutting the fronds at a certain height from their attachment point, leaving behind the rest of the plant for regrowth [83]. Techniques used in wild harvesting range from manually harvesting (hand pulling, using rakes or other tools that remove the seaweed from rocks or the bottom where they grow) to mechanical harvesters. Harvesting cultivated seaweeds and bringing them to land is a key and relatively costly aspect of sea farming compared to wild harvesting. Based on the scale of the operation, harvesting methods are different. For instance, on a large seaweed farm, harvesting operations use various mechanical harvesters, including winches and cranes in which cranes may be used to unload the harvested material. Depending on the crop being farmed or the cultivation technique, harvesting may be total or partial [83].

2.8 Environment Impact and Ecosystem Services

The world is facing serval environmental challenges, namely, deforestation, air pollution, global warming, water pollution, natural resource depletion, etc. [84]. Literature has contributed to how seaweed farming has benefited the improvement of the environment and ecosystem. Studies have shown that the seaweeds industry has played many functions along with the coastal countries, helping to gain sustainable development of the local economy and society.

2.8.1 Improving the world climate

Studies have pointed to the great benefit of the world seaweed industry in improving the climate, which has been assumed as an effective alternative for solving the worsened problem of climate change. For instance, the seaweed aquaculture industry and technologies have contributed significantly to the world climate. Algal materials could effectively be used to produce renewable products such as paper, fertilizer and biofuel, saving many other natural resources such as forests and fossil fuels. This implies that the world industries would significantly cut pollution and environmental deterioration using seaweeds as an effective alternative [85]. The World Bank Group has also done fruitful research on the use of seaweed materials in contributing to climate change improvement. According to a study by the World Bank, an annual harvest of 500 million dry tons of seaweeds with 50% carbohydrate content could produce about 1.25 billion megawatt-hours, which implies the saving of millions of tons of fossil fuels for replacement. This is an excellent contribution to protecting the worsened world climate in this age of fast growth of the world industries [86].

2.8.2 Contribution to ecosystem services and eco-diversity

Seaweed aquaculture has been viewed as an alternative or complement to terrestrial biomass production, as seaweed farming does not require fresh water and arable land, and in most cases, fertilization is not needed [87]. Besides the potential contribution to economic activity, seaweed cultivation also provides ecosystem services, such as oxygenation, carbon sequestration, uptake of nutrients, and habitat protection for humans and marine organisms, which improve conditions of the coastal waters for the benefit of other living organisms and the environment [88,89]. The application of IMTA creates a more balanced ecosystem by combining the fed aquaculture (e.g., fish or shrimp) with the extractive aquaculture (seaweed and shellfish) by recycling waste and absorbing excess nutrients created by the farmed fish species. Specifically, seaweeds absorb nitrogen, phosphorus and carbon dioxide, which are used to grow and produce energy storage products. When seaweeds are harvested, these nutrients are also removed from the water [85,90]. Several studies show that large-scale seaweed cultivation can help purify the water from potentially harmful nutrient evaluation by rapid and effective nutrient absorption in the water [91–93]. Hence, seaweed can be used as an extractive component to remove inorganic nutrients and mitigate potentially adverse environmental impacts.

During the past decades, with the fast development and expansion of the world industries, there has been significant damage to the world ecosystem, causing the extinction of a wide range of species worldwide. Scientists have conducted many types of research to study both the direct and indirect reasons for the cause of ecological deterioration, and most of them have concluded that industrial development is one of the significant reasons. However, the development of the seaweeds industry has provided a possible solution to this problem by improving the ecosystem. For instance, an experiment conducted on the sea areas off southern Norway has found that the seaweed industry could contribute to various effects that are important in protecting the natural ecosystem, including the enhanced benthic oxygen flux benefiting the marine lives locally [94]. Similar research done by another research team has found the benefits of seaweed materials as food materials for a wide range of sea species contributing to the diversity of sea-based lives [95].

2.9 Current Regulations and Policy in Canada

In Canada, it is by and large the provincial governments that regulate the cultivation and harvesting of seaweeds, though the federal government sometimes issues non-targeted but relevant polices, such as environmental and ecological policies. Initially, seaweed harvesting was an open industry around the 1950s and 1960s without a limit on the number of harvesters. However, with the emerging risk of disturbing and destroying the aquatic ecological balance of the Canadian sea area, a series of policies have been made by provincial governments [96]. For instance, BC adopted a license granting policy in the local fish and seafood licensing regulation, requiring individuals and organizations to grow and harvest seaweed plants with government-granted licenses [97]. Similarly, in NB and NS, similar policies have been made to guard against the over-exploitation of seaweed and other aquatic resources to preserve ecological balance [6].

The license granting system in Canada has been firmly based on scientific and industrial research results, which have pointed to the risk of over-exploitation of sea plants in the Canadian sea areas. For instance, In NS and NB, studies on the value of Rockweed (*Ascophyllum nodosum*) as the Habitat for tide-pool fishes resulted in issuing policies that prescribed the maximum exploitation rate and protected areas [96]. At the federal level, precautionary policies were made to limit seaweed overexploitation after investigating the potential risks of over-exploitation of seaweeds and other aquatic plants in Canada. With the adoption of the limitation policy, positive changes have occurred in Eastern Canadian coastlines with the increased rockweed beds for the habitation and prosperity of tide-pool fishes and other species of aquatic life [98].

Overall, the provincial and federal policies have played a crucial role in regulating the sustainable development of the Canadian society and aquatic regions with the limitation of seaweed cultivation and harvesting nationwide. The policies have been adopted to protect the ecological balance as well as the benefits of the seaweed consumers. For the time being, a wide range of measures have been taken by the provincial and federal governments to guard against overexploitation of seaweeds [99]. Apart from the license granting system, the policy of lease areas has also been adopted, both of which are very effective for controlling the cultivation and exploitation of aquatic resources in Canadian sea regions.

2.10 Market Analysis

2.10.1 Current seaweed market in Canada

Traditionally Canada is not the main seaweed production area because of the eating habits of the local population. According to the latest report by Mordor Intelligence about the commercial market size of the world, Canada belongs to the lower-sized markets of the world to produce seaweed foods [100]. Compared with Asia, Oceania and Europe, North America has a lower capacity of seaweed production [1]. However, as one of the world's nations with the longest coastal line, Canada has been focusing on the increased development of the seaweed industry. Various Canada-based seaweed companies have been established and developed steadily, which helps maintain a comparatively big seaweed market both domestically and overseas [101]. Currently, the seaweed market of Canada is mainly located and distributed on the Eastern coast of Canada [6,102]. However, the federal and local governments deliberately limited the market size of seaweed agriculture in fear of disturbing the natural balance of marine life. In this way, it usually takes a very long time to secure a license for seaweed products from the federal and provincial governments in Canada, which has served as the main obstacle to the expansion of seaweed market development domestically [102].

2.10.2 Commercial seaweed products in Canada

With the world's longest coastlines Canada is highly rich in seaweed raw materials. For instance, in Eastern Canada, there is abundant Irish moss, which has broad market potential in producing seaweed food [1]. However, the lower capacity of seaweed production has substantially limited seaweed products in the world market. The seaweed products produced in Canada are mainly for the overseas markets (especially the market of Eastern Asian nations) [103]. A company based in BC produces a dried brown algae product known as BC Kelp which has been very popular among Western Asian consumers. The product series (**Figure 2.1**) are considered highly nutritious and sustainable edibles with premium quality. It is very rich in vitamins and minerals, which are of great benefit to people's health. In Atlantic Canada, rockweed (*Ascophyllum*), Irish moss (*Chondrus*) and dulse (*Palmaria*) are the most well-known commercial seaweed crops [104]. In retail stores (e.g., Atlantic Superstore) in Halifax, seaweed sheets (nori, for sushi) and dry dulse (**Figure 2.2**) are the common seaweed products. The sales of the Acadian Seaplants's Hana Tsunomata® seaweed products (**Figure 2.3**) are booming all over Canada and the U.S. through traditional and online distribution channels [101].

Figure 2.1: Canadian Kelp Series of Dried Seaweed (products of BC Kelp). Source from: http://canadiankelp.com/shop/

Figure 2.2: Seaweed Products at Retail Store. Left: seaweed sheets for sushi, right: dry dulse. Picture of seaweed sheet: https://www.atlanticsuperstore.ca/seaweed-sheets/p/20750367_EA

Figure 2.3: Food Items with Hana Tsunomata®**.** Acadian Seaplants Photography courtesy of Acadian Seaplants Limited. Source from: https://www.acadianseaplants.com/hana-tsunomata/

2.10.3 Seaweed marketing in Canada

In Canada, the federal and provincial governments have deliberately controlled and limited seaweed marketing. The limitation has been based on the total consideration of the balanced aquaculture development and the abundance of the wild sea life resources along the Canadian coastline [105]. The over-development of seaweed production would disturb the balance of the aqua ecology, posing an additional danger to the marine environment. However, despite the limitation, the lure of a big world seaweed market has promoted the local seaweed companies in the private sector. Acadian Seaplants Limited is a top-rated seaweed company in Nova Scotia. The company has been recognized globally for processing seaweed-based food products with highly advanced biochemical and agricultural technologies [103]. The company is primarily known for the large sum of resources spent researching seaweed formulas to produce tasty and nutritious seaweed-based foods. Acadian Seaplants Limited has also been known for the development of expertise in a wide range of seaweed processing technologies that have the potential to produce seaweed foods with bioactive components. Another well-known company is B.C.'s Cascadia Seaweed, which was established in 2019 and is now the largest seaweed provider in the North American regions [106]. Overall, these companies belong to the private sector of the Canadian economy, proving to be flexible in adjusting their product according to the ever-changing trends of seaweed food products across the world market.

2.10.4 Potential impact of commercial seaweed on Canadian economy growth

Though aquaculture and seaweed production are not fully developed because of the limitations of governmental policies and people's dietary habits, Canada has witnessed increasing seaweed industry development and a bigger seaweed market domestically. This has dramatically impacted Canadian economic growth in recent decades [105]. For instance, in some areas in Canada, algae farming has become more active in coastal provinces, making the industry more and more influential on the local levels. Currently, seaweed cultivation is a potential new industry in Canada, especially since the 2010's with the full integration of the Canadian economy into the world market. Currently, seaweed harvesting is a big industry along the coastal provinces, totalling roughly 800 to 1,000 MT every year, contributing enormously to the domestic economy of Canada [107].

2.11 Seaweeds Consumed as Human Food and Its Contribution to Food Security

Seaweeds have not been popular because of its salty taste, fishy smell and slimy texture. As shown in the section above, seaweeds have been used in various ways in industry. However, the most common seaweed usage comes from human consumption.

Seaweeds used as human food dates back to 12,000 BCE in southern Chile, where seaweed remnants were discovered in hearths and other features in Monte Verde. This discovery proves that the early settlement of South America originated along the Pacific coast and included seaweeds as a part of their diet and health [108]. The use of seaweed as food, especially in Asia, can be traced back 2,500 years ago. The first written description of seaweed dates back to 600 BCE in China. The Chinese philosopher, Sze Teu, wrote that seaweed was for "most honorable guest, even the king himself" [1,109,110]. Food remains containing a mixture of brown seaweeds, shellfish, and fish have been found on Japanese prehistoric archeological sites [111]. Up-to-date, about 21 species of seaweeds are used in everyday cooking in contemporary Japan, six of them since the 8th century [109]. Among central and southern Pacific Ocean areas, many types of seaweed have been an essential part of the diet in Hawaiian and other Polynesian islands for thousands of years. European historical sources document that seaweeds have been used as a food ingredient in Norway, Iceland, Scotland, Ireland, Brittany (France), and Wales for centuries [112]. Seaweed was regarded as a seasonal food item or was marketed locally seasonally [112]. For example, dulse (*Palmaria palmata*) has been used as human food in Norway; traditional preparations of laver (Porphyra spp.) in Wales include boiling it and serving it with cockles and bacon or frying it with oats to make laverbread; in fifth-century Ireland, dulse was used as a condiment with bread, butter, and milk [112–114]. Ireland still maintains a rich tradition of using algae in soups, stews, bread, and salads [112]. In some North American regions, such as British Columbia, Nova Scotia, and California, seaweeds have been used as a food source due to the migratory movements in the mid-nineteenth century that established significant Asian (Chinese, Japanese, and Korean) and European (Irish and Scottish) communities [115]. Among indigenous communities across North America, seaweed have been traditionally consumed for millennia. For instance, the Inuit of Greenland consumed cooked bladderwrack (*Fucus spiralis*), knotted wrack (*Ascophyllum nodosum*), winged kelp (*Alaria esculenta*), and dulse (*Palmaria palmata*) as a staple food during the winter. In times of famine, along the northwest coast of North America, the Kwakwake'wakw, Haida, Heiltsuk, and Tsimshian, etc., relied on red laver (*Porphyra/Pyropia*) to provide essential vitamins and minerals. Other than red laver, fresh fronds of the brown seaweed bull kelp (*Nereocystis luetkeana*) were cooked and used in stews and soups as the traditional dishes by indigenous people along the northwest coast of North America. Northeast indigenous people of the Iroquois, Wampanoag, and Arcadian communities consumed and preserved food with sea lettuce and red laver, from which they obtained necessary salts and trace minerals for survival [73,116].

Today, China, Japan, and the Republic of Korea are the largest seaweed consumers in forms of raw, dried, or cooked, such as salads, soups, and main dishes, including sushi, as well as in processed forms such as flavorings in chips and snacks [1]. Especially in Japan, the daily amount of seaweed consumption is estimated at 4–8 g per capita (dry weight), which is served in approximately 21% of meals [111,117]. The main seaweed species that have been used as a food source in these countries are kombu (*Saccharina*), nori (*Porphyra* spp.) and wakame (*Undaria*) [118]. Another example of seaweed that consumers highly appreciate in Asia (Indo-Pacific region) is *Caulerpa*, with the species *C. lentillifera* and *C. racemosa* var. *turbinata*, commonly known as "green caviar" [119]. The purple laver, also known as nori, is primarily used in sushi. Other than making sushi, toasted "nori" can be cut into small pieces and sprinkled over boiled rice or noodles or added to soups, bread, and salad. In China, it is mainly used in soups and for seasoning fried foods. In the Republic of Korea, a popular snack with beer known as "Hoshi-nori" is quickly fried in a pan with a bit of oil [110,112].

In Europe and North America, dulse (*Rhodymenia palmata*) is the most common of edible seaweeds, which is harvested in Ireland, on the eastern coast of Canada, and Maine [110,120]. Dried dulse is frequently offered as a salty cocktail snack at bars in Nova Scotia and Maine. In Ireland, it is consumed either uncooked, chewed like tobacco, or cooked with potatoes in soups and seafood meals. Dulse can be added to bread, fish meals, fish and vegetable soups, toasted and eaten as a snack, or it can be fried crisp to replace fried bacon in modern cuisine [110,112]. Several small-scale businesses in Ireland currently harvest, process, and package edible seaweeds for sale as health foods. France was the first European country where seaweeds were approved for human consumption, which opened new opportunities for the food industry. Fresh seaweeds from Brittany are consumed in France. Seaweeds are sold in various ways, including raw goods, condiments, and spreads [113].

With the increasing world population, the world today is confronted with the massive pressure of food shortage. One in seven people worldwide cannot access sufficient diet and nutrition properly. In this way, the world's food production capacity does not match the rising demand by the world's population, which has led to a large-scale food crisis. Thus, the whole world urgently needs to find complementary nutrition resources to meet the increasing food demands, and seaweeds are an essential alternative of food materials available in many coastal areas [121]. As mentioned above, the increased world population has led to the worsened problem of food shortage. The crisis is also caused by world climate change and economic slowdown by economic recession, and unexpected disasters. For instance, the current Covid-19 pandemic is an important cause of food recession with the decreased food production capacity worldwide. In addition, with the worsened world economy, many people in the underdeveloped regions have been affected by the lower capacity to purchase food products, which has led to large-scale famine in many places. For instance, more than 200 million people in the Sub-Saharan areas are undernourished. This is a severe situation confronted by the world [122,123].

With the increasing food shortage problem, the world urgently needs to find alternative food materials as complementary supplies to increase nutrition. Seaweeds as nutritious food materials have been widely accepted in some coastal regions such as East Asia [121]. However, seaweeds are under-exploited in many other regions such as Europe and North and South Americas. This implies a significant potential for further exploitation since many seaweed species are edible and nutritiously abundant. In some Asian countries such as China and Japan, seaweeds are important ingredients of the local cuisine. It is expected that seaweeds can be used as important alternative materials for food production and improve the state of nutrition of the world's population [95]. In addition, the production and consumption of seaweeds are expected to promote the development of marine culture as an important economic sector, which could be crucial to altering the economic recession impacted by the current Covid-19 pandemic.

To summarize, seaweeds as nutritious materials are important food alternatives for the world's population. This has a latent significance in tackling the problem of food security and food crisis worldwide. Especially in the vast coastal regions in Europe and North America where seaweeds are not fully cultivated, it is expected that seaweed plantation and production will become a potentially crucial industrial sector that would contribute to the local economic development. In addition, the people in these regions would improve their nutrition by including seaweeds in their diets.

2.12 Acceptance of Seaweed in Western Countries

Compared to other Asian countries, seaweed consumption is limited to specific coastal populations in the western world (e.g. some coastal Atlantic communities such as Ireland, Brittany, Maine and Nova Scotia) and is not a traditional food in the Western diet [124,125]. However, seaweed is becoming more popular in western societies due to the cosmopolitan development of the human palate, the public's understanding of nutritional science, and their desire for healthy alternatives. It is moving away from the traditional discourteous attitudes and becoming recognized as an edible, tasty, and healthy addition to people's diets and may have the potential for becoming more central elements in Western human diets than currently realized. East Asian diet worldwide has gradually increased public interest and acceptance of seaweed as a food source, partly due to their suggested health benefits. Consequently, consumption of seaweed and seaweed-based products is rising, similar to the trend observed with fresh fruits and vegetables [118].

2.12.1 Factors influencing consumer acceptability of food

Factors influencing people's desire to consume a type of food are usually highly complicated. On the one hand, habitual factors are closely related to people's conventions and taboos, and on the other hand, sensory factors and familiarity with food are essential in influencing people's appetite and attitude toward food [126]. Tradition plays a significant role. Most Westerners think seaweed is a highly daunting food material that causes intrinsic neophobia [125]. The lack of knowledge about cooking with and incorporating seaweed into the diet, as well as a lack of access to many seaweed-containing food items, may contribute to the limited seaweeds consumption [127]. Overall, people have a variety of intrinsic factors, such as innovativeness, belief and knowledge of foods, that influence their emotions toward foods. People are most reluctant to accept a new type of food just because they are unfamiliar with it. Moreover, as long as people understand more about the food, their attitude toward it may change for better acceptance [128].

While research has provided evidence for the abundant nutrition contained in seaweed foods, there has been a sense of neophobia toward seaweed food in Western society. Food neophobia refers to the intrinsic fear or horror of eating new and unfamiliar food. A study conducted among Spanish consumers revealed that the reluctance of the Spanish to try seaweed foods is due to its high degree of novelty that has strongly impacted people's consuming behaviors toward seaweed foods [129].

With the continued pressure of population growth, there has been a tendency to study the broader scope of food materials globally. Some protein alternatives, such as insects, cultured meat, pulses and algae, contain rich nutrients; however, consumers showed a negative attitude towards those novel food ingredients. Sometimes the concern comes from the worry that these alternative food substances may contain harmful substances to people's health [130]. For instance, the public's concern about seaweed as a nutrition alternative comes from the sodium content, which might cause high blood pressure and heart diseases [131]. The educational interventions on the benefits of consuming seaweeds have resulted in gradually changed prejudice and attitude toward seaweeds in consumers [131,132]. Research has concluded the rising popularity of seaweed by Nordic consumers in North Europe [131]. In France, many consumers are also becoming habitual in eating foods containing seaweed materials. Because of less fat content, the acceptability of seaweed has also been increasing among specific social groups such as vegetarians and bodybuilders [133]. Dried seaweed showed the greatest purchase-intent scores among other seaweed-containing food items and consumers believed seaweed could be added to fish, savoury, and cereal grains-based foods [134].

To summarize, in Western society, though there has been remarkable progress in the studies of nutrition and benefits of seaweed foods for people, there are still substantial social and psychological barriers that stop people from trying seaweeds as food. There is still a long way to go to have a fundamental change. For the time being, a better solution to change people's attitude toward seaweeds in Western nations is to popularize the benefits with the scientific and medical elucidation of the nutritional substance contained.

On the other hand, improving the sensory and taste of seaweed foods to increase the acceptability of seaweeds by Western society, in general, is also important.

2.13 Incorporating Seaweed into the Canadian Diet

It might be challenging to determine what exactly counts as "Canadian food" as Canada is a multi-ethnic, restaurant-heavy country. Most Canadians generally have a predominantly "western" diet with a major emphasis on processed grains and dairy products, farm-grown cattle and chicken, limited cooked or fresh fruits and vegetables, and high salt and sugar levels [135,136]. According to the most updated Canada's food guide, Canadians are encouraged to consume various vegetables, fruits, whole grains, and protein-rich foods with an emphasis on plant-based protein sources, drink plenty of water and limit highly processed foods [137]. On the other hand, seaweeds are high in biologically active components such as carbohydrates, proteins, minerals, oils, lipids, polyunsaturated fatty acids, and antioxidants (polyphenols, tocopherols, vitamin C, mycosporine-like amino acids) [118]. There has been a renewed interest in seaweeds in Norway, Iceland, and Ireland, although it is a traditional component of the diet [138], due to its image as health or "superfood," increased demand for snack foods, and the growing popularity of Asian cuisine, seaweed consumption is booming in these countries [1].

Seaweeds are considered novel food ingredients by many Canadians; therefore, incorporating seaweed ingredients into Canadian's daily diet faces several challenges. Some challenges include a lack of awareness of seaweed's potential health benefits; concerns about the pollution of seaweed with industrial waste, heavy metals, etc.; sensory changes in the product containing seaweed ingredients, and changes in the physicochemical properties of food [139]. Seaweed can be used to provide taste, texture, and additional nutrients to bread, soups, salads, and even ice cream. Salsas, sauces, salads, pasta, snack bars/chips, seasonings, and flavoured products are some examples of value-added edible seaweed products [110]. The most practical and quickest technique to increase intake in the Western diet is to incorporate seaweed flakes or powder into the dough, for instance, bakery and pasta products, the most extensively consumed food products all over North America [139,140].

Seaweed hydrocolloids are increasingly being sought in the baking industry for their capacity to improve dough handling capabilities and improve the quality of fresh bread by minimizing moisture loss and crumb dehydration during storage. Furthermore, alginate has an anti-staling effect during storage, slowing crumb hardening and extending the shelf life of stored bread [141,142]. A study on the effect of brown seaweed powder (*Fucus vesiculosus*) addition on physical and textural properties of wheat bread indicated that a maximum of 4 % addition significantly modified wheat dough and bread properties without affecting the density or crumb quality [143]. However, researchers found that the addition of seaweed powder dramatically increased the bread crust's green colour, which could negatively impact consumer acceptance [143]. Another study also found that the addition (2–8%) of red seaweed powder *Kappaphycus alvarezii* increased the water absorption capacity of the dough, decreased stickiness but increased firmness of the bread [141]. Another study demonstrated seaweed's acceptance in a Western community and indicated that brown seaweed (*Ascophyllum nodosum*) and red seaweed (*Chondrus crispus*) were found to be most acceptable as components in bread at the levels of 4% and 2%, respectively [144].

Pasta is a source of carbohydrate that is gaining popularity due to its nutritious characteristics, particularly its low glycemic index (GI) [139]. Moreover, it is a staple food in many countries, and pasta products are well-accepted worldwide. However, pasta is low in protein and essential amino acids [145]; thus, recent research has attempted to enhance its nutritional characteristics by adding supplements from various high-protein sources. A study showed that adding 10% Wakame (*Undaria pinnatifida*) increased pasta's protein, fibre and fat content, and meanwhile had no negative impact on customer acceptance [146]. Another study indicated that adding Indian brown seaweed up to 2.5% to enrich the pasta may improve bio-functionality/quality and the utilitarian value of seaweeds as food products [145]. Seaweed could be used as a rich source of carotenoids, including astaxanthin and fucoxanthin, as well as dietary fibre, and can be incorporated into pasta products with little sensory impact. Wakame seaweed was shown to have considerable potential to enhance for fucoxanthin content in pasta [145].

Seaweed can also act as a source of salt to be added to dairy or meat products. Sensory investigation revealed that yoghurts containing brown seaweed extracts showed positive acceptability, and there was no effect on shelf-life properties, such as pH, microbiology, or whey separation [147]. Similarly, based on sensory analysis, water-prepared seaweed extracts were shown to be more acceptable as functional components in milk than ethanolic extracts [148]. Researchers from Norway indicated that dulse was easily combined with Nordic dishes and food items, such as ice cream or fresh cheese, and did not appear dominant in the flavour spectrum [131,149]. High quantities of K and Mg, as well as favourable Na to K ratios, are found in seaweeds, which provide a good balance of minerals for human nutrition [118]. The salty flavor of seaweeds presents an appealing option for decreasing Na intake in the regular diet, thereby lowering the risk of developing high blood pressure [118]. For example, nori sauce has significant potential as a salt substitute due to its high total nitrogen, potassium content, and unique free amino acid composition [150]. Meat products containing seaweeds or seaweed extracts may act as potential functional foods for consumers due to the bioactive compounds from seaweeds. The addition of brown seaweed to sausage resulted in the production of calcium-rich, low-sodium, high-fibre sausage with an improved lipid profile [151].

In today's environment, being aware of healthy eating habits is critical. Seaweed products include whole seaweed meal, fresh or dried, processed seaweed extracts, bioactive compounds, homogenates, and fermented seaweeds. Algae can be used to provide excellent nutritional value and act as a source of salt, natural colors, and flavors. They can keep the food from deteriorating its qualities and extend its shelf life. Seaweeds can also substitute some of the traditional ingredients in food production. Seaweeds as the food ingredients have a significant potential to be supplemented into the Canadian diet. However, to successfully integrate seaweed as a food ingredient in Canada, multiple factors, such as innovation, cultivation, and niche markets, should be combined, resulting in a broader acceptance of seaweed products. In addition, to encourage individuals to include seaweed as part of their diet, more information about seaweeds and their availability should be made available.

2.14 Nutritional Composition of Edible Seaweeds

Seaweed records the maximum content of carbohydrates, proteins, vitamins, minerals, fat, fiber, ash, and moisture compared to vegetables, fruits, pulses and cereals. It has been shown that, on average, per 100g of red seaweed contains 4989 mg potassium [152] which is approximately 30 times more than bananas [153]. Red seaweed also comes with 200 times more iron than beetroot, twice the protein of meat, and twice the calcium of full-cream milk [153]. Although most seaweed species are edible, not all are safe or acceptable for human consumption. Seaweed species, time of year, harvesting and processing procedures, age, and light all affect the seaweed's chemical composition and amount of nutrients [91,154,155], especially for the protein content, which is low for many brown seaweeds (<15% of dry weight) and high for some red seaweeds (up to 47% of dry weight) [156]. The protein composition of several species varies dramatically with the seasons. Brown seaweed dulse, for example, has a protein content of 8% dry weight at the end of summer and 25% dry weight at the beginning of spring [111,157]. In addition, it was shown that brown seaweed and red seaweed had the highest biomass in the summer, while green seaweed had the highest biomass in fall [158]. Although seaweed's chemical composition varies at different times throughout harvest season [159,160], in general, seaweeds come with high nutritional value, such as their content of proteins and amino acid profiles; lipids, including polyunsaturated fatty acids (PUFA); soluble carbohydrates; ash; minerals (iodine, iron, zinc); vitamins (B12, A, K); pigments; as well as polyphenols and their antioxidant properties [118]. Depending on the species, water makes up approximately 68%-94% of the weight of fresh seaweed [161]. Seaweed is considered a low-calorie food with approximate nutrient composition proportions consisting of 12% - 71% complex carbohydrates, 4% - 47% protein, 0.9% - 4% fat, and a large number of vitamins and minerals, up to 36% dry weight [162,163]. Seaweed also contains polysaccharides, soluble and insoluble dietary fibre, and polyphenols. Furthermore, seaweeds are well-known for containing high levels of important elements like calcium and magnesium. For example, in the raw product, the brown seaweed *Ascophyllum nodosum's* calcium content is 5.75 g/kg wet weight and 1.15 g/kg for whole milk [164]. The composition of dried and rehydrated red seaweed *Chondrus crispus* is listed in the table below (**Table 2.1**) [165].

Table 2.1: Nutrient composition of raw *Chondrus crispus*

Nutrient name	**Dried *Chondrus crispus*** value per 5 g/60ml edible portion	**Rehydrated *Chondrus crispus*** value per 42 g/100ml edible portion
	Amount	
Moisture (water) (g)	0.70	37.85
Ash (g)	1.15	1.18
Protein (g)	0.76	0.79
Total Fat (g)	0.07	0.07
Carbohydrate (g)	2.30	2.38
Energy (kcal)	12.75	13
Other Carbohydrates		
Fiber, total dietary (g)	1.86	1.9
Minerals		
Calcium, Ca (mg)	14.71	15
Iron, Fe (mg)	3.30	3.41
Magnesium, Mg (mg)	34.31	36
Phosphorus, P (mg)	12.75	14
Potassium, K (mg)	146.08	151
Sodium, Na (mg)	215.69	222
Zinc, Zn (mg)	0.13	0.13
Copper, Cu (mg)	0.02	0.025
Manganese, Mn (mg)	0.29	0.300
Vitamins		
Beta carotene (µg)	242.16	250
Retinol activity equivalents, RAE (µg)	20.59	21
Folacin, total (µg)	9.80	10
Folate, naturally occurring (µg)	9.80	10
Dietary folate equivalents, DFE (µg)	9.80	10
Niacin (mg)	0.19	0.194
Niacin equivalents (NE)	0.32	0.335
Riboflavin (mg)	0.08	0.080
Thiamin (mg)	0.02	0.025
Vitamin B-6 (mg)	0.01	0.013
Vitamin B-12 (µg)	0.11	0.11
Vitamin C (mg)	1.47	1.5
Vitamin D (µg)	6.27	0.2
Tocopherol, alpha (mg)	0.27	0.28
Lipids		
Total saturated fatty acids (g)	0.02	0.021
Total monounsaturated fatty acids (g)	0.01	0.004

Total polyunsaturated fatty acids (g)	0.04	0.038

Information obtained from Canadian Nutrient File, 2015. Food code: 7134 (Dried Chondrus crispus); 7135 (Rehydrated Chondrus crispus)

2.14.1 Fibers and carbohydrate contents

The typical algae carbohydrates are not digestible by the human gastrointestinal tract and, therefore, they are dietary fibers. The average total dietary fiber content in edible seaweed ranges between 25% and 75% of dry weight, of which 51% - 85% is soluble fiber, which is situated between cells and binds them together [166]. This fiber consists of complex polysaccharides such as alginate, carrageenan, fucoidan, and laminarin. The total polysaccharide concentrations in the seaweed species of interest range from 4 to 76 % of the dry weight [167]. Polysaccharides comprise 55%-66% of *Chondrus crispus'*s dry weight [168]. Of those, carrageenan is the dominant polysaccharide, making up to 71% of the total polysaccharides [167]. Other polysaccharides from *Chondrus crispus* include agar, xylans, floridean starch, water-soluble sulphated galactan, and porphyrin [167]. Carageenans as seaweed-derived food hydrocolloids are used for textural functionality in various food applications, including dairy products, jellies, confectionary and cooked processed meat products [169]. Carageenans are recognized as safe by Joint FAO/WHO Expert Committee on Food Additives as their level in food varies from 0.1 to 2% or at concentrations of 1,000 mg/L in infant formula or in the food for special medical purpose [169]. While there is no defined serving size for dehydrated seaweed in Canada, the reference amount for seaweed is 15g [170] and one of the available serving sizes indicated in the Canadian Nutrient File for cultivated dehydrated *Chondrus crispus* is 5.1g or 60 ml [165]. Seaweeds are abundant in soluble fibres, with the highest amounts found in *Ascophyllum*, *Porphyra*, and *Palmaria*; however, other green species, such as Ulva, has contents of up to 65 % of dry weight [157]. Insoluble fiber, derived from the plant's rigid cell walls, constitutes between 15% - 49% of the total dietary fiber [166]. According to Canadian Dietary Reference Intakes for total fiber intake, it is recommended that Canadian women need 25 grams of fiber per day and men need 38 grams of fiber per day [171]. However, most Canadians only get about half that much [172]. Consuming a five-gram serving of brown, red, or green seaweed would contribute up to 14.3%, 10.6%, or 12.1% of daily dietary fiber intake, respectively [162]. Compared to other vegetables, this is a significant amount of fibre; it also suggests that seaweed could be a beneficial fibre source as a dietary component.

2.14.2 Protein

Seaweed is getting significant attention due to its protein content as people are demanding healthy foods and facing the challenges of improving food security from sustainable protein sources. Moreover, Canada's dietary guidelines recommend that people choose plant protein foods more often. As discussed above, the protein content of seaweed varies from species to species. It is low in brown seaweeds (3 ± 15% of dry weight), moderate in green seaweeds (9 ± 26% of dry weight), and high in red seaweeds (up to 47% of dry weight [156,173]. *Chondrus crispus*, on the other hand, protein content ranges from 15.7% to 35.2 % of the dry weight [174,175]. Per 100g of dried *Chondrus crispus* come with 15.34g of protein, which is comparable with the protein content of salmon (22.1g), egg (11.8g), cereal (13g), soybean (16.64g) [165].

The level of digestibility of seaweeds proteins is negatively related to the amount of soluble fiber in the seaweeds [164], study has indicated that breaking down the fibers by physical processes or fermentation [176], or the use of digestive enzymes can significantly increase the bioavailability of seaweed proteins [177,178]. The *in vitro* digestibility of seaweed protein is comparable with other terrestrial plant proteins [174]. In addition, due to the high anti-nutritional substances (phenolic content) of the brown and green seaweeds, protein availability *in vivo* is constrained. Red seaweeds have a low anti-nutritional substances concentration; thus, the red seaweed protein is shown to have higher digestibility [179,180]. However, a relatively low *in vitro* digestibility is reported for *Chondrus crispus* (45%) [181].

In general, seaweeds have abundant amino acids such as aspartic acid, glycine, arginine, alanine, and glutamic acid [164]. Glutamic acid, found in *L. digitata*, is the main component of the umami flavour [164]. The initial source of the flavour enhancer monosodium glutamate was Kombu (*Laminaria japonica*), which is closely linked to *L. digitata* [156]. Many seaweeds, such as dulse (*Palmaria palmata*) and sea lettuce (*Ulva* spp.), provide essential amino acids (EAAs) such as histidine, leucine, isoleucine, and valine [156]. The high amounts of protein (up to 47%) in many red seaweed species are comparable to those protein-rich plant-based foods, such as beans (20.9%), lupine (30.5%), chickpeas (24.7%), linseed (26.35%), peanuts (29.59%), and rice (9.57%) (177), as well as the presence of EAAs, make it a desirable source of protein [174,182].

2.14.3 Lipids and fatty acids

Generally, the lipid content is very low in all seaweed species: between 0.9% and 4% of dry weight [163], much of this lipid content is made up of polyunsaturated fatty acids (PUFA) in the form of omega-3 and omega-6 [156,183]. Depending on the species and season, omega-3 and omega-6 make up approximately 31% - 54% of the total fat content [184]. Essential fatty acids must be obtained from the diet as the human body does not produce them. The Western diet's ratio of omega-6 to omega-3 fatty acids is significantly higher (20:1 or higher). Increased consumption of omega-6 fatty acids may be related to the increase in chronic systemic inflammation and obesity [185]. Studies suggested that a healthy omega-6 to omega-3 fatty acids ratio appears to be between 1:1 and 4: 1 [186,187]. Seaweeds provide an appropriate ratio of fatty acids within this optimal ratio.

2.14.4 Vitamins

Vitamins are organic micronutrients that an organism cannot produce in adequate amounts and thus must be obtained from the foods [155]. The public also commonly uses multivitamin supplements to fulfil recommended daily intakes. However, seaweeds may be an excellent source of fat- and water-soluble vitamins, including vitamins A, B, C, and E [162]. However, as mentioned above, seaweed species, environmental and seasonal factors and harvesting and processing methods can impact the chemical composition and amount of nutrients in the seaweed. For example, there are significant differences in the amounts of β-carotene and vitamin C in *Ulva fasciata* samples taken from various locations [161]. Vitamin C, B2, B1, and A concentrations in *Eisenia arborea* (a dominant species of kelp) were found to be lowest in the summer months (June, July, and August) and highest in April and September (for vitamins C, B2, B1) and January (for provitamin A) [188]. Vitamin contents of seaweed vary from species to species. Seaweeds such as laver (*Porphyra umbilicalis*), sea spaghetti (*Himanthalia elongata*), and *Gracilaria changii*, have vitamin C levels comparable to tomatoes and lettuce [155]. The vitamin C level for brown seaweed *Eisenia arborea* is comparable to mandarin oranges [155]. For the vitamin A content, a 5-g of dried seaweed varies from 14.5 μg (1.6% of RDA for adult males, 2% of RDA for adult females) in *Ulva rigida* to 70.5 μg (7.8% of RDA for adult males, 10% of RDA for adult females) in *Fucus spiralis* [162]. In general, seaweeds are an excellent source of B-group vitamins (especially B1, and B12), lipophilic vitamin A and vitamin E (tocopherol). Kelp can have amounts of α-tocopherol (the most biologically active form of vitamin E) comparable to those found in plant oils such as palm, sunflower seed, and soybean oils. Moreover, the levels of β-carotene in the seaweeds *Codium fragile* and *Gracilaria chilensis* could be higher than those found in carrots [155]. It should be noted that seaweed is one of the few non-animal sources of vitamin B12, which may provide an alternate source of vitamin B12 for vegetarians or vegans. *Ulva lactuca* can provide this vitamin above the recommended dietary reference intake for Canadian of 2.4 μg/day with 5 μg in 8g of the dry form [171]. However, there is an ongoing debate about whether vitamin B12 from seaweed is bioavailable to human [162,189,190]. According to recent studies, seaweed and microalgae species provide a plant source of accessible vitamin B12. The highest amount and bioavailability of vitamin B12 were found in red seaweed and microalgae species such as nori, *Spirulina*, *Chlorella*, and *Dunaliella*. However, even within comparable algal species, there were considerable differences between the investigations [191,192]. It has also been reported that drying *Porphyra* spp. inactivates vitamin B12; therefore, processing methods may impact vitamin bioavailability [162].

2.14.5 Minerals

Seaweeds are a rich source of minerals due to their maritime environment and the diversity of the minerals they absorb from the ocean. However, the mineral contents of seaweed also vary based on seaweed species, environmental and seasonal factors. For example, brown, green and red seaweeds from Indonesia contain high potassium, calcium, and sodium levels. However, when it comes to the iron and zinc contents, they are much lower than the seaweed species in Japan [193]. Generally, most seaweeds are high in calcium, magnesium, potassium, sodium, iron and iodine [155,162,164]. Seaweed trace minerals include zinc, copper, manganese, selenium, molybdenum, and chromium [164,194]. Important minerals, such as calcium, are far more abundant in some seaweeds than in terrestrial foods. For example, an 8 g serving of *Ulva lactuca* (sea lettuce) has 260 mg of calcium, contributing to approximately 26% of daily reference intake for adults [164,195]. Calcium levels in *U. pinnatifida* and *S. polycystum* are even more significant than in milk, and the sodium/potassium ratio in Laminaria species is ideal for humans [8,196]. Seaweeds also have a higher level of iron than several well-known terrestrial sources of minerals such as meats and spinach [164]. *Sargassum* spp. contains 156.9 mg of iron per 100 g of dry weight, a study showed that adding this seaweed to wheat and maize-based bread boosted the amount of absorbed iron [197]. Moreover, seaweeds are considered a rich source of magnesium. *L. digitata*is rich in magnesium with six times higher contents than carrots and tomatoes (mg/100g of dry weight) [198]. However, the bio-accessibility of magnesium varies between seaweeds. In a vitro study, under simulated gastrointestinal conditions, bio-accessible magnesium contents from *Ulva pertusa*, *Laminaria japonica*, and *Gloiopeltis furcate* were 41.8%, 60.8%, and 72.5% respectively [199]. Iodine, rich in most seaweeds, is a critical nutrient for metabolic regulation and growth patterns. The amount of iodine in seaweed varies considerably depending on the location, harvesting method, and species. Kelps, for example, can concentrate iodine up to 100,000 times that of the surrounding seawater and exceed the minimum dietary requirement of 150 μg when consumed [140,195]. Iodine levels in seaweeds range from 0.06 mg/100 g dry weight (*Ulva lactuca*) to 624.5 mg/100 g dry weight (*Laminaria digitata*) [162].

2.15 Seaweed Consumption and Human Health

In addition to macro-and micronutrients, seaweed contains bioactive components such as antioxidants, polyphenols, sterols, and other phytochemicals. Seaweed has been used as food and medicine by coastal peoples worldwide for thousands of years. Seaweed has been used as a medicine in ancient China for at least 5,000 years when Emperor Shen Nung included it in his classic pharmacopeia, the Pen Ts'ao. Traditional Chinese medicine has employed seaweeds to treat goitre, scrofula, swelling and pain of testes, and edema. In addition, several seaweed species have been used to treat arteriosclerosis, skin diseases, high blood pressure, hepatosplenomegaly, neurosis, angina pectoris, acute esophagitis, and chronic bronchitis in modern Chinese medicine [200]. The recent surge in interest in seaweed is fueled by interest in its bioactive components, which have shown a significant potential with antioxidant, anti-infective (antiviral, antimicrobial activity: bacterial and fungal), antitumor, anticancer, antiproliferative, anti-inflammatory, anticoagulant, phytoestrogenic and endocrine modulating properties (thyroid) [118]. Therefore, seaweeds have potential applications in the functional food and nutraceutical industries, with a focus on reducing the risk of specific metabolic syndromes (obesity, dyslipidemia, diabetes, hypertension), cardiovascular diseases (coronary heart disease), weight management, cancer [1,9,118,162]. Epidemiological studies have shown an association between the dietary intake of seaweeds and a reduced prevalence of chronic diseases [9].

2.15.1 Seaweed and cardiovascular health

For centuries, seaweeds have been considered as part of a healthy diet in East Asian countries, such as China, Japan, and South Korea. Japanese, particularly the Okinawans, have the world's longest life expectancies, possibly due to their high intake of fish, soy, and seaweed in their diet. In addition, mortality from cardiovascular disease and all cancers is low in Japan [9]. Seaweed consumption may prevent diet-related CVDs due to its abundant fiber content, which could act as an alternative source of dietary fiber. Other bioactive components from seaweed, such as antioxidants and proteins, may also contribute to CVDs prevention. Moreover, seaweed consumption has been linked to reducing the incidence of various CVDs risk factors, including obesity, hyperlipidemia, diabetes, and hypertension [36].

Seaweed intake has been associated with protection against CVDs in collaborative cohort research looking at nutrition's effect on mortality in Japan [201]. A prospective cohort study also demonstrated that the typical Japanese dietary pattern, including seaweed consumption, has decreased CVDs risk [202]. Seaweed powder (5 g/day) added to the diet for up to 10 weeks showed a considerable reduction in cholesterol [203]. However, due to a lack of essential information in the reported data, conclusions on the relevance of these findings are challenging to draw. A retrospective study indicated that consuming seaweed associated with other legumes presented a significantly lower total cholesterol level in Japanese men [204]. Researchers concluded that adding seaweed to the diet may minimize cardiovascular disease risk factors in type II diabetic patients by lowering blood lipids while increasing HDL cholesterol levels and enhancing antioxidant enzyme activity. However, more research is needed to confirm these effects and establish dietary recommendations for patients with type II diabetes [205]. A recent perspective study (surveyed 40,707 men and 45,406 women) examined the dietary intake of seaweed and its impact on stroke and ischemic heart disease risk among a Japanese population found that high seaweed intake may prevent middle-aged men and women from cardiovascular disease [206].

The link between seaweed consumption and a lower risk of CVDs may be due to the anti-inflammatory, antiangiogenic, anticoagulant, and antiadhesive properties of seaweed components [207]. In addition, seaweeds contain a large number of soluble polysaccharides functioning as dietary fiber and might be considered beneficial to CVDs risk factors. Mechanisms associated with potential health benefits have broadly been identified *in vitro* or in animal models. The pigment fucoxanthin present in wakame lowered blood pressure and stroke risk factors [208], while tropical seaweeds reduced cholesterol, low-density lipoprotein, triglycerides, lipid peroxidation, and erythrocyte glutathione peroxidase levels [209]. Carrageenan from *Chondrus crispus* has a long history of use in traditional medicine as a cough and cold remedy, and *in vitro* and animal models have indicated that it may inhibit platelet aggregation and have anticoagulant characteristics [9].

Moreover, fucoidan, derived from brown seaweeds such as *Laminaria* and *Ascophyllum* spp., has shown anticoagulant effects that may help prevent CVDs. Furthermore, laminarin, which can also be found in *Laminaria* and *Ascophyllum* spp., has been shown to have antioxidant, anti-inflammatory, and anticoagulant properties [9]. In addition to other bioactive substances found in many seaweed species, lipids from seaweeds may have therapeutic qualities related to heart disease. As documented previously, although the lipid content is very low for all seaweed species [163], much of this lipid content is made up of PUFA, most of which occur in the form of long-chain omega-3 (n-3) PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [156,183].

2.15.2 Seaweed and cancer

Seaweeds have been advocated for a function in cancer prevention, tumour advancement, and even health recovery after radio- or chemotherapy for decades [36]. Even though there is limited human clinical intervention trial investigating the potential benefits of seaweed consumption on certain cancers, some observational evidence and a wealth of *in vitro* and *in vivo* studies have demonstrated clear anti-cancer properties of seaweed and seaweed components [210]. In previous studies, edible seaweeds have been shown to protect against mammary, cutaneous, cervical, and intestinal cancers [210]. Seaweed consumption was linked to decreased lung cancer mortality in both men and women and lower pancreatic cancer mortality in men [201].

Breast cancer is the most common cancer globally, with approximately 2.26 million cases reported in 2020 [25]. *Porphyra* spp. consumption was linked to a lower risk of breast cancer in premenopausal women but not in postmenopausal women, and there was no link between *Undaria pinnatifida* consumption and breast cancer risk [211]. According to a large prospective study in Japan, investigators reported that after nine years of follow-up, miso soup (made from seaweed) was the food most closely associated with low breast cancer risk among other foods investigated [212]. A case-control study involving 362 Korean women with histologically confirmed breast cancer and their controls reported that gim (a kind of nori) consumption was inversely associated with breast cancer risk in premenopausal women [213]. The anti-cancer properties of gim may also be attributable to the iodine content of this seaweed [213]. Other epidemiological studies indicated that consuming miso soup at least five times per week was associated with a reduced rate (13%) of breast cancer among women in Japan [200]. The association between seaweed consumption and protection against breast cancer development may be because seaweed consumption changed the estrogen and phytoestrogen metabolism by lowering serum estradiol (E2) level and increased urinary excretion of 2-hydroxyestrogen (2-OHE), the ratio of 2-OHE:16a-hydroxyestrone (16aOHE1) and equol excretion [214].

Bioactive compounds from seaweed, such as fucoidan, alginate, fucoxanthin, and polyphenols (catechins, flavonols, and phlorotannins) [215], may have unique health-promoting qualities that could be used in human health applications such as cancer prevention and treatment [9]. The phenolic content in seaweeds varies from 1352 μg/ g to 386 mg/g on a dry weight basis [179], with lower concentrations in green and red seaweeds, and higher concentrations in brown seaweeds [216]. Phlorotannins (fucolls, phlorethols, fucophlorethols, fuhalols and halogenated and sulphited phlorotannins) comprise the major phenolic compounds in brown seaweeds [179], bromophenols, phenolic acids, and flavonoids are the most abundant polyphenols in red and green algae [215].

Anti-cancer activity has been evaluated on various crude extracts or partially purified substances from brown, green, and red seaweeds. Compounds derived from seaweeds have been found to be efficient anti-cancer agents in several studies [217]. The anti-cancer effects of seaweed components are mediated by various pathways antioxidation, tumoral cell apoptosis, and immune stimulation [210]. Tumours generate a large number of free radicals, which are often accompanied by a lack of DNA repair mechanisms [218]. The most critical component for DNA damage is reactive oxygen species (ROS), which also causes high amounts of oxidative stress [210]. Chlorophyll, fucoxanthin and phycoerythrobilin from edible seaweeds showed the most robust antioxidant activity by inhibiting the progression of abnormal cells to cancer [219]. At a dosage of 200 μg/ml, the *Ulva fasciata* extract could inhibit the development of HCT116 human colon cancer cells by 50% by activating apoptosis [220].

Polyphenols from red edible seaweeds show anti-cancer properties by triggering apoptosis, reducing oxidative stress, and inhibiting the manufacture of endogenous estrogen [221]. Sulfated polysaccharides isolated from seaweeds increased the production of specific and OVA-specific antibodies in mice, demonstrating the immunomodulatory effects of seaweeds [222]. Fucoidan has been shown to stimulate T cell production of Interferon-gamma (IFN γ) and alter natural killer (NK) and T helper 1 (Th1) cell responses in leukaemia mice [223]. Tumours need a high supply of oxygen and nutrients which can only be achieved by increased angiogenesis in the growing mass. Evidence indicated that sulfated polysaccharides such as fucoidan and laminarin, might have anti-angiogenesis effects [210]. *In vitro* and *in vivo* studies showed that fucoidan extracted from brown seaweed reduced vascular endothelial growth factor (VEGF) and other pro-angiogenic cytokines, contributing to a substantial reduction in the tumour size of 4T1 tumours [224,225]. Although various pathways mediate the anti-cancer effects of seaweed components, no consensus has yet been achieved in this area, and further research is needed to understand these mechanisms better.

2.15.3 Seaweed and metabolic syndrome (MetS)

Metabolic syndrome (MetS) is a group of metabolic abnormalities that include abdominal obesity, high blood pressure, high fasting plasma glucose, high triglycerides, and decreased high-density lipoprotein cholesterol (HDL-C), all of which increase the risk of CVDs, type 2 diabetes mellitus (T2DM), and all-cause mortality. MetS is one of the world's major public health concerns, with an estimated one-quarter of the world's population suffering from it [226]. The US Food and Drug Administration (FDA) has not authorized any drug to treat MetS to date; nevertheless, an insulin-sensitizing agent, such as metformin, is currently commonly used in patients with MetS who are beginning hyperglycemia treatment. Although these medications can be beneficial, most of them have side effects, and their effectiveness may be decreased or lost due to long-term use [227]. As a result, there is growing interest in using natural products to reduce the risk of MetS and its progression. Seaweeds, due to their bioactive compounds (e.g. polyphenols, sulfated polysaccharides, carotenoids, and polyunsaturated fatty acids), have been used in nutraceuticals and functional foods for the management of MetS and MetS-related diseases. The inhibition of digestive enzymes (e.g., α-amylase, α-glucosidase, lipase, PTP1B) reduces dietary fat and glucose absorption [228]. Much epidemiological evidence has shown that nations where seaweeds are regularly taken within the diet (for example, Japan) have a lower incidence of obesity and food-related disease than Western countries, further confirming the effectiveness of seaweeds in reducing and managing MetS [9,229,230]. The Nutrition and Health Study in Taiwan revealed that various foods, including seaweeds, may help prevent MetS [231]. Research in animal models also suggested a role for algae in the treatment and prevention of MetS [232].

2.15.4 Impacts on appetite, energy intake and weight management

Seaweed carbohydrates exhibit various biological characteristics, including anti-obesogenic properties due to the high content of sulphated polysaccharides in seaweed, such as alginate, carrageenan, fucoidan, and laminarin. Moreover, alginates, fucoidan, and laminarin remain undigested in the upper digestive tract of animals. Therefore, seaweed is considered a rich source of dietary fiber that may associate with the effect of seaweed consumption on appetite control, and energy intake [233,234]. Most human research on the health benefits of seaweed-derived dietary fiber components has focused on potential anti-obesogenic effects such as improved satiation, delayed nutrient absorption, and gastric emptying, but human research on whole seaweed consumption appears to be under-investigated [162,235]. A recent acute study shows increased sensation of satiety and reduced feelings of hunger after seaweed consumption which suggested the two tested brown seaweed species (*Laminaria digitata* and *Undaria pinnatifida*) could be excellent candidates for lowering energy intake for several hours [235]. The effect could be owing to the polysaccharide, alginate, and perhaps other soluble dietary fibers from seaweed having a satiating effect, which could be caused by bulking or a slower stomach emptying rate [235].

Alginate ingestion has been proven to significantly impact appetite and food intake in humans in several placebo-controlled intervention trials. A crossover study reports that daily energy intake was reduced by 135 kcal when participants consumed alginate (1.5 g/100 mL) prior to meals [236]. An acute study showed that an alginate drink increased self-reported satiety and decreased the feeling of hunger in a dose-dependent manner compared to a placebo [237]. Another parallel study in healthy overweight males (n=12) found that consuming an *Ascophyllum nodosum*–enriched (4%) bread reduced energy intake by 109 kcal at 4 hours and 506 kcal at 24 hours, respectively, when compared to an isocaloric placebo [238]. In another study (10 men and 9 women), consumption of a high-volume (15.0 g alginate in 500 ml) alginate-based preload drink resulted in increased satiety, and low-volume preload (9.0 g alginate in 330 ml) lowered energy intake by 44 kcal (8%) after an *ad libitum* lunch compared to the placebo [15]. However, there was no substantial effect of a preload alginate drink on energy intake or satiety hormone concentrations in overweight/obese people [16]. Alginates appear to alter appetite and food intake, but further research is needed to clarify their function by looking at the relationship between structure and function, especially molecular weight, and the ratio of guluronate to mannuronate, as well as the significance of alginate's gelling capacity. Given that alginate does not appear to impact stomach emptying, more research into its mechanism of action is needed [15,16].

2.15.5 Impacts on controlling blood glucose levels

Dietary carbohydrates are the major source of blood glucose. Before being absorbed in the small intestine, these carbohydrates are hydrolyzed by pancreatic α-amylase and α-glucosidase. The role of seaweed in metabolic control suggests that seaweed extracts affect blood glucose control by inhibition of α-amylase and α-glucosidase activities [239,240] or by increasing insulin sensitivity [241]. The extract of 500 mg of brown seaweeds (*Ascophyllum nodosum* and *Fucus vesiculosus*) reduced the insulin iAUC by 12.1% and increased the Cederholm index of insulin sensitivity by 7.9% when compared to placebo [241]. In addition to seaweed extract, consumption of whole brown seaweed indicated lower postprandial blood glucose, insulin and C-peptide response [235]. A recent randomized crossover trial involving 26 participants with untreated type II diabetes mellitus found that participants who consumed 200 g of rice with 4 g of dried wakame at each meal had significantly lower blood glucose and insulin levels at 30 minutes compared to the control group [242].

There is also considerable interest in the effect of alginate on glycemic control, particularly its impact on postprandial glucose absorption. Alginate is the predominant polysaccharide of brown seaweed, comprising 14–40 % of its dry mass [155]. Alginate fiber forms viscous fluids by binding Ca2+ ions and stomach H+ ions due to the presence of guluronic acid residues [243,244]. Consumption of viscosity-raising fiber may lower postprandial glucose reactions, slow stomach emptying, and impede macronutrient absorption [243,245]. Postprandial blood glucose absorption has been shown to be reduced following alginate consumption due to intragastric gelation, nutrient entrapment in the gel matrix, and altered nutrient absorption in the gastrointestinal tract at relatively low concentrations compared to other soluble fibers [246]. Due to the greater viscosity polysaccharide in *L. digitata*, which could alter glucose absorption and insulin response in the intestine, alginates from *L. digitata* dramatically lowered blood glucose absorption balance (50 %) and insulin responses in pigs over eight hours [247]. When sodium alginate (1.5 g) was added to a glucose-based beverage resulted in a substantial reduction in postprandial blood glucose response and a larger incremental peak insulin response [248]. Another study in healthy individuals showed that 500 mL 3% sodium alginate beverage lowered glucose incremental area under the curve (iAUC) by 40% without lowering insulin responses compared to the control beverage [249]. A similar effect of alginates restoring glucose uptake was also found in overweight and obese individuals [250].

2.15.6 Impacts on blood lipids

Obesity and dyslipidemia represent two risk factors for MetS and coronary disease development. Lipid disorders could exacerbate atherosclerosis, leading to chronic heart failure [228]. A considerable amount of evidence has indicated seaweed intake is associated with lowering serum lipids such as triglyceride, low-density lipoproteins, and total cholesterol. A study showed that at the end of a 6-week intervention period, a 5% significant reduction in triglyceride levels was observed in the treatment group compared to the placebo group. However, the effect was not seen after three weeks of intervention, implying that it takes at least six weeks of supplementation to be effective. Researchers also found a non-significant trend in reduced total cholesterol and low-density lipoprotein-cholesterol (LDL-C) and increased high-density lipoprotein-cholesterol (HDL-C) in both groups [251].

The effects of seaweed intake on blood lipids may be due to the bioactive compounds of seaweed, such as carrageenan (seaweed polysaccharide), fucoxanthin (a seaweed carotenoid) and polyphenols. A study showed that the carrageenan diet considerably reduced serum total cholesterol and triglyceride levels while significantly increasing HDL-C levels. These findings suggest that eating a carrageenan-rich diet can help with lipid balance [252]. In a mouse model of diabetes, fucoxanthin administration has shown effects on improving glucose and lipid metabolism and reducing plasma triglycerides and total cholesterol levels and lowering insulin resistance [253]. The evidence showed that supplementing marine polyphenol-rich extracts may have small-to-medium beneficial effects on fasting blood glucose, total cholesterol, and LDL-C levels. However, more research is needed [254].

Brown seaweeds and their derivatives have dominated scientific investigations and studies with human participants, owing to their commercial abundance and perceived sustainability. Despite the nutritional benefits of red seaweeds such as nori (*Porphyra* spp.) and dulse (*Palmaria palmata*), which have a high protein content, there have been few studies on red seaweeds as a source of bioactive components [162]. Despite many clinical studies investigating the effect of brown seaweeds in MetS comorbidities, most were using seaweed extract as the treatment; there was litter research using whole seaweed as the intervention. To fully understand whether the same effects can be seen using whole seaweed as the treatment, more research is needed.

2.15.7 Seaweed and digestive tract health

More and more evidence has revealed that gut microbiota plays a crucial role in maintaining intestinal homeostasis and improving metabolic health [255]. Seaweeds may benefit gut health through various mechanisms, including anti-inflammatory properties, immunomodulation, and lipid peroxidation. The high fiber content of seaweeds is most likely responsible for these effects [36]. Although some viscous fibers, such as alginate, may have a direct protective and coating effect, the most likely mechanism of fibers is through the prebiotic activities of seaweed polysaccharides [9,256]. Low-molecular-weight polysaccharides have been studied for their possible prebiotic effects in several studies. Seaweed fibre appears to differ from higher plant carbohydrates in terms of chemical, physicochemical, and fermentation properties; however, nothing is known about its properties in the human gut or the bacteria it may influence [257].

Marine-derived products such as alginates, laminarin, and agar, as well as seaweed structural components, are emerging as new sources of prebiotic carbohydrates. Alginate, xanthan gum, and carrageenan gum have been shown to increase probiotic survival as they provide living bacteria a physical barrier against unfavourable digestive conditions [258,259]. Overall, there is a significant information gap regarding seaweed bioactive compounds and their significance in human digestive system health. There might be some potential for these compounds to be developed as prebiotics or components that help probiotics survive. However, the data from human investigations on seaweed prebiotic carbohydrates is insufficient to draw any conclusions. In this growing field of study, ongoing research employing human studies may help to improve the understanding of potential pathways and specific substances of interest.

2.15.8 Other health benefits of seaweed intake

Other potential health benefits associated with seaweed consumption include improved bone health, lower prevalence of some neurological diseases and potential antiviral agents [9,36]. As observed from *in vitro* and animal research, marine and algal compounds may have the potential to combat osteoporosis. Possible mechanisms include the mineral content of seaweed that helps prevent bone mineral loss; compounds that may promote osteoblast differentiation; and some bioactive compounds that may inhibit osteoclast differentiation [36]. The antiviral properties of seaweed may be due to the sulfated polysaccharides from various seaweed species. Carrageenans, fucoidans, and sulfated thamnogalactans have been found to have significant antiviral potential against enveloped viruses in both *in vitro* and *in vivo* models with animals [9]. Although the *in vitro* evidence is encouraging, preclinical studies and clinical trials are needed to evaluate seaweeds and their compounds.

Various *in vitro* and *in vivo* animal experiments have contributed to the current understanding of seaweed's health-promoting properties. Although many studies have reported the potential nutritional or bioactive content of different seaweeds, there are significant demands on quantifying the bioavailability of nutrients and phytochemicals from seaweed foods. More well-designed clinical trials with human participants are needed to promote seaweed or seaweed-containing food or seaweed extracts as functional foods or natural health products.

Chapter 3: Rationale, Objectives, and Hypothesis

3.1 Rationale

Previous studies have indicated the effects of seaweed consumption on appetite control and energy intake. However, most investigations used seaweed extracts, or the bioactive compounds isolated from seaweed. Studies investigating the effects of consuming whole seaweed on satiety and short-term food intake in humans are limited. Research gap exists regarding whether consuming whole red seaweed *Chondrus crispus* will have the same appetite control properties as observed in the previous studies. Edible seaweeds are rich sources of nutrients, including minerals, polysaccharides, soluble and insoluble fibers and trace elements. Additionally, seaweed intake has shown significant health potential; thus, edible seaweeds are considered as functional foods in many countries. However, edible seaweed consumption in western countries, including Canada, is very limited. The major reasons for limited consumption include their unique taste and flavor, unfamiliarity with using seaweeds in traditional foods, unavailability of ready-to-use processed foods with seaweeds and lack of information regarding their health benefits. Other than the health benefits of edible seaweeds, seaweeds aquaculture also showed great potential for economic growth and provided several inter-sectorial benefits. Even though many products from Asia are starting to be marketed, and some domestic products are emerging, the seaweed industry in most western societies such as Canada is underdeveloped. Therefore, it is necessary to investigate the sensory acceptability of seaweed-containing foods and their association with appetite sensation in consumers to enhance the development and consumption of seaweed products in Canada. Given the extensive Canadian coastline and its long-established high biodiversity of seaweed varieties, there appears to be abundant opportunities to exploit the global trend of seaweed consumption. However, very little is known about consumers' perceptions of seaweed as a food product and their demand. The current project would result in a better understanding of the design/development of acceptable seaweed products with improved health benefits.

3.2 Objectives

3.2.1 Primary objective

- To determine the effect of a meal formulated with *Chondrus crispus* on subjective appetite and short-term food intake in young adults.

3.2.2 Secondary objective

- To evaluate the sensory characteristics of foods formulated with seaweed (*Chondrus crispus*);
- To examine the effect of consuming a meal formulated with seaweed (*Chondrus crispus*) on subjective physical comfort over two hours.

3.3 Hypothesis

It was hypothesized that:

1) The addition of 5g dried *Chondrus crispus* to a mixed meal (omelette) will result in a lower subjective appetite and subsequent food intake compared to a mixed meal without the seaweed.
2) Adding seaweed to omelette will result in acceptable sensory characteristics due to a partial masking of seaweed flavour.

Chapter 4: Methodology

4.1 Study Design

The study followed a within-subject, randomized, controlled, single-blinded, cross-over design as recommended by Health Canada's guidance document [260]. Three treatments were given in a random order to each participant on different session days with one-week washout between the sessions. The randomization of the treatments was performed using Compusense software (Compusense Inc., Guelph, ON, Canada). Three treatments included a mixed meal (omelette) prepared without (N) or with (S) *Chondrus crispus* seaweed, or water as an energy-free control (W). The treatments were labeled with 4-digit identifying codes and arranged according to the randomized serving order for each participant. The participants were unaware of a type of an omelette they have received.

4.2 Participants

The study was conducted according to the principles set in the Declaration of Helsinki [261], and the study protocol was approved by the Mount Saint Vincent University Research Ethics Board (protocol # 2021-001). All participants gave their written informed consent to participate.

Thirty healthy young males aged 19-35 with Body Mass Index (BMI) of 20.0-24.9 kg/m2 were recruited for this study. The study was powered to detect the difference in food intake between the treatments using the data from a previously published study with male participants [262], allowing detection of 10% difference in food intake with an alpha-level of 0.05, and a beta-level of 0.8. This sample size is also sufficient to detect a 10mm difference (10%) in appetite sensation using visual analogue scales [263].

Exclusion criteria were as follows: having any diseases, smoking (including e-cigarettes), consuming cannabis, skipping breakfast regularly, disordered eating patterns (e.g., focusing too much on body weight, body imaging, dieting, self-limiting the choice of foods, performing specific food rituals, feel anxious or guilty when eating or after eating, having bulimia, anorexia or binge eating disorder), taking certain medications that may affect the central and peripheral mechanisms of food intake regulation [264], cognitive performance and sedative medications, having intellectual disabilities that would affect their ability to participate in the study as required. Individuals with known food allergies and aversion to seaweeds were also excluded. Exclusion criteria aligned with Health Canada's Draft Guidance Document - Satiety Health Claims on Food, which states that participants of the study must be considered healthy in order to substantiate a functional health claim [260]. Considering the differences in sensory food perception between genders [265], as well as the effect of menstrual phase on nutrient intake, appetite and food craving [266], only males were recruited to the study.

4.2.1 Study participant recruitment & selection

This study was advertised via word of mouth primarily, but not limited to, the MSVU community, specifically students and employees. The recruitment cards and flyers were circulated in local grocery stores, libraries, and community places and posted on the Kijiji website, the Appetite Lab Facebook page and other social media platforms.

All participants were screened over the phone (Appendix 2) to do the initial eligibility check and invited to an information session for the final enrollment. Their restrained eating patterns were evaluated with the Restraint Scale [267]. The past year's physical activity was assessed using Past Year Total Physical Activity Questionnaire adopted from Cancer Epidemiology and Prevention Research Cancer Control Alberta, Alberta Health Services [268]. Their height, weight and body composition were measured using bioelectrical impedance analysis using a Tanita Body Composition Analyzer TBF-300A and Stadiometer HR-200 (Tanita Corporation of America, Inc, Arlington Heights, IL). Eligible participants were scheduled for experimental sessions one week apart between the sessions.

4.3 Study Protocol

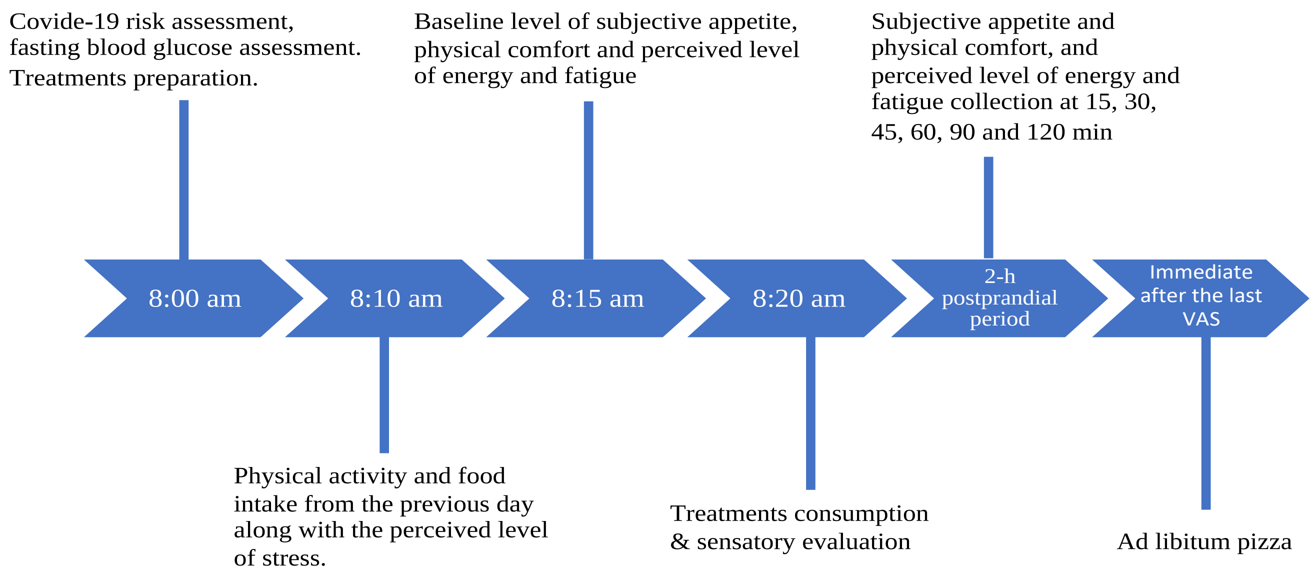

Figure 4.1: Experimental Protocol

Participants were asked to fast for ten hours overnight before each session and abstain from alcohol, caffeinated or energy drinks, sweetened beverages, seaweed products, and intense physical activity. Participants were allowed to drink water until one hour before arriving at the laboratory. The day before data collection occurred, a researcher called participants who were scheduled to participate and completed the COVID-19 Risk Assessment for Research (See Appendix 3a). During the day of the study, all participants completed a COVID-19 Risk Assessment for Research form (Appendix 3a), reviewed an Information Letter: COVID-19 Risks (Appendix 3b), and an Information for Contact Tracing Participants form (Appendix 3b). These forms were sent to the MSVU Research Office on the day of the study.

Participants arrived at the laboratory between 8:00 am and 10:00 am. Each participant arrived at the same chosen time for each session. Upon arrival, participants had their fasting blood glucose level measured using a sterile lancet device and portable glucose meter HemoCue® Glucose 201 (HemoCue AB, Ängelholm, Sweden). Participants completed questionnaires on their physical activity and food intake from the previous day, along with their perceived level of stress. Participants with fasting blood glucose levels higher than 6mmol/L, or if they had experienced stress, unusual food intake, alcohol consumption, did not sleep enough or had extra physical activity on the day before their sessions were rescheduled for another day.

Upon completion of baseline measurements, participants were taken to the individual feeding cubicles and were asked to complete the visual analogue scales (VAS) for the motivation to eat (MTE) and physical comfort (PC) (Appendix 5) immediately before being served with a treatment. Once the treatment was served, participants were asked to consume the whole amount. Time 0 was marked when participants took the first bite of S or N, or the first sip of W. The sensory characteristics of the treatment were assessed immediately after ingestion. VAS for MTE and PC were collected at 15, 30, 45, 60, 90 and 120min after the treatment. At 120 min, participants were served with a pizza meal (Giuseppe Pizzeria, Dr. Oetker Canada Ltd., Mississauga, ON) and water, and were instructed to eat until they feel "comfortably full" as reported earlier [269]. The new tray with sliced pizza and new bottle with spring water was provided every 8 minutes, and the previous tray and water bottle with the leftover were taken away. Once participants have finished their meals, they rated their SA and PC at 145 min, and the session was completed.

In between sessions, participants were free to continue their usual lifestyle, eating and exercise habits. All subjects were free to withdraw at any time during the study.

4.4 Dietary Treatments

Chondrus crispus seaweed (**Figure 4.2b**) was provided by Acadian Seaplants Ltd. The nutrient profile of *Chondrus crispus* seaweed is listed in the Table 4.1. Other food ingredients were purchased from the local grocery store (**Figure 4.2a**) (**Table 4.2**).

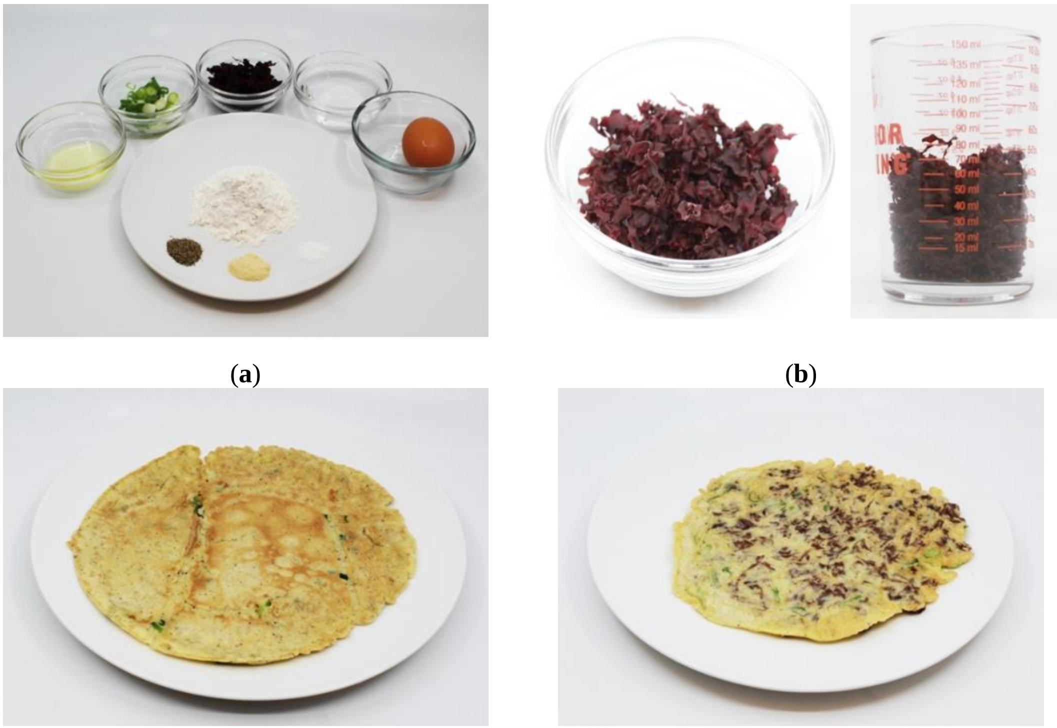

Figure 4.2: Dietary treatments. (a) treatment ingredients; (b) dehydrated *Chondrus crispus* seaweed (5g, 60ml); (**c**) Omelettes without *Chondrus crispus* seaweed; (d) Omelettes with *Chondrus crispus* seaweed

Table 4.1: Macronutrient composition of dried *Chondrus crispus* (value per 5 g of edible portion)[1]

Nutrient Name	60ml / 5 g
Moisture (g)	0.70
Ash (g)	1.15
Protein (g)	0.76
Total Fat (g)	0.07
Carbohydrate (g)	2.30
Energy (kcal)	12.75
Other Carbohydrates	
Fiber, total dietary (g)	1.86

[1] information obtained from Canadian Nutrient File, 2015. Food code: 7134.

Table 4.2: List of ingredients

Ingredients	CNF Food Code	Quantity
Egg (Large)	125	1 large
All Purpose Flour	4484	30g
Salt	214	1/4 teaspoon
Green Onion	2144	10g
Onion Powder	194	1/4 teaspoon
Black Pepper	198	1/4 teaspoon
Oil	5622	1 tablespoon
Water	2933	1/3 cup
Seaweed, dried	7134	5g

According to the recommendations from Health Canada, the studies investigating the effect of food on satiety should include at least three experimental treatments: the test food, the control food or the reference food, and the energy-free control [260]. Participants were served the following treatments in a random order with one treatment per session with one week washout between the sessions: (1) the omelette with added 5g seaweed (S) (test food, per 193 g, 318 kcal) (**Figure 4.2d**), (2) the omelette without seaweed (N) (control food, per 188 g, 309 kcal) (**Figure 4.2c**), or (3) 350mL spring water (W) (0 kcal, energy-free control) for a total of three sessions with at least one week apart. To obtain a more accurate energy values, energy content of the treatment was calculated by the Atwater method [270] which applies energy conversion factors to the macronutrients carbohydrate, fat, protein and fiber. The Atwater general factor includes energy values of 4 kcal per gram (kcal/g) for protein, 4 kcal/g for carbohydrates, 9 kcal/g for fat and 2 kcal/g for dietary fibre [271]. Nutritional composition for the dietary treatments can be found in **Table 4.3**. An average weight difference (29.4 ± 2.8 g) between the cooked food weight and the weight of the ingredients stated on in the **Table 4.2** was observed due to the water loss during the cooking process. Details of the test meal preparation can be viewed in **Figure 4.3.**

Table 4.3: Nutritional Composition of Dietary Treatments

Basic Components	Non-seaweed Meal	Seaweed Meal
Energy (Kcal)	309	318
CHO (g)	25	27
Available CHO (g)	24	23
Dietary Fibre (g)	1	3
Protein (g)	10	10
Fat (g)	19	19
Sodium (mg)	650	870
Iodine (mcg)	121	781[1]
Weight	188	193

Information obtained from Canadian Nutrient File, 2015. Available CHO: Available carbohydrate, calculated as the difference between total carbohydrate and dietary fibre. [1] Iodine content for eggs was obtained from USDA FoodData Cetral and for *Chondrus crispus* from Acadian Seaplant Ltd.

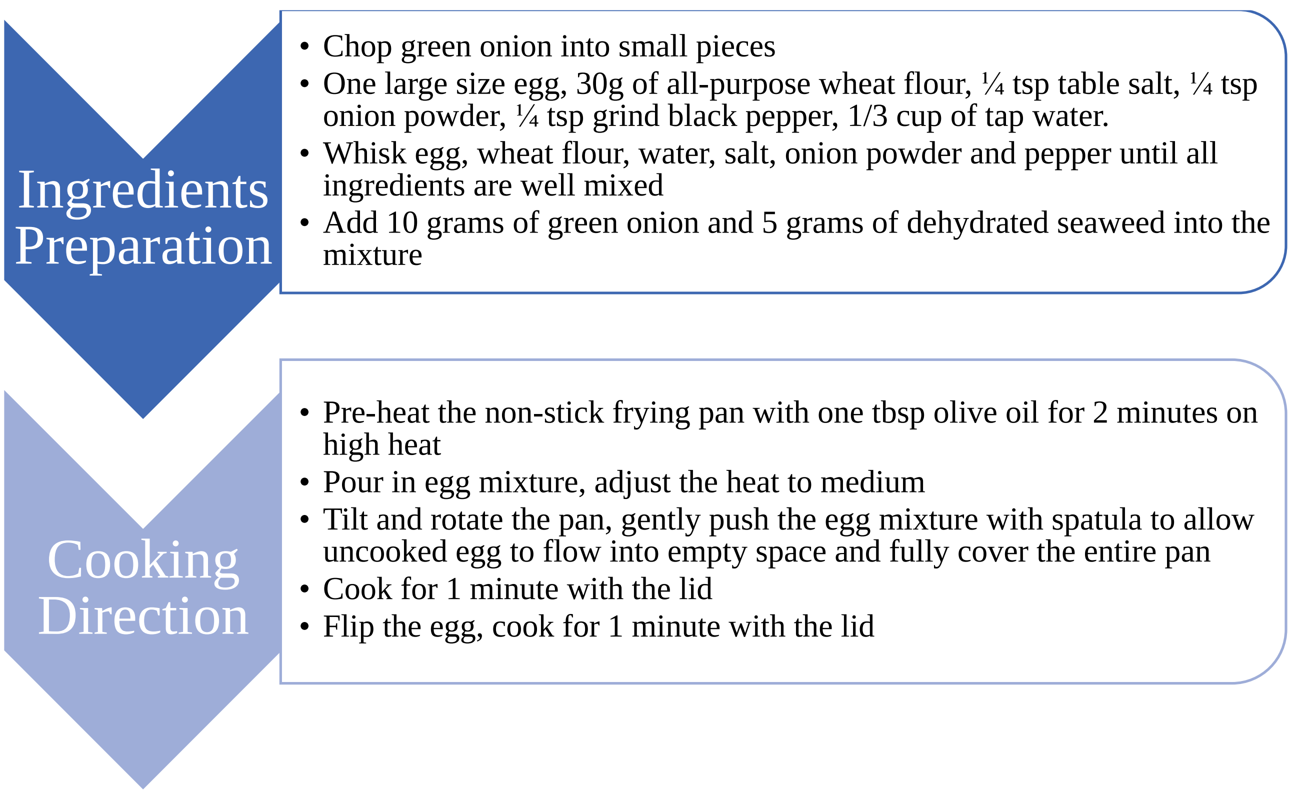

Figure 4.3: Details of the test meal preparation

Chapter 5: Data Analysis

5.1 Data

5.1.1 Subjective appetite and physical comfort

The subjective appetite (SA) was assessed using the 100 mm motivation to eat (MTE) visual analogue scales (VAS) questionnaire [272,273]. The baseline levels of SA and physical comfort were collected before the treatment consumption. The following SA dimensions were assessed using MTE VAS: (1) Desire to eat (DTE) (“Very weak”, 0mm to “Very Strong”, 100mm); (2) Hunger ("Not Hungry At All”, 0mm to “As Hungry As I Have Ever Felt”, 100mm); (3) Fullness ("Not Full At All, 0mm to “”Very Full”, 100mm); (4) Perspective Food Consumption (PFC) ("Nothing At All”, 0mm to “A Large Amount”, 100mm); (5) Thirst ("Not Thirsty At All”, 0mm to “As Thirsty As I Have Ever Felt”, 100mm).

Participants were asked to make a mark across 100mm line corresponding to their feelings. Quantitation of the measurement was done by measuring the distance from the left end of the line to the mark. VAS are used in appetite studies to assess different appetite sensations and have shown excellent reproducibility and validity for measuring appetite sensations [263]. Average appetite scores were calculated using the formula:

Average appetite score = [desire to eat + hunger + (100 –fullness) + prospective consumption]/4 [274].

The satiety quotient (SQ) for caloric treatments was calculated using the following formula:

SQ (mm/kcal) = [(fasting AS - mean 2-hour post meal AS) / (energy content of the treatment)] × 100 [275].

The physical comfort was assessed using 100mm VAS for: (1) Energy (“Not At All”, 0mm to "Very Energetic”, 100mm); (2) Fatigue (“Not At All”, 0mm to "Very Tired”, 100mm); (3) Nausea (“Not At All”, 0mm to "Very Much”, 100mm); (4) Stomach Discomfort (“Not At All”, 0mm to "Very Much”, 100mm); (5) Wellness (“Not WellAt All”, 0mm to "Very Well”, 100mm); (6) Gas (“Not At All”, 0mm to "Very Much”, 100mm); (7) Diarrhea (“Not At All”, 0mm to "Very Much”, 100mm).

5.1.2. Sensory evaluation

Sensory evaluations: the sensory acceptance of the treatments were assessed using 9-point hedonic scales (9-pt HS) for pleasantness, taste, flavour, texture, saltiness, chewiness, mouthfeel, aftertaste and appearance [276]. The intensity of saltiness (“Not Salty At All”, 0mm to "Very Salty”, 100mm), chewiness (“Not Chewy At All”, 0mm to "Very chewy”, 100mm), bitterness (“Not Bitter At All”, 0mm to "Very Bitter”, 100mm), seaweed flavour (“No Seaweed Flavour”, 0mm to "Strong Seaweed Flavour”, 100mm) and prospective purchasing (“Would Not Purchase”, 0mm to "Would purchase”, 100mm) was assessed using VAS. The palatability of pizza meal served at 120min was assessed with VAS (“Not At All Pleasant”, 0mm to “Very Pleasant” 100mm).

5.1.3 Ad libitum food intake (FI) at 120min

At the screening session, the participants indicated their preference of a pizza meal selecting either “Pepperoni”, or “Three Cheese”. The pizza used in the study had no outer crust and therefore had a uniform energy density allowing the accurate estimation of food intake by subtracting the energy of pizza leftover from the initial weight of pizza in each tray, as reported earlier [262]. The same type of pizza meal was used for the same participant over three sessions. At 120 minutes, the participants were served a pizza meal and asked to eat until comfortably full. The weight of ingested pizza was converted in energy (kcal) using the manufacturer’s information (**Table 5.1**). Cumulative FI (cFI) over two hours was calculated by summing the energy of the treatment and pizza meal consumed over two hours. *Ad libitum* water intake (WI) was measured by weighing the amount of consumed water served with each tray of pizza meal.

Caloric compensation as the ability to compensate for the energy consumed in a meal by consuming less energy in the subsequent meal [277] as calculated from the following equation:

*[kcal (pizza meal after control treatment) – kcal (pizza meal after caloric treatment)/kcal (caloric treatment – kcal (control treatment))*100* [278].

Table 5.1: Nutrient Composition of Pizza Meal[1]

	Pepperoni (per 191 g)	Three Cheese (per 187 g)
Calories (kcals)	460	440
Fat(g)	15	13
Sodium (mg)	970	860
Carbohydrate (g)	61	61
Fibre (g)	3	3
Sugar (g)	10	10
Protein (g)	19	18

[1] Information obtained from Dr.Oetker Giuseppe Pizzeria Nutrition Facts Table..

5.2 Statistical analysis

A two-way analysis of variance (ANOVA) was used to determine the effects of a treatment, time, and a treatment by time interaction for subjective appetite scores and physical comfort scores over 120 minutes. A one-way ANOVA was used to determine the effect of treatment on FI, total area under the curve (tAUC) for subjective appetite, and sensory evaluation of treatments and palatability of pizza meal at 120min. To determine the order effect, a session was included in ANOVA. Tukey's post hoc tests were used to analyze the differences between treatments. The data were presented as mean ± standard deviation (SD). Correlation analyses of dependent measures were conducted by using Pearson's correlation coefficients. Statistical analyses and graph plotting were conducted using SAS 9.4 (Statistical Analysis Systems, SAS Institute, Cary, NC, USA) and GraphPad Prism version 9.4.0 (GraphPad Software, San Diego, CA, USA).

Chapter 6: Results

6.1 Subject Characteristics

Thirty male participants completed all study sessions (**Figure 6.1**). The demographics of the participants were diverse. Asians made up most of the participants (70%), including East Asian (27%), Southeast Asian (20%), South Asian (17%), Central and West Asian (3%, 3%, respectively). The rest were Caucasian (14%), Biracial (10%), Middle Eastern (3%) and African Canadian (3%) (**Figure 6.2**). Participant characteristics are shown in **Table 6.1**.

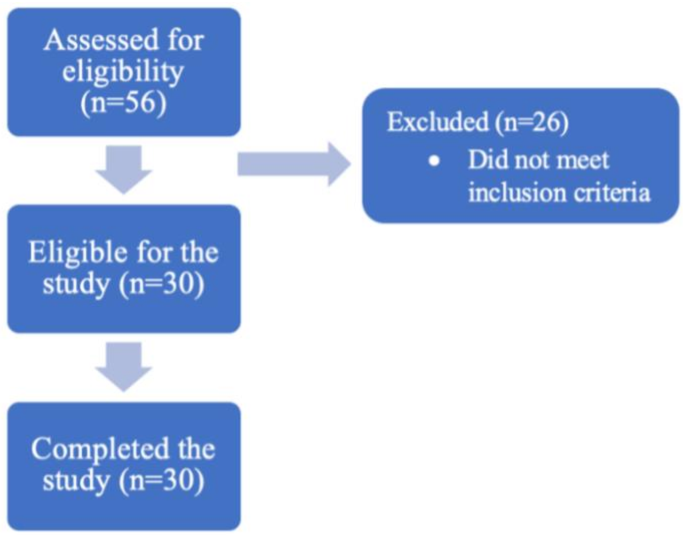

Figure 6.1: Participants' enrollment to the study according to the CONSORT 2010 Flow Diagram [279]

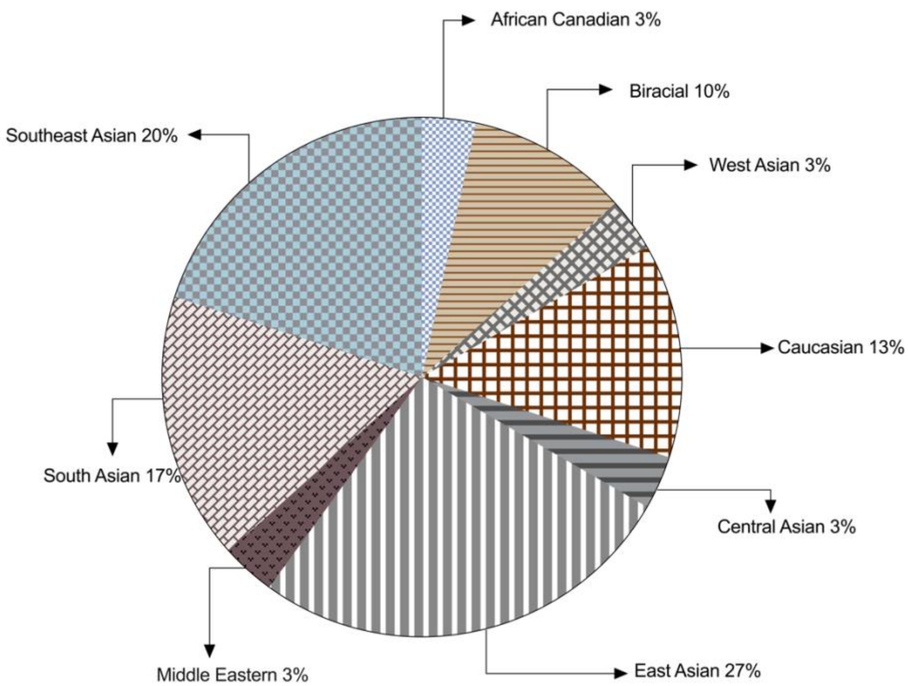

Figure 6.2: Participant Ethnic Background

Table 6.1: Participant Baseline Characteristics

Subject Characteristics	Mean (n=53)
Age (years)	24.3 ± 4.5
Height (cm)	174.7 ± 7.4
Weight (kg)	70.7 ± 8.0
BMI (kg/m2)	23.1 ± 1.6
Body Fat %	16.1 ± 3.7
FM (kg)	11.5 ± 3.4
FFM (kg)	59.1 ± 5.9
TBW (kg)	43.3 ± 4.3
PYTPA (hours/week)	7.7 ± 4.0
MET PYTPA (hours/week)	24.7 ± 11.0
Restrained Eating Score	10.1 ± 3.6
Stress in Past 24 hrs (mm)	45.5 ± 16.2
FI in Past 24 hrs (mm)	47.5 ± 14.7
PA in Past 24 hrs (mm)	45.7 ± 15.1
FBG (mmol/L)	5.5 ± 0.4

Means ± Standard Deviation, n=30. Abbreviations: BMI, body mass index; FM, fat mass; FFM, fat-free mass; TBW, total body water; PYTPA, past year's total physical activity; MET, metabolic equivalents; FI, food intake; PA, physical activity; FBG, fasting blood glucose.

6.2 Food and Water Intake

The was an effect of treatment on *ad libitum* food intake (FI) at 120 min (P<0.0001) (**Table 6.2**) (**Figure 6.3**). Both caloric treatments resulted in lower FI compared to W (P<0.05) while S led to a lower FI compared to N (P=0.05). There was an effect of a treatment on cFI over two hours (P=0.001). S and W resulted in a similar cFI (P=0.2) while cFI was higher after N compared to W (P=0.0008) and tended to be lower after S compared to N (P=0.08) (**Table 6.2**) (**Figure 6.3**). There was no effect of a session on *ad libitum* FI (P=0.2). There was no treatment (P=0.4) or session (P=0.7) effects on *ad libitum* WI or cumulative WI (**Table 6.2**). The caloric compensation after S (74.9±82.1%) was higher than after N (40.0± 92.6%) (P=0.02).

Table 6.2: Food and Water Intake

Treatment	*Ad Libitum* FI (kcals)	Cumulative FI (kcals)	*Ad Libitum* Water Intake (g)	Cumulative Water Intake (g)
W	1058.8 ± 468.0[c]	1058.8 ± 468.0[b]	389.3 ± 203.7	739.3 ± 203.7
S	820.6 ± 371.4[b]	1138.6 ± 371. 4[ab]	362.7 ± 181.6	712.7 ± 181.6
N	935.5 ± 457.0[a]	1244.5 ± 457.0[a]	369.7 ± 166.5	719.8 ± 166.5

Means ± SEM, n=30. One-way ANOVA with a Tukey Kramer post-hoc test. Values with different superscript letters are significantly different (P≤0.05).

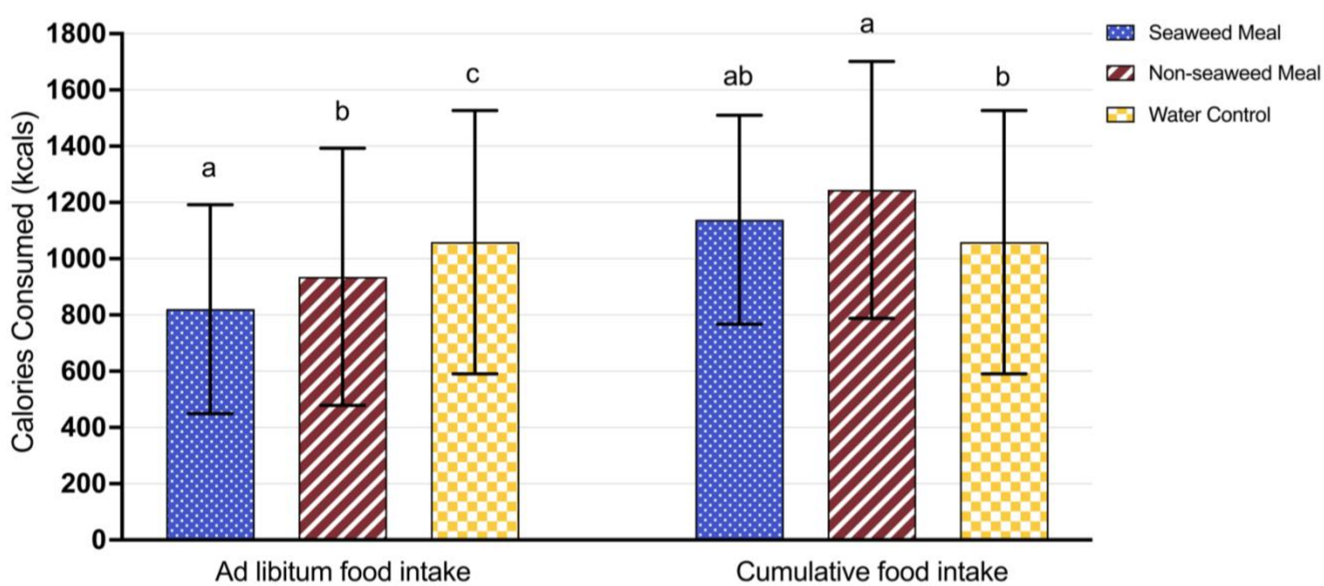

Figure 6.3: *Ad Libitum* and Cumulative Food Intake (kcals). Means ± SD, n =30. A one-way ANOVA with Tukey Kramer post-hoc test. Bars with different superscript letters are significantly different (P≤0.05).

6.3 Subjective Appetite

6.3.1 Average appetite (AA) over two hours

Average appetite scores were calculated using the formula: appetite score = [desire to eat + hunger + (100 –fullness) + prospective consumption]/4.

There was no difference in AA among the treatment at the baseline (0 min, P>0.05). There was, however, an effect of time (P<0.0001), treatment (P<0.0001), time by treatment interaction (P<0.0001), and session by treatment by time interaction (P=0.03) detected. Both S and N led to a reduced AA in comparison with W (P<0.0001) throughout the study. AA was decreased after S and N ingestion (15 min), and steadily increased between 15 and 120min, and started to decrease when pizza meal was served at 120 min (**Figure 6.4**). Both S and N resulted in the similar AA score (p>0.05) over two hours (**Table 6.3**) (**Figure 6.4**). Differences were shown between W and S, between W and N at 15 (P<0.0001), 30 (P<0.0001), 45 (P<0.0001), 60 (P<0.0001), 90 (P<0.0001) and 120min (P=0.0001, P=0.0003) where W led to an increased AA score (**Figure 6.4**). In addition, there was an effect of a treatment on the mean AA over two hours (P<0.0001) (**Table 6.3**) (**Figure 6.5**) and AA tAUC (P<0.0001) (**Table 6.3**) (**Figure 6.5**), where two-hour mean AA (**Figure 6.5**) and tAUC were decreased after S and N, compared with W (P<0.0001) (**Table 6.3**) (**Figure 6.6**).

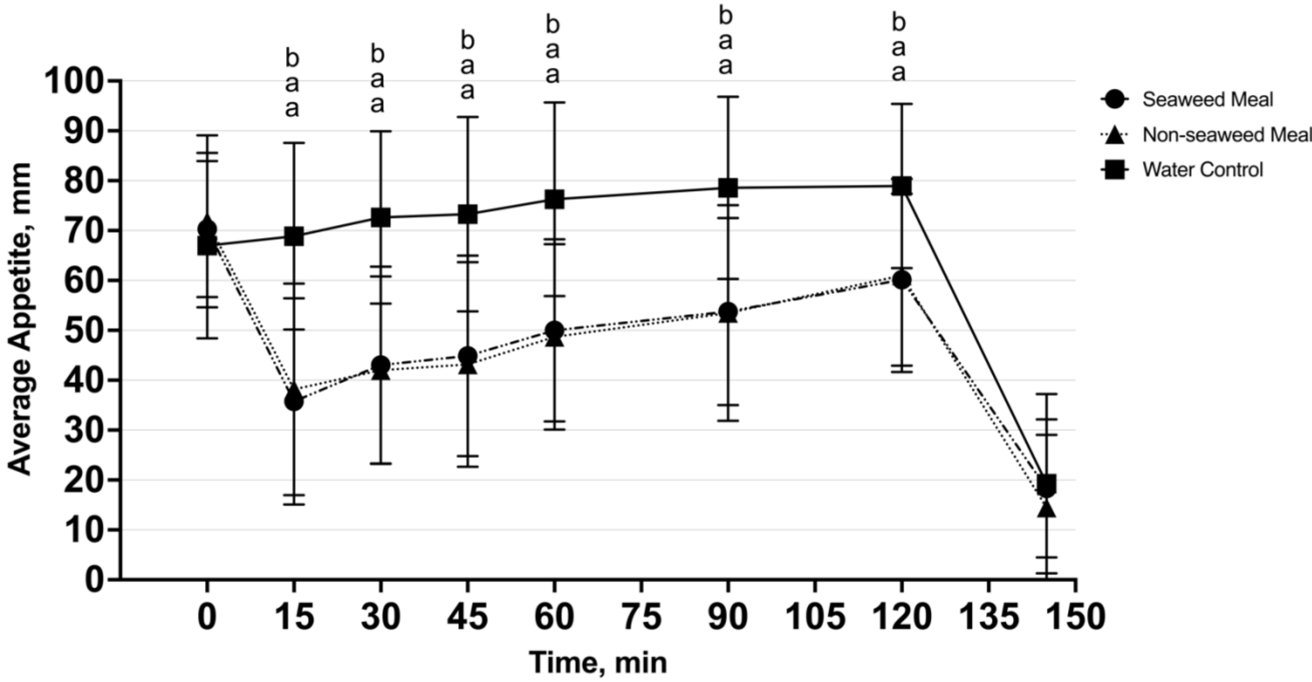

Figure 6.4: Average Appetite Score over Two hours. Means ± SD, n=30. Two-way ANOVA with a Tukey Kramer post-hoc test. Values with different superscript letters are significantly different (P≤0.05).

Table 6.3: Subjective Appetite Measures over Two Hours

Subjective Appetite	N	S	W
AA (VAS, mm)	46.6 ± 24.7[a]	47.0 ± 23.1[a]	66.9 ± 25.8[b]
DTE (VAS, mm)	46.7 ± 27.9[a]	49.5 ± 27.6[a]	66.8 ± 28.1[b]
Hunger (VAS, mm)	41.4 ± 28.3[a]	41.0 ± 25.3[a]	64.7 ± 29.6[b]
Fullness (VAS, mm)	50.1 ± 27.9[a]	51.9 ± 26.7[a]	29.8 ± 28.8[b]
PFC (VAS, mm)	47.2 ± 26.6[a]	48.6 ± 26.3[a]	65.5 ± 26.8[b]
tAUC AA (mm × min)	5980.4 ± 2112.8[a]	6022.5 ± 1900.3[a]	8982.3 ± 1981.9[b]
tAUC DTE (mm × min)	6112.2 ± 2560.8[a]	6505.7 ± 2521.5[a]	8905.5 ± 2412.3[b]
tAUC Hunger (mm × min)	5292.4 ± 2500.3[a]	5204.6 ± 1968.9[a]	8696.4 ± 2470.0[b]
tAUC Fullness (mm × min)	5603.0 ± 1958.43[a]	5896.3 ± 1897.8[a]	2592.5 ± 1875.6[b]
tAUC PFC (mm × min)	6117.8 ± 2512.8[a]	6235.8 ± 2432.3[a]	8828.1 ± 1987.8[b]

Means ± SD, n=30. Two-way ANOVA with Tukey Kramer post-hoc test. Values with different superscript letters are significantly different (P≤0.05). Abbreviations: AA, average appetite; DTE, desire to eat; PFC, prospective food consumption; tAUC, total area under the curve; VAS, visual analogue scale.

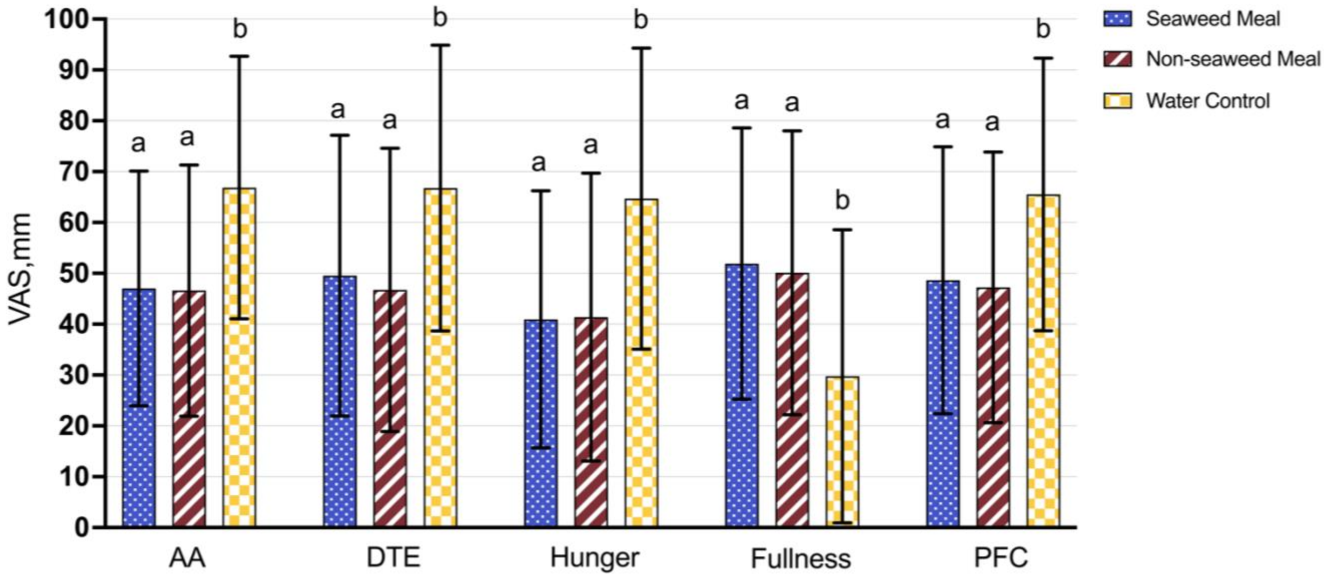

Figure 6.5: Subjective Appetite Measures over Two Hours. Means ± SD, n=30. One-way ANOVA with a Tukey Kramer post-hoc test. Values with different superscript letters are significantly different ($P \leq 0.05$). Abbreviations: PFI, Prospective food intake.

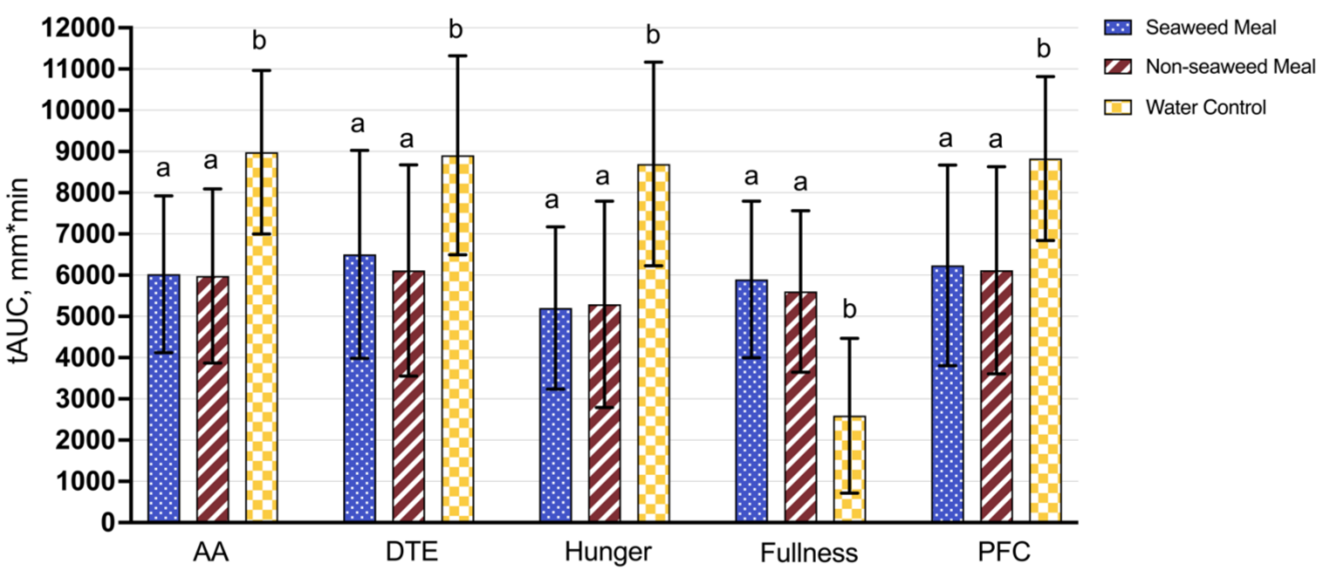

Figure 6.6: Two-hour Postprandial tAUC Subject Appetite Measures. Means ± SD, n=30. One-way ANOVA with a Tukey Kramer post-hoc test. Values with different superscript letters are significantly different ($P \leq 0.05$). Abbreviations: PFI, Prospective food intake.

6.3.2 Desire to eat (DTE)

There was no difference in DTE at baseline (0 min, P>0.05) between the treatments. There was an effect of a treatment (P<0.0001), time (P<0.0001), a treatment by time interaction (P<0.0001), and a session by treatment interaction (P=0.01) on DTE throughout the session. Both S and N showed similar DTE suppression abilities (P>0.05) and resulted in reduced DTE over two hours compared with W (P<0.05). DTE was suppressed after the ingestion of S and N at 15 min, and was gradually increasing between 30 and 120 min, and was suppressed after ad libitum FI at 120 min (**Figure 6.7**). There was an effect of a session on DTE (P=0.05); however, the post-hoc analysis did not find any pairwise differences between the sessions (P>0.05). There was an effect of a treatment on the mean DTE over two hours (P<0.0001) (**Table 6.3**) (**Figure 6.5**) and DTE tAUC (P<0.0001) (**Table 6.3**) (**Figure 6.6**). Two-hour mean DTE (**Figure 6.5**) and tAUC (**Figure 6.6**) were reduced after S and N, compared with W (P<0.0001) (**Table 6.3**).

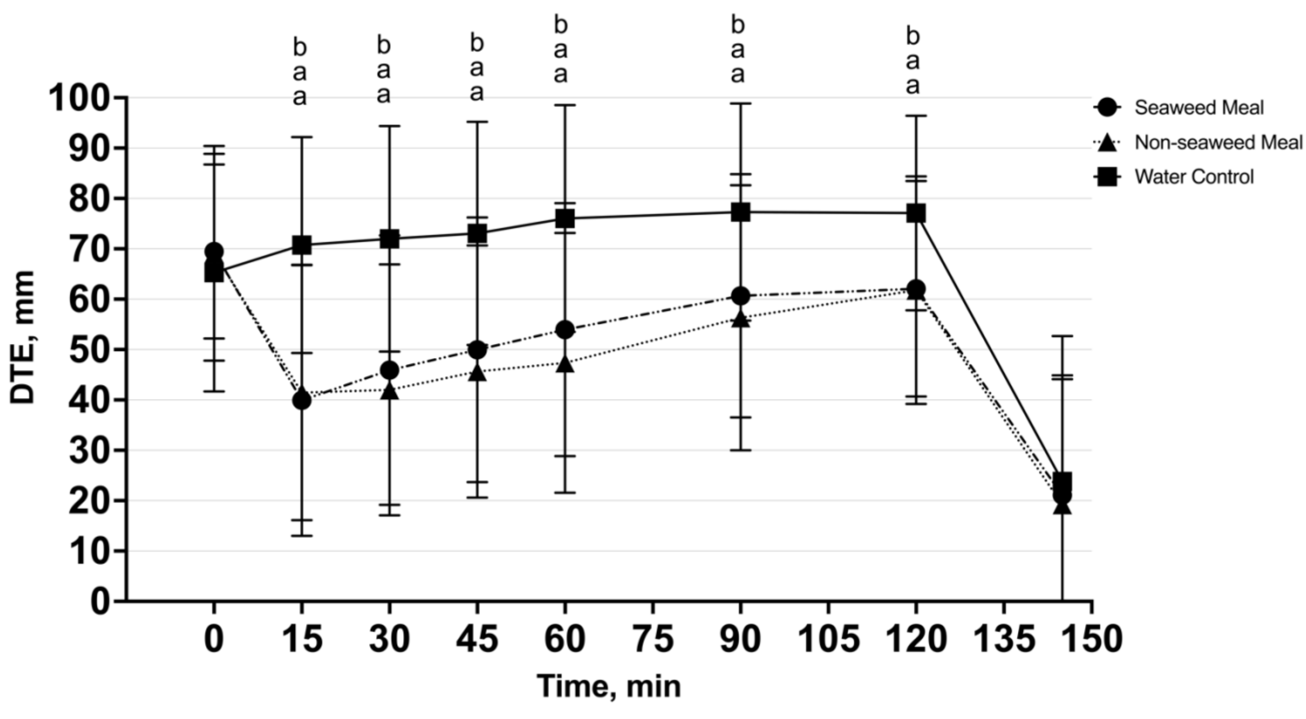

Figure 6.7: Desire to Eat over Two Hours. Means ± SD, n=30. Two-way ANOVA with a Tukey Kramer post-hoc test. Values with different superscript letters are significantly different (P≤0.05).

6.3.3 Hunger

There was no difference in perceived level of hunger at baseline (0 min, P>0.05) between the treatments. There was a treatment (P<0.0001), time (P<0.0001), and time by treatment interactions (P<0.0001) effect shown on hunger ratings over two hours. Hunger scores fell immediately following S and N consumption and continued to rise from 15 min until the pizza meal was served at 120 min (**Figure 6.8**). Both S and N suppressed subjective hunger compared to W (P<0.0001). The hunger suppression ability between S and N was not different at any time point (P>0.05). Similar with DTE, there was an effect of a treatment on the mean hunger over two hours (P<0.0001) and hunger tAUC (P<0.0001). Two-hour mean hunger (**Table 6.3**) (**Figure 6.5**) and tAUC (**Table 6.3**) (**Figure 6.6**) were reduced after S and N, compared with W (P<0.0001).

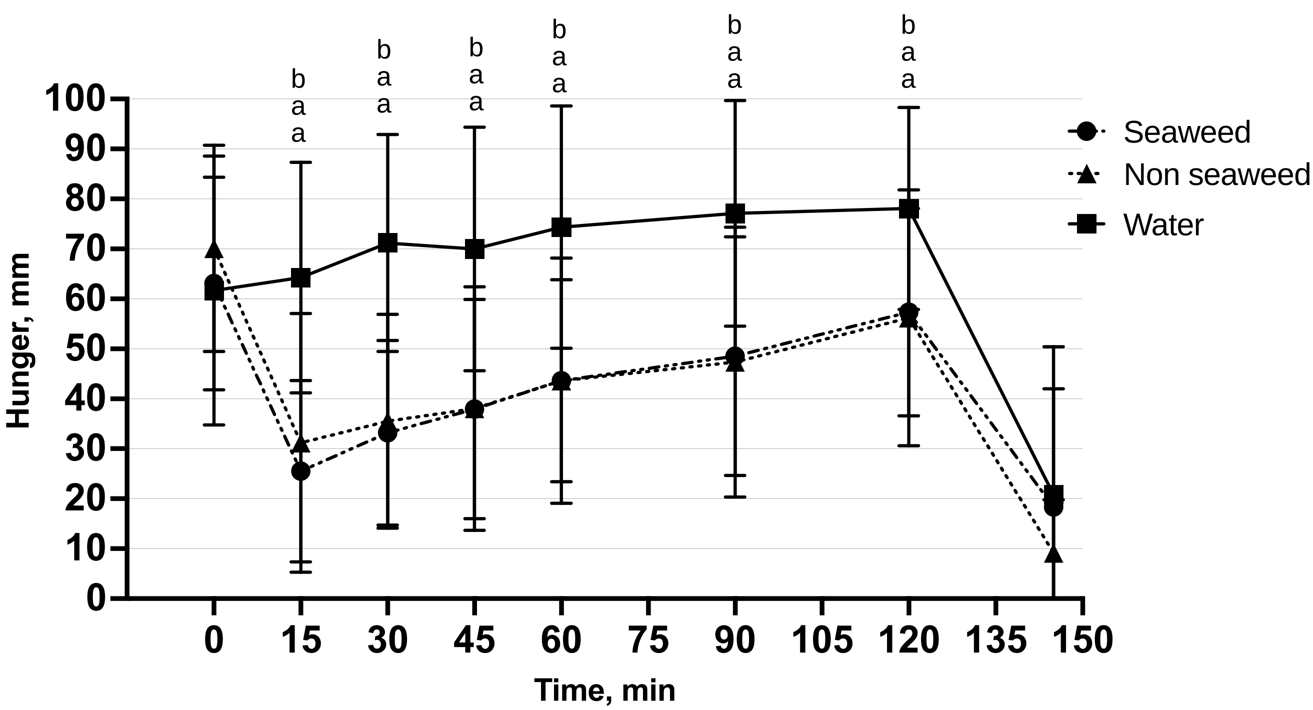

Figure 6.8: Hunger over Two Hours. Means ± SD, n=30. Two-way ANOVA with a Tukey Kramer post-hoc test. Values with different superscript letters are significantly different (P≤0.05).

6.3.4 Fullness

Fullness scores were similar at baseline (0 min, P>0.05) among three treatments. An effect of time (P<0.0001), treatment (P<0.0001), and time by treatment integration (P<0.0001) was observed on fullness ratings over two hours. Fullness scores increased immediately following the S and N consumption and decreased gradually after the treatments were consumed (**Figure 6.9**). In comparison with W, S and N both showed higher fullness ratings at 15 (P<0.0001), 30 (P<0.0001), 45 (P<0.0001), 60 (P<0.0001), 90 (P<0.0001) and 120min (P<0.0001, P=0.0019, respectively) (**Figure 6.9**). There was no difference in fullness score detected S and N at any time point. Additionally, there was an effect of a treatment on the mean fullness over two hours (P<0.0001) (**Table 6.3**) (**Figure 6.5**) and fullness tAUC (P<0.0001) (**Table 6.3**) (**Figure 6.6**), where two-hour mean fullness and tAUC were increased after S and N, compared with W (P<0.0001).

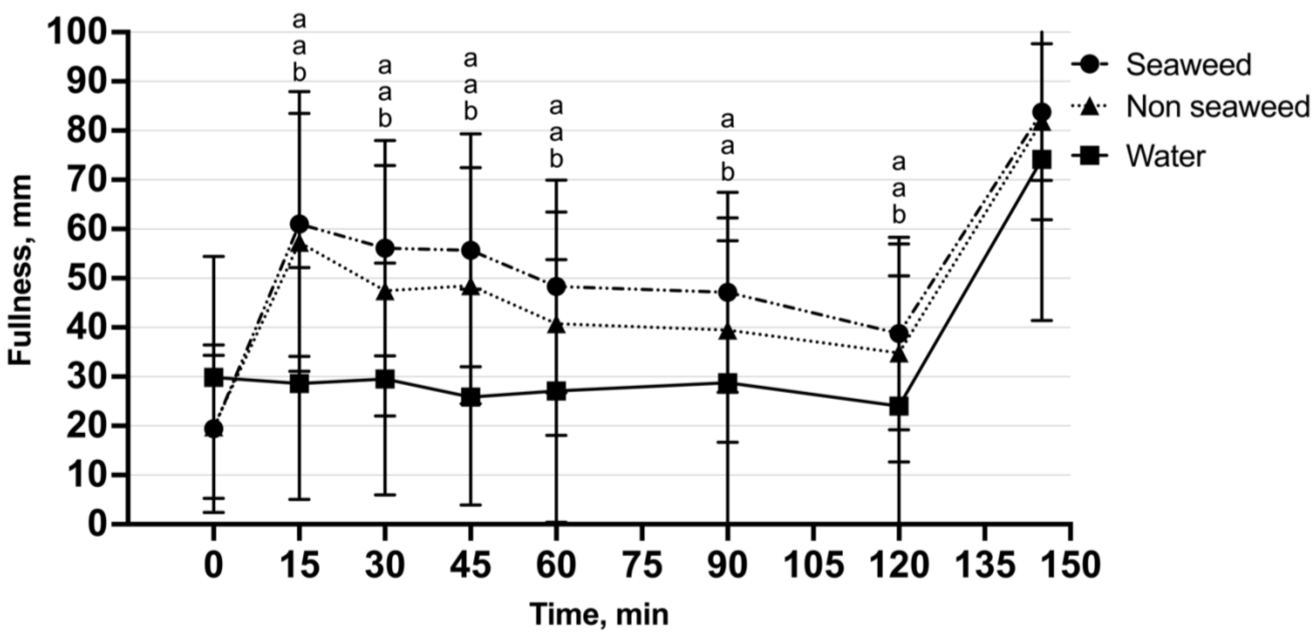

Figure 6.9: Fullness over Two Hours. Means ± SD, n=30. Two-way ANOVA with a Tukey Kramer post-hoc test. Values with different superscript letters are significantly different (P≤0.05).

6.3.5 Prospective food consumption (PFC)

There was no difference in PFC at baseline (0 min, P>0.05) between the treatments. There was an effect of time (P<0.0001), treatment (P<0.0001), time by treatment interaction (P<0.0001), session by treatment interaction (P=0.04), session by treatment by time interactions (P=0.05) observed over two hours. PFC scores fell immediately after S and N and steadily increased over 120 minutes (**Figure 6.10**). No difference was shown between the two caloric treatments (P>0.05). No difference was shown between S and N for PFC scores at any time point (**Figure 6.10**). However, differences detected between W and S, between W and N where W resulted in a higher PFC ratings at 15 (P<0.0001, P=0.0005), 30 (P=0.0037, P<0.0001), 45 (P<0.0001), 60 (P<0.0001), 90 (P=0.0002, P=0.0012) and 120min (P=0.0023, P=0.0119) (**Figure 6.10**). Moreover, there was an effect of a treatment on the mean PFC over two hours (p<0.0001) (**Table 6.3**) (**Figure 6.5**) and fullness tAUC (P<0.0001) (**Table 6.3**) (**Figure 6.6**), where two-hour mean fullness and tAUC were decreased after S and N, compared to W (P=0.02). In addition, there was an effect of session (P=0.02), session by treatment interaction (P=0.02) observed for tAUC of PFC where session two showed a higher tAUC value than session one (P=0.02).

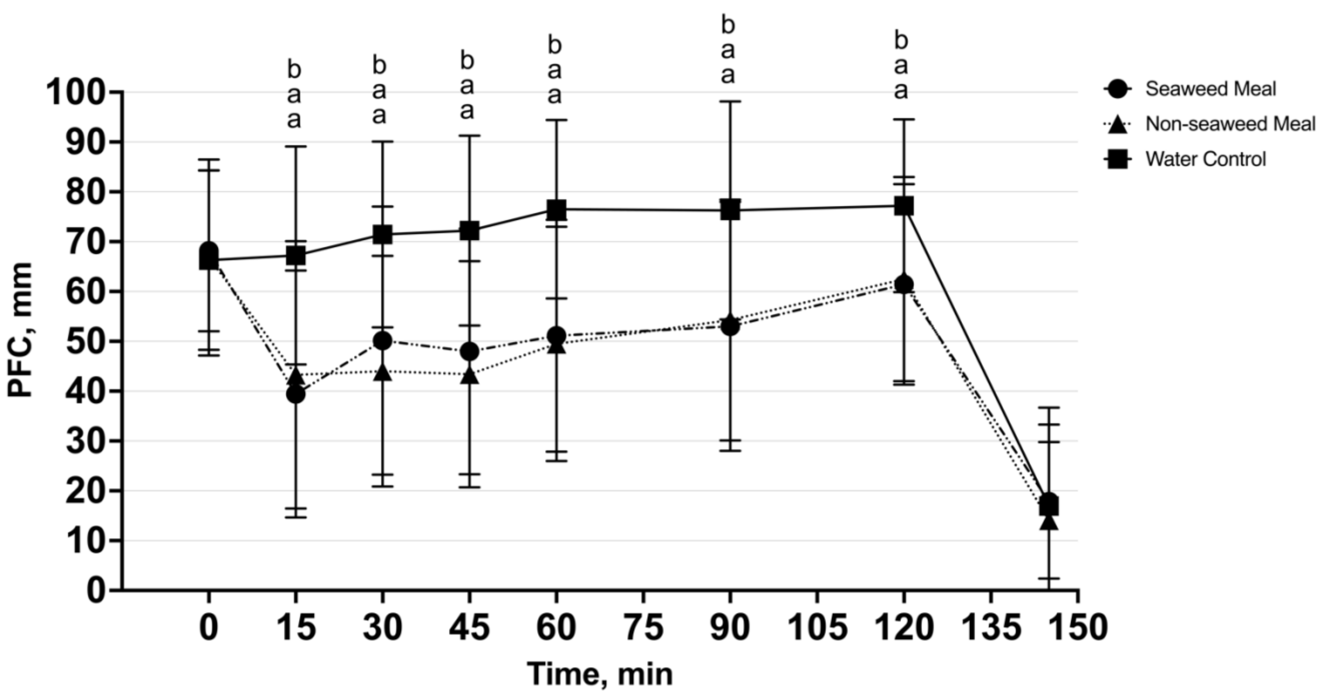

Figure 6.10: PFC over Two Hours. Means ± SD, n=30. Two-way ANOVA with a Tukey Kramer post-hoc test. Values with different superscript letters are significantly different (P≤0.05).

6.3.6 Satiety quotient

The satiety quotient (SQ) for each appetite sensation (AS) was calculated using the equation:

SQ (mm/kcal) = [(fasting AS - mean 2-hour post meal AS) / (energy content of the treatment)] *100. Compared with non-seaweed treatment, SQ for DTE, hunger and PFC from seaweed treatment were lower, and SQ for fullness was higher (**Table 6.4**). However, such differences between the two groups were not statistically significant (DTE, P=0.5; hunger, P=0.1; fullness, P=0.8; PFC, P=0.9).

Table 6.4: Satiety Quotient for Appetite Sensations over Two Hours

Variables (mm/kcal)	N	S	P value
SQ for DTE	6.45 ± 6.97	5.28 ± 8.21	0.5
SQ for Hunger	9.07 ± 6.97	6.68 ± 7.37	0.1
SQ for Fullness	-10.00 ± 6.71	-9.77 ± 7.36	0.8
SQ for PFC	5.58 ± 6.72	5.36 ± 5.79	0.9

Means ± SD, n=30. Abbreviations: SQ, satiety quotient; DTE, desire to eat; PFC, Prospective food consumption.

6.3.6 Subjective appetite scores per 100kcal of caloric treatments

In order to further explore whether each calorie from S (318 kcal) and N (309 kcal) has the same power to suppress appetite sensations, the VAS scores for each appetite measure were converted into the scores per 100 kcal of consumed treatments (**Table 6.5**). Although, per 100 kcal S showed lower DTE, hunger, PFI and AA scores compared with N over 2 hours, such differences were not statistically significant (DTE, P>0.05; hunger, P=0.2; PFC, P=0.4, AA, P=0.1). Moreover, no treatment by time interactions for all appetite sensations were shown at any time point between two caloric treatments.

Table 6.5: Subjective Appetite Scores over Two Hours (per 100kacl of caloric treatments)

Scores (mm VAS)	N	S
DTE	15.1 ± 8.0	15.0 ± 8.4
Hunger	13.4 ± 9.1	12.4 ± 7.7
Fullness	16.2 ± 9.0	15.7 ± 8.1
PFC	15.2 ± 8.6	14.7 ± 8.0
AA	15.0 ± 8.0	14.3 ± 7.0

Means ± SD, n=30. Abbreviations: DTE, desire to eat; PFC, Prospective food consumption; AA, Average appetite.

6.4 Energy, Fatigue, Physical Comfort and Thirst over Two Hours

6.4.1 Energy

There was a treatment effect observed on perceived energy level (P=0.006). Compared with W, S resulted in a higher perceived energy level (P=0.005) (**Table 6.6**). There was also a time effect observed on energy level (P<0.0001), with energy scores being lower at all time points prior to the pizza meal in comparison with energy scores after the pizza meal (**Figure 6.11**). No differences in perceived energy levels were shown neither between N and S (P=0.6) nor between N and W (P=0.09). In addition, no treatment by time interaction effect was observed on perceived energy levels (P=0.07).

Table 6.6: Subjective Energy, Fatigue, Physical Comfort and Thirst over Two Hours

Measures	N	S	W
Energy (mm VAS)	66.0 ± 20.2^{ab}	69.2 ± 18.6^{a}	59.6 ± 22.1^{b}
Fatigue (mm VAS)	28.6 ± 19.9^{a}	30.5 ± 20.6^{ab}	35.7 ± 20.3^{b}
Nausea (mm VAS)	5.9 ± 15.2	4.0 ± 9.9	3.6 ± 9.7
Gas (mm VAS)	4.6 ± 10.2	6.1 ± 12.8	6.2 ± 14.7
Stomach pain (mm VAS)	4.1 ± 9.3	3.7 ± 7.9	6.0 ± 13.2
Wellness (mm VAS)	75.1 ± 26.1	74.8 ± 25.2	70.1 ± 26.6
Diarrhea (mm VAS)	3.4 ± 11.2	3.0 ± 9.8	2.3 ± 7.6
Thirst (mm VAS)	42.6 ± 28.8	44.3 ± 29.0	31.2 ± 27.9

Means ± SD, n=30. Two-way ANOVA with Tukey Kramer post-hoc test. Values with different superscript letters are significantly different (P≤0.05). Abbreviations: VAS, visual analogue scale.

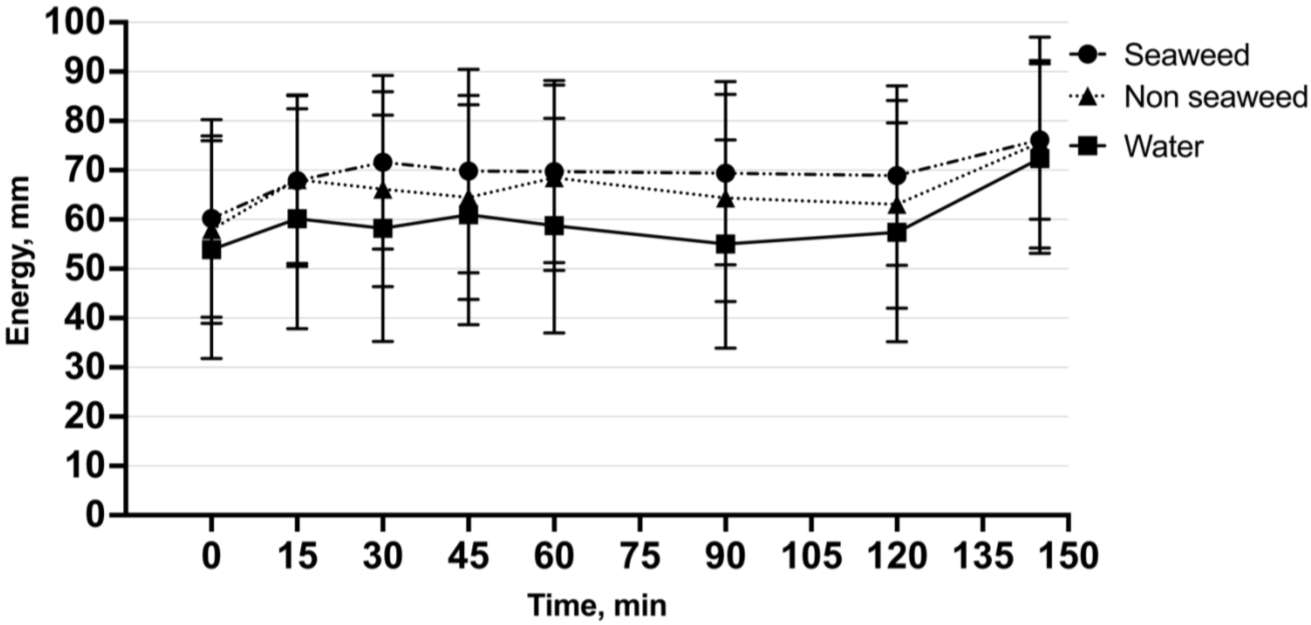

Figure 6.11: Average Energy Score over Two Hours. Means ± SD, n=30. Two-way ANOVA with a Tukey Kramer post-hoc test.

6.4.2 Fatigue

There was an effect of treatment (P=0.03) observed, where N resulted in lower ratings of fatigue compared with W (P=0.02) (**Table 6.6**). No time by treatment interaction effect was observed on fatigue ratings over 2 hours (P=0.07). At the same time, there was a time effect observed (P<0.0001), where higher perceived fatigue scores were shown prior to the treatment consumption in comparison with after the pizza meal (P<0.0001) (**Figure 6.12**).

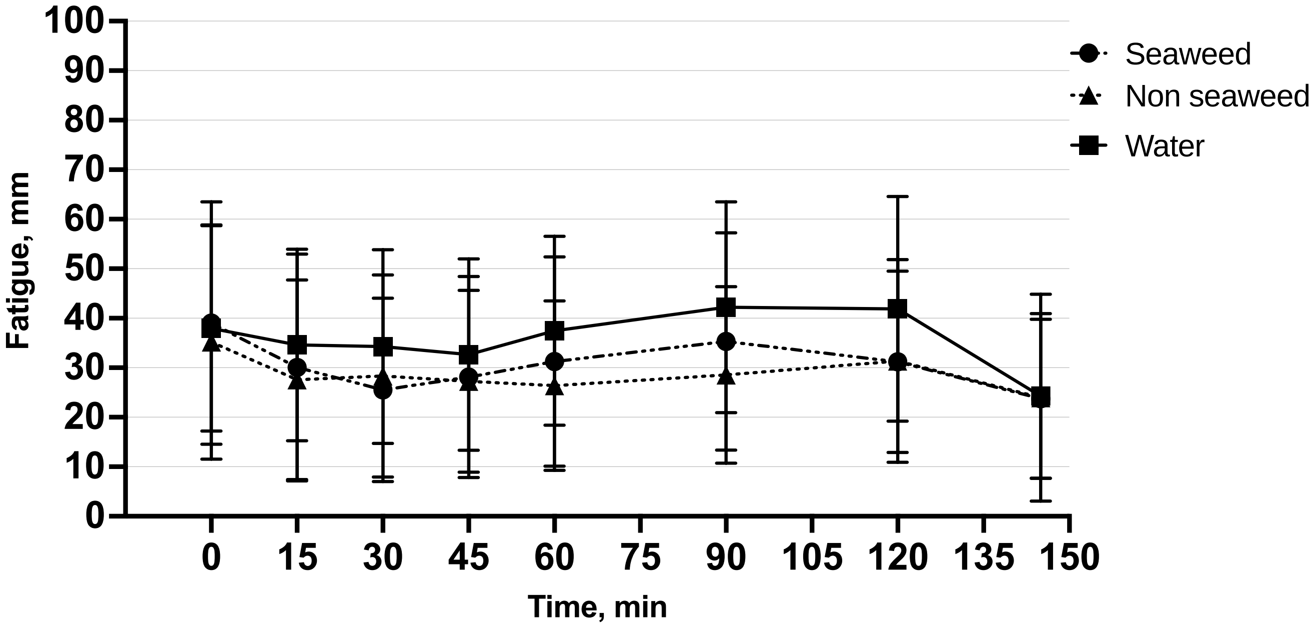

Figure 6.12: Average Fatigue Score over Two Hours. Means ± SD, n=30. Two-way ANOVA with a Tukey Kramer post-hoc test.

6.4.3 Physical comfort

Nausea. There was an effect of time (P=0.03) but no effect of a treatment (P=0.3) or treatment by time interaction (P=0.6) on perceived feeling of nausea. The scores for nausea were higher before the consumption of the treatments compared to the ranking at 90 min (P=0.03) and after the pizza meal (P=0.03) (**Figure 6.13a**) (**Table 6.6**).

Gas. There was no time (P=0.4), treatment (P=0.6) and time by treatment interactions (P=0.97) on subjective ratings of flatulence (**Table 6.6**) (**Figure 6.13b**).

Stomach pain. There was no effect of treatment (P=0.4), time (P=0.2) or time by treatment interactions (P=0.4) on stomach pain scores observed throughout the study (**Table 6.6**). Overall, participants' subjective ratings on stomach pain were lower among three treatments (**Figure 6.13c**).

Wellness. There was an effect of time (P=0.03) but no treatment effect (P=0.3) or time by treatment interaction (P=0.1) on subjective ratings of wellness. The higher wellness ratings were observed after the *ad libitum* pizza meal at 120 min compared to the earlier time points (P=0.01) (**Table 6.6**) (**Figure 6.13d**).

Diarrhea. There were no treatment (P=0.1), time (P=0.07) or time by treatment interaction (P=0.6) effects observed on subjective perception of diarrhea feeling throughout the study (**Table 6.6**) (**Figure 6.13e**).

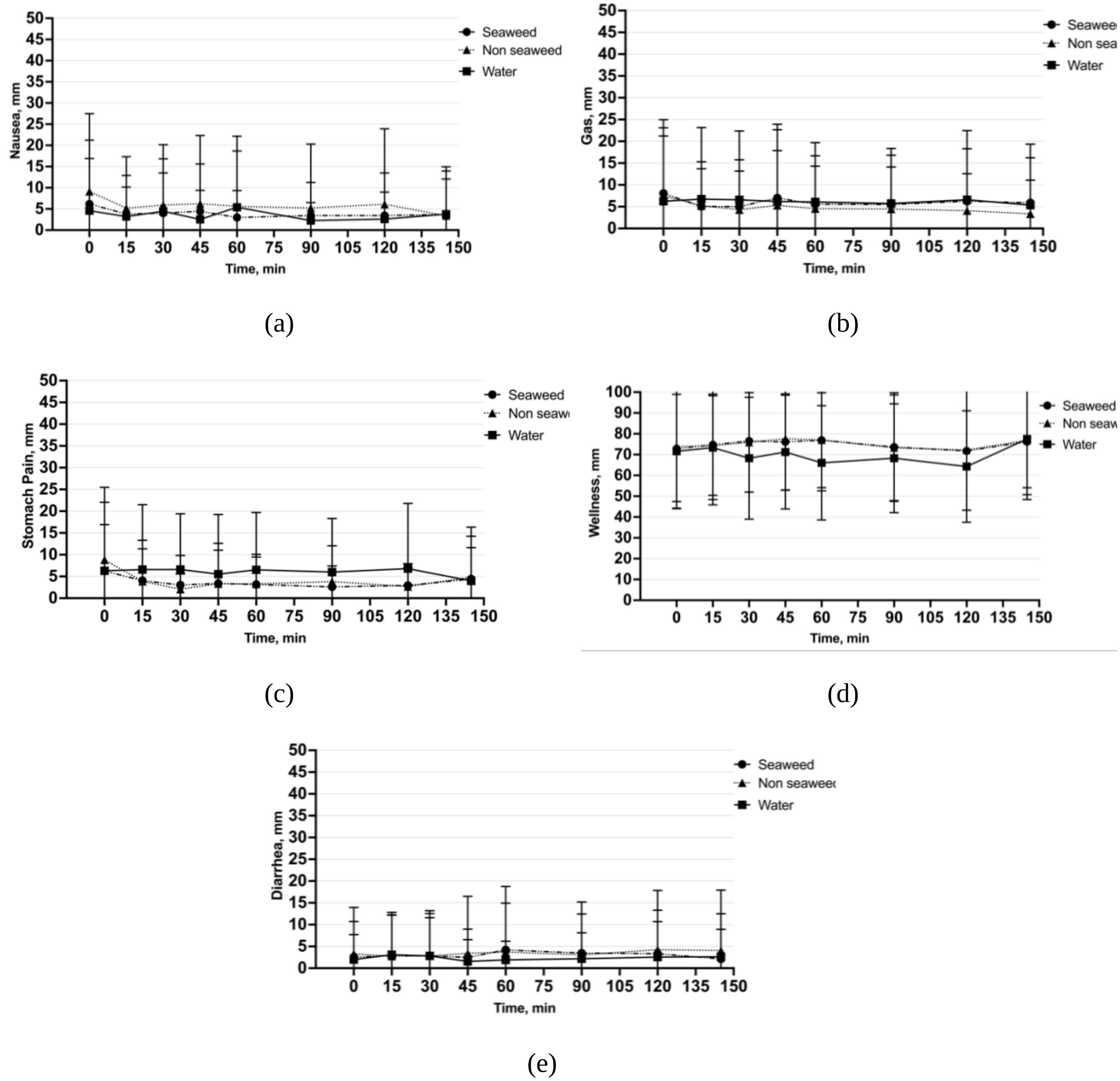

Figure 6.13: Physical Comfort over Two-hour. (a) Nausea; (b) Gas.; (c) Stomach Pain; (d) Wellness; (e) Diarrhea. Means ± SD, n=30. Two-way ANOVA with a Tukey Kramer post-hoc test.

6.4.4 Thirst

There was an effect of time (P<0.0001) but no treatment effect (P=0.1) or time by treatment interaction (P=0.2) on the subjective feeling of thirst. The thirst temporarily decreased following the treatments and continued to increase up to the pizza meal at 120 min (**Table 6.6**) (**Figure 6.14**).

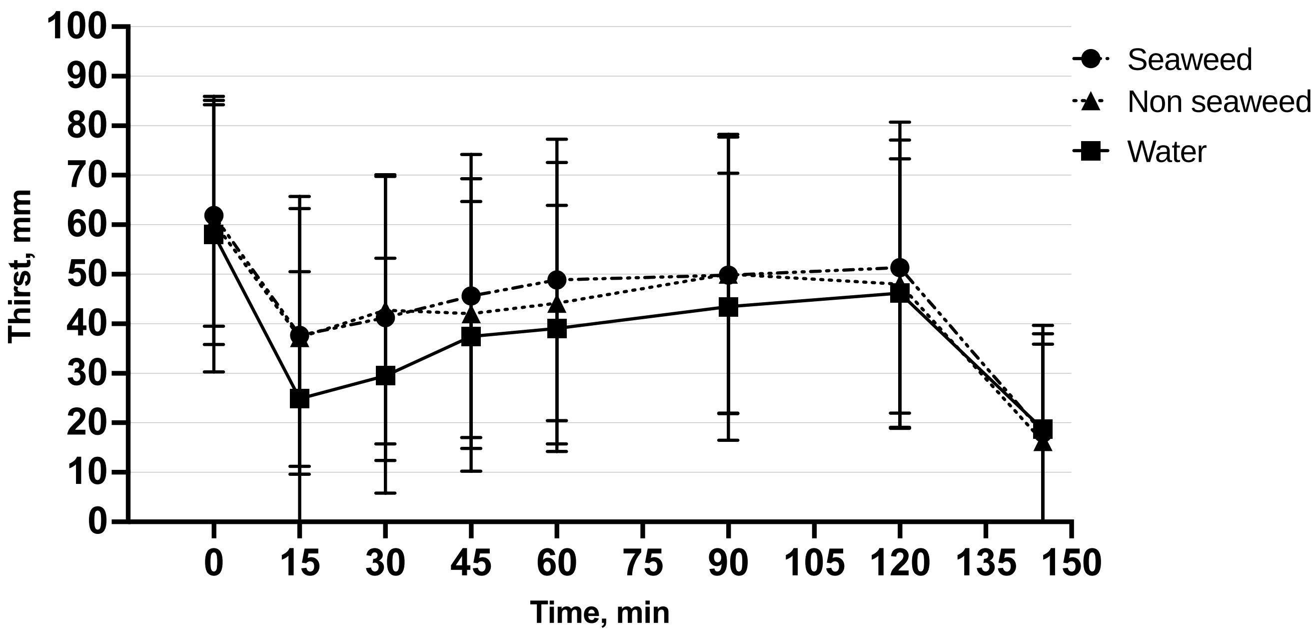

Figure 6.14: Thirst over Two Hours. Means ± SD, n=30. Two-way ANOVA with a Tukey Kramer post-hoc test.

6.5 Relations between Variables

All average subjective measures of appetite over 120min, including DTE, hunger, PFC and average appetite were associated with FI at 120min. Subjective fullness over 120 min and fullness score at 120min were inversely associated with FI at 120min (**Table 6.7**).

The average past year's total physical activity for participants was 7.3 ± 4.0 hours per week and equal to 24.7 ± 11.0 metabolic equivalents (MET) per week. There was a weak positive trend for the association between PYTPA MET hours/week and food intake (**Table 6.7**). There was no relationship between food intake and BMI. However, there was a strong positive relationship between fat-free mass (FFM) and food intake (**Table 6.7**).

Table 6.7: The relationship between variables and food intake at 120 minutes.

Variable	Food Intake (kcal) at 120min	P-value
DTE at 120min	r=0.13	0.225
DTE over 120min	r=0.39*	0.0002
Hunger at 120min	r=0.14	0.197
Hunger over 120min	r=0.42*	<0.0001
Fullness at 120min	r=-0.24*	0.025
Fullness over 120min	r=-0.41*	<0.0001
PFC at 120min	r=0.31*	0.003
PFC over 120min	r=0.42*	<0.0001
AA at 120min	r=0.23*	0.03
AA over 120min	r=0.44*	<0.0001
tAUC DTE	r=0.34*	0.001
tAUC Hunger	r=0.39*	<0.0001
tAUC Fullness	r=-0.39*	<0.0001
tAUC PFI	r=0.38*	<0.0001
tAUC AA	r=0.45*	<0.0001
SQ DTE	r=-0.37*	0.004
SQ Hunger	r=-0.36*	0.005
SQ Fullness	r=0.50*	<0.0001
SQ PFC	r=-0.23	0.075
PYTPA (hours/week)	r=0.17	0.117
PYTPA (MET hours/week)	r=0.20	0.059
FFM	r=0.38*	0.0003
BMI	r=0.08	0.472
Palatability of pizza (mm, VAS)	r=0.19	0.075

Pearson Correlation Coefficients (r). *Indicates significance ($P \leq 0.05$). Abbreviations: DTE, desire to eat; PFC, Prospective food consumption; AA, Average appetite; SQ, satiety quotient; PYTPA, past year's total physical activity; MET, metabolic equivalents; FFM, free fat mass; BMI, body mass index

6.7 Sensory Evaluation

There was an effect of a treatment on pleasantness, taste, texture, saltiness, chewiness, bitterness, seaweed flavour, mouthfeel, flavour, aftertaste, appearance, prospective purchasing (**Table 6.8**). S and N resulted in a similar pleasantness, taste, texture, saltiness, bitterness, flavor, mouthfeel, aftertaste and appearance ratings (P>0.05); however, the sensory perception of both caloric treatments was different compared with W (P≤0.05) (**Table 6.8**). Although hedonic perception of chewiness was similar for S and N treatments, the intensity of chewing was higher with S compared to N treatments (P=0.0003). There was an effect of a treatment on the pleasantness of pizza meal served at 120 min (P=0.03). The pleasantness of pizza was lower after S compared to N and W treatments (P≤0.05) (**Table 6.8**). The sensory attributes of the treatments and the palatability of the pizza meal are illustrated below in **Figures 6.15 - 6.30**.

Table 6.8: Sensory Evaluation of the Three Treatments

Sensory Characteristics	Treatments			P-value (treatment)
	S	**N**	**W**	
Pleasantness (9-pt HS)	7.1 ± 1.1^{a}	7.6 ± 1.0^{a}	5.9 ± 1.9^{b}	P=0.0001
Pleasantness (VAS, mm)	72.8 ± 15.5^{a}	78.2 ± 13.6^{a}	52.2 ± 24.4^{b}	P=0.03
Taste (9-pt HS)	7.20 ± 1.1^{a}	7.5 ± 0.9^{a}	5.5 ± 1.5^{b}	P<0.0001
Texture (9-pt HS)	6.90 ± 1.6^{a}	7.3 ± 1.6^{a}	5.8 ± 1.6^{b}	P=0.002
Saltiness (9-pt HS)	6.40 ± 1.9^{a}	6.6 ± 1.6^{a}	5.1 ± 1.2^{b}	p<0.0001
Saltiness (VAS, mm)	55.5 ± 22.8^{a}	51.4 ± 20.3^{a}	6.4 ± 17.1^{b}	p<0.0001
Chewiness (9-pt HS)	6.9 ± 1.3^{a}	6.8 ± 1.5^{a}	5.1 ± 1.5^{b}	p<0.0001
Chewiness (VAS, mm)	62.8 ± 21.0^{a}	44.9 ± 23.9^{b}	5.0 ± 17.4^{c}	p<0.0001
Bitterness (VAS, mm)	14.9 ± 17.2^{a}	16.2 ± 19.5^{a}	4.8 ± 12.7^{b}	p=0.01
Flavour (9-pt HS)	6.8 ± 1.5^{a}	7.3 ± 1.5^{a}	5.3 ± 1.3^{b}	p<0.0001
Mouthfeel (9-pt HS)	6.7 ± 1.5^{ab}	7.1 ± 1.3^{a}	5.8 ± 1.7^{b}	P=0.009
Seaweed Flavour (VAS, mm)	46.1 ± 26.6^{a}	24.6 ± 26.6^{b}	6.1 ± 17.3^{c}	p<0.0001
Aftertaste (9-pt HS)	6.3 ± 1.7^{a}	6.9 ± 1.5^{a}	5.1 ± 1.3^{b}	p<0.0001
Appearance (9-pt HS)	6.8 ± 1.6^{ab}	7.0 ± 1.4^{a}	5.8 ± 1.6^{b}	p=0.01
Prospective Purchasing (VAS, mm)	65.5 ± 18.1^{a}	71.9 ± 21.9^{a}	42.0 ± 35.2^{b}	p=0.0005
Pizza meal				
Pleasantness (VAS, mm)	72.0 ± 22.4^{a}	77.7 ± 17.6^{b}	79.6 ± 23.1^{b}	p=0.03

Means ± SD, n=30. 9-point hedonic scale: 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely. One-way ANOVA with a Tukey Kramer post-hoc test. Values with different superscript letters are significantly different (P≤0.05).

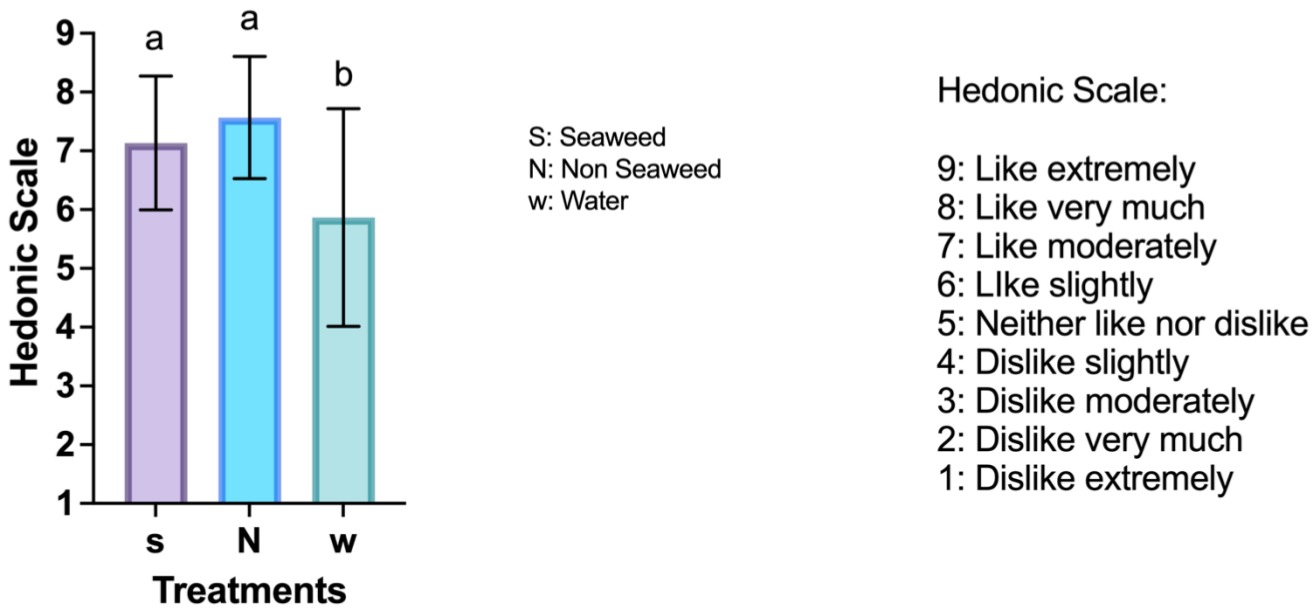

Figure 6.15: Treatment Pleasantness (9-point hedonic scale). Means ± SD, n=30. One-way ANOVA with a Tukey Kramer post-hoc test. Values with different superscript letters are significantly different (P≤0.05).

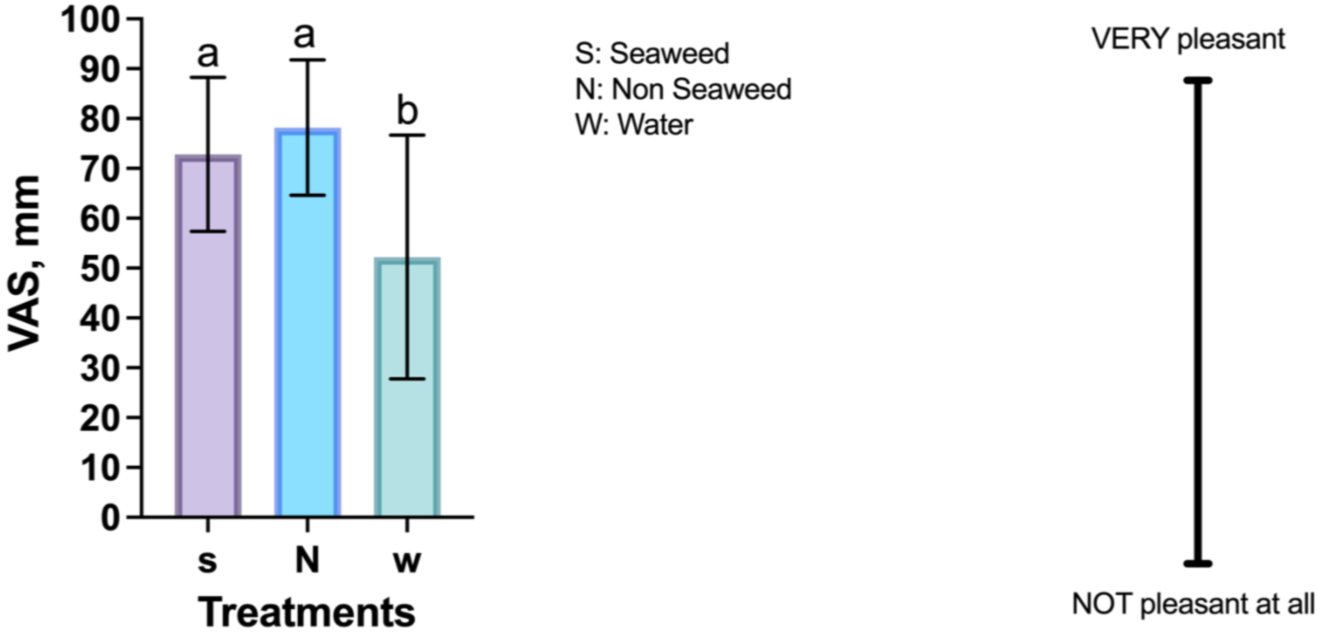

Figure 6.16: Treatment Pleasantness (VAS). Means ± SD, n=30. One-way ANOVA with a Tukey Kramer post-hoc test. Values with different superscript letters are significantly different (P≤0.05).

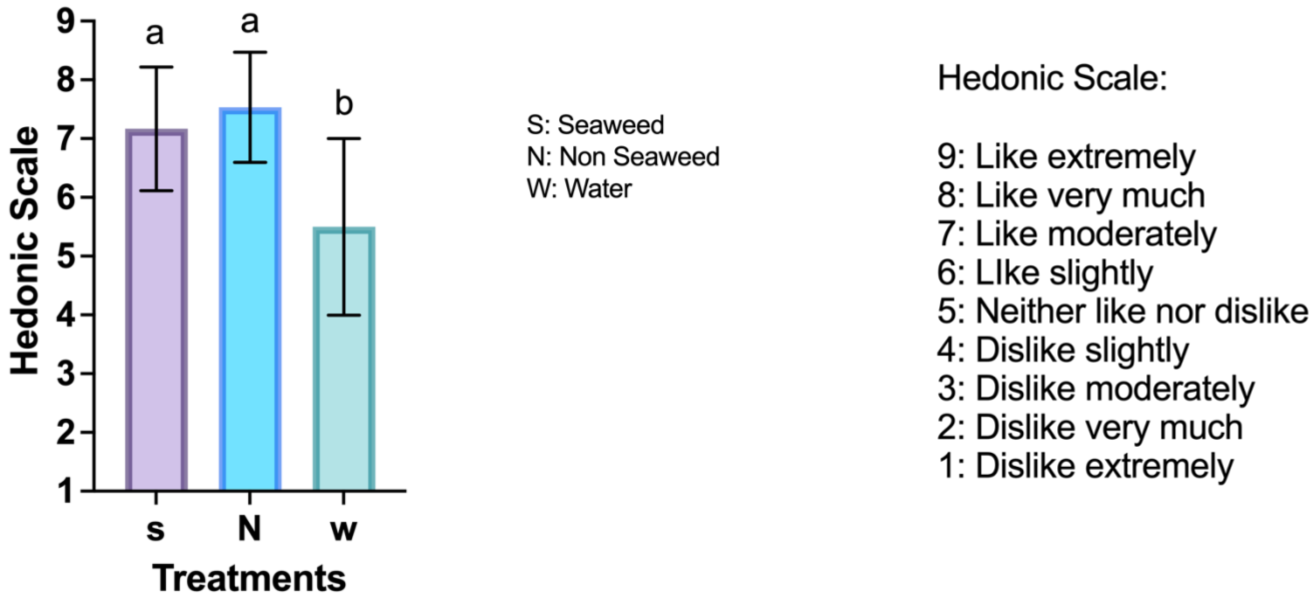

Figure 6.17: Treatment Taste (9-point hedonic scale). Means ± SD, n=30. One-way ANOVA with a Tukey Kramer post-hoc test. Values with different superscript letters are significantly different ($P \leq 0.05$).

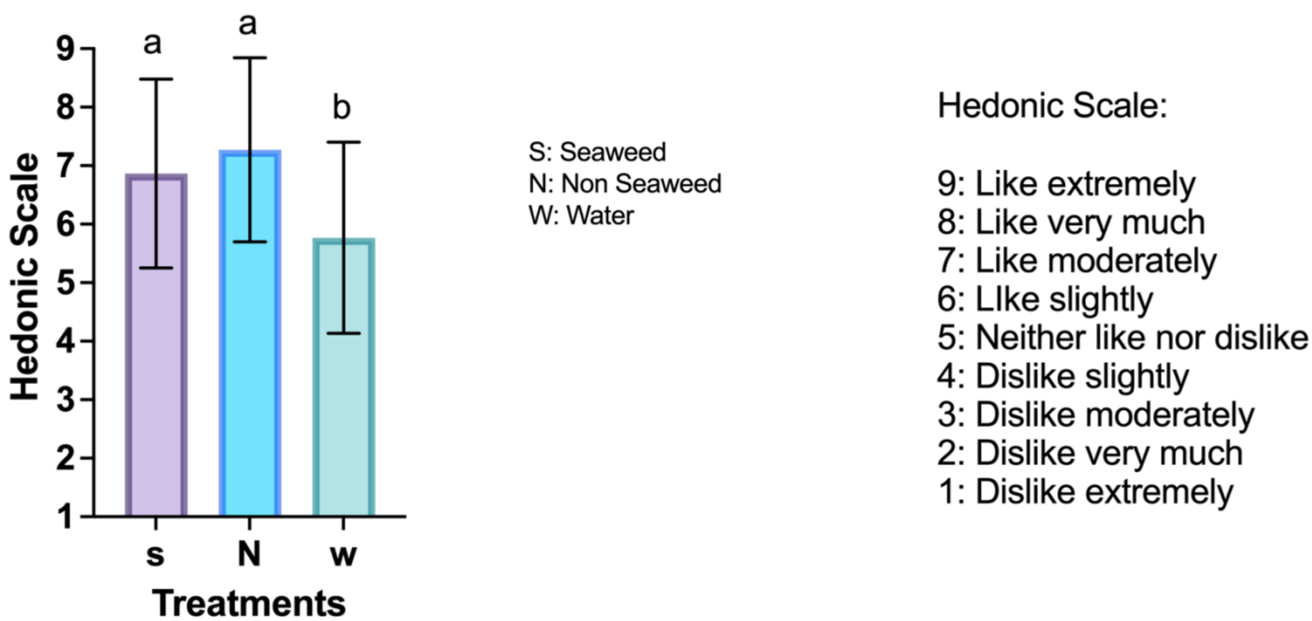

Figure 6.18: Treatment Texture (9-point hedonic scale). Means ± SD, n=30. One-way ANOVA with a Tukey Kramer post-hoc test. Values with different superscript letters are significantly different ($P \leq 0.05$).

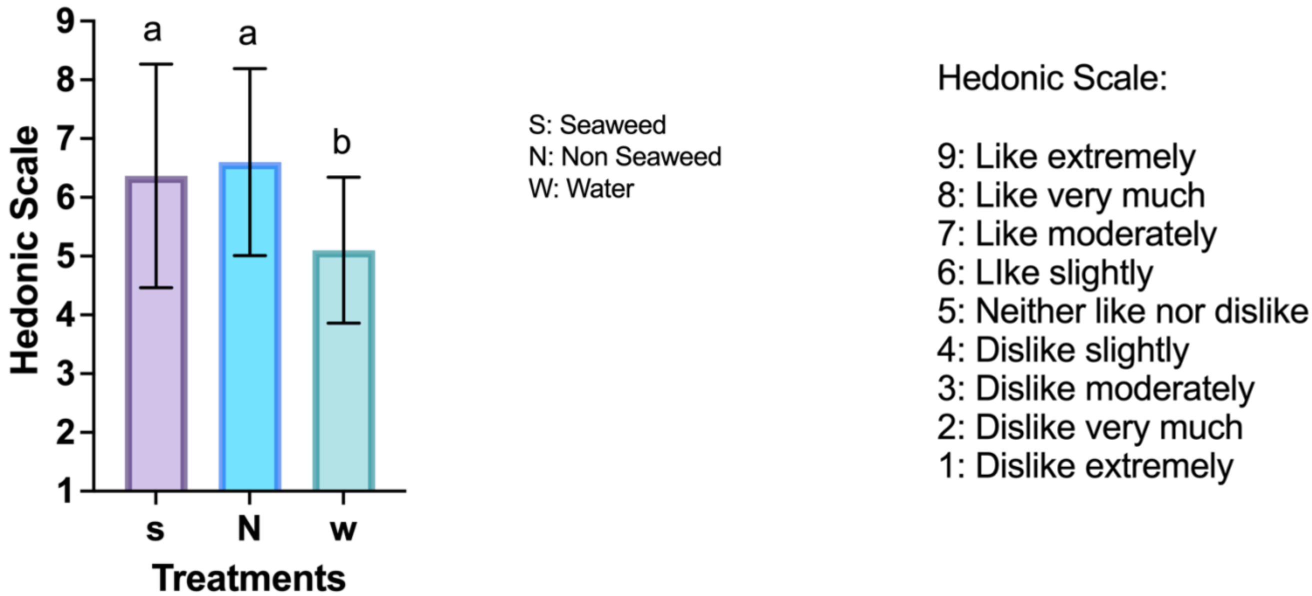

Figure 6.19: Treatment Saltiness (9-point hedonic scale). Means ± SD, n=30. One-way ANOVA with a Tukey Kramer post-hoc test. Values with different superscript letters are significantly different (P≤0.05).

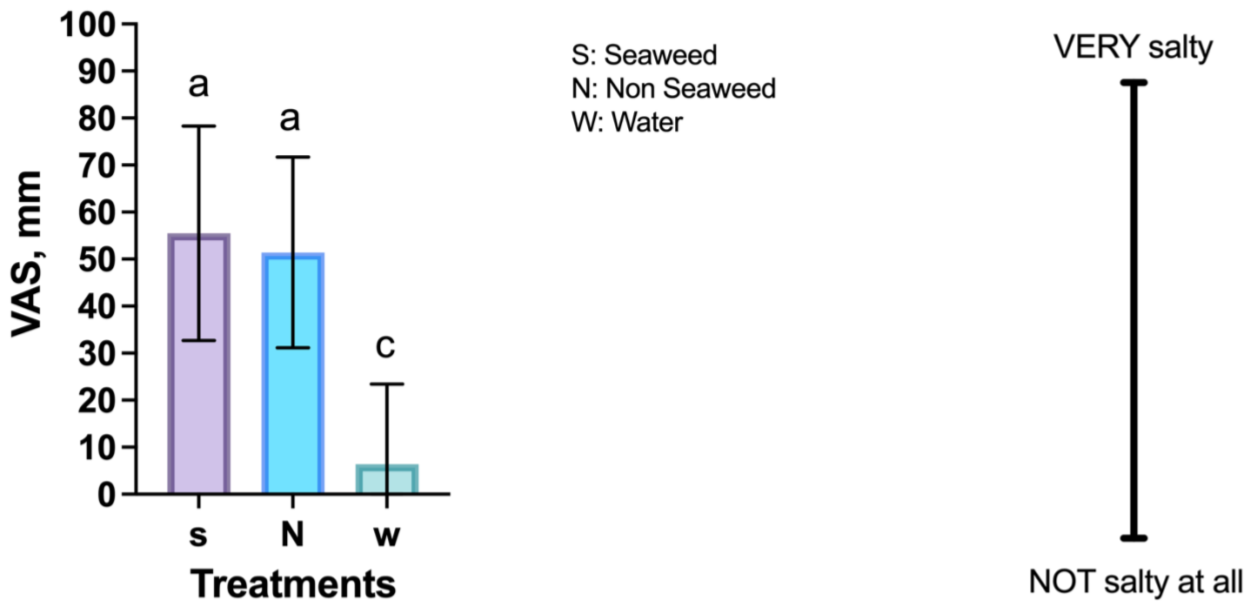

Figure 6.20: Treatment Saltiness (VAS). Means ± SD, n=30. One-way ANOVA with a Tukey Kramer post-hoc test. Values with different superscript letters are significantly different (P≤0.05).

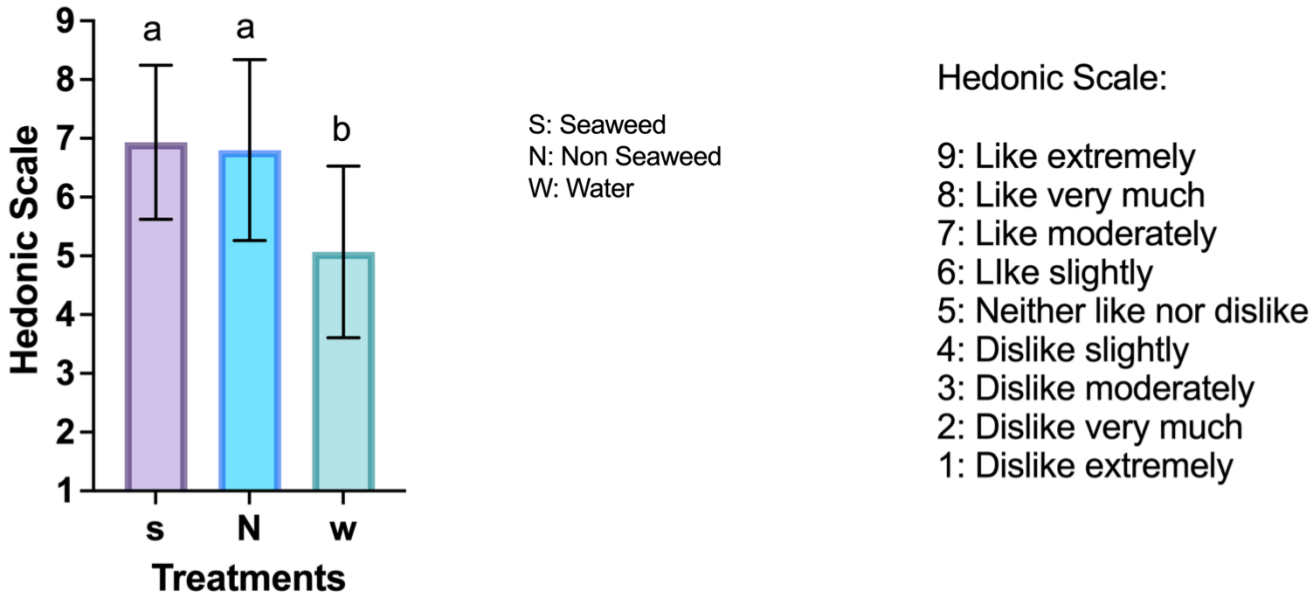

Figure 6.21: Treatment Chewiness (9-point hedonic scale). Means ± SD, n=30. One-way ANOVA with a Tukey Kramer post-hoc test. Values with different superscript letters are significantly different (P≤0.05).

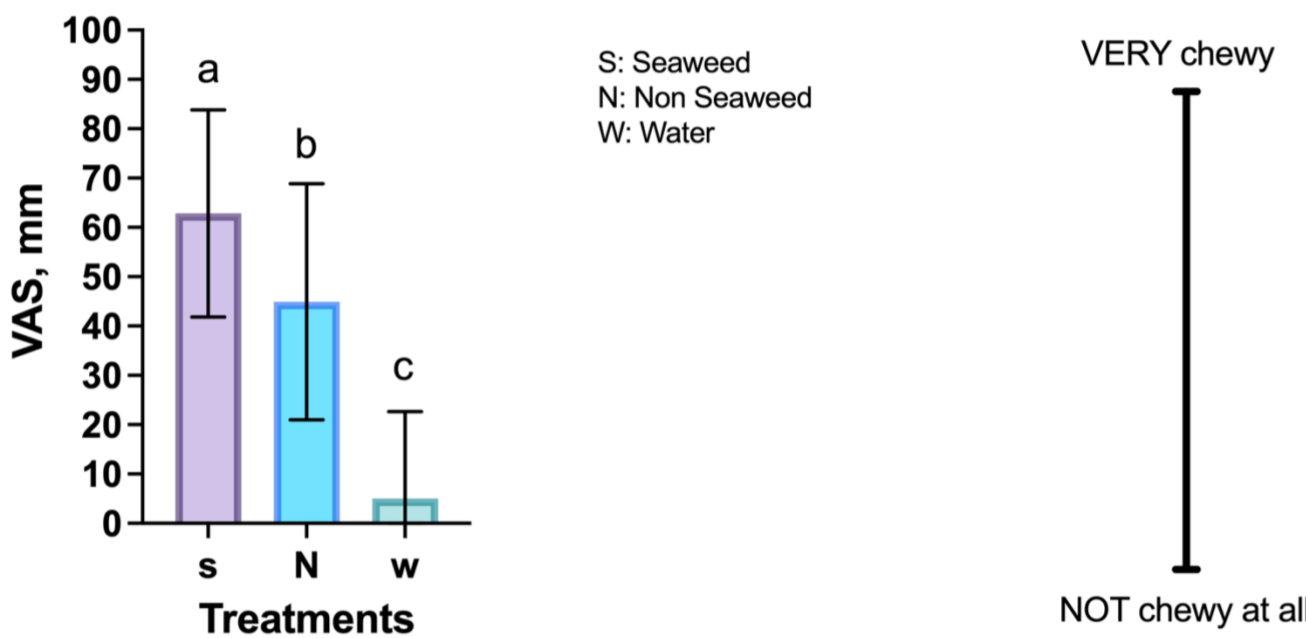

Figure 6.22: Treatment Chewiness (VAS). Means ± SD, n=30. One-way ANOVA with a Tukey Kramer post-hoc test. Values with different superscript letters are significantly different (P≤0.05).

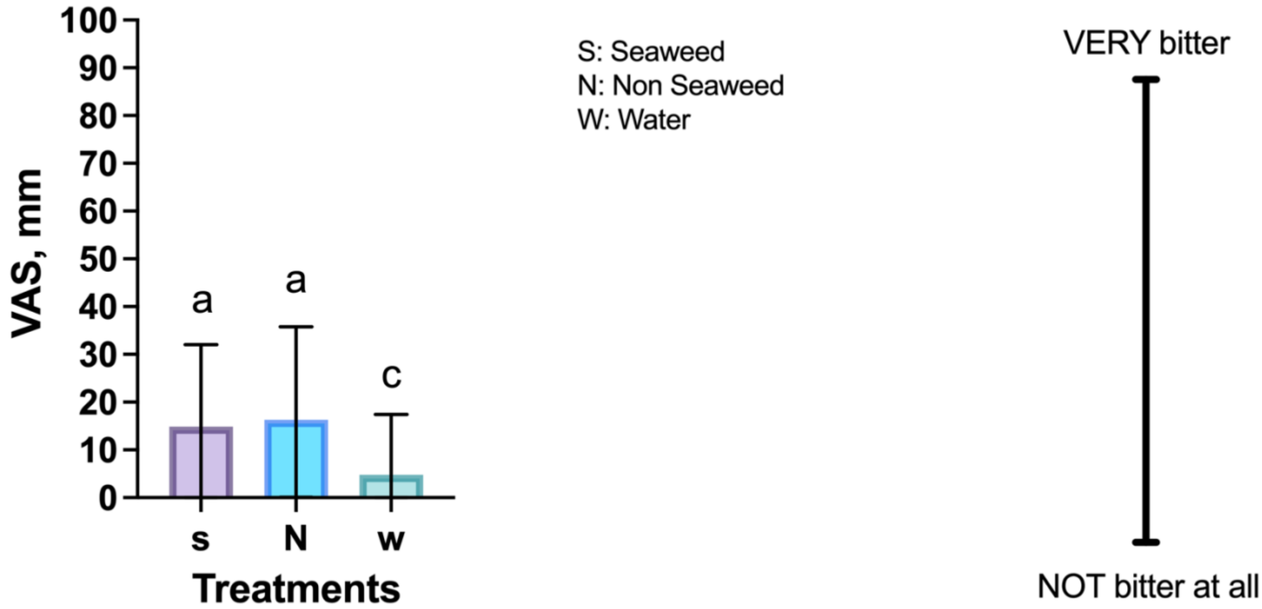

Figure 6.23: Treatment Bitterness (VAS). Means ± SD, n=30. One-way ANOVA with a Tukey Kramer post-hoc test. Values with different superscript letters are significantly different (P≤0.05).

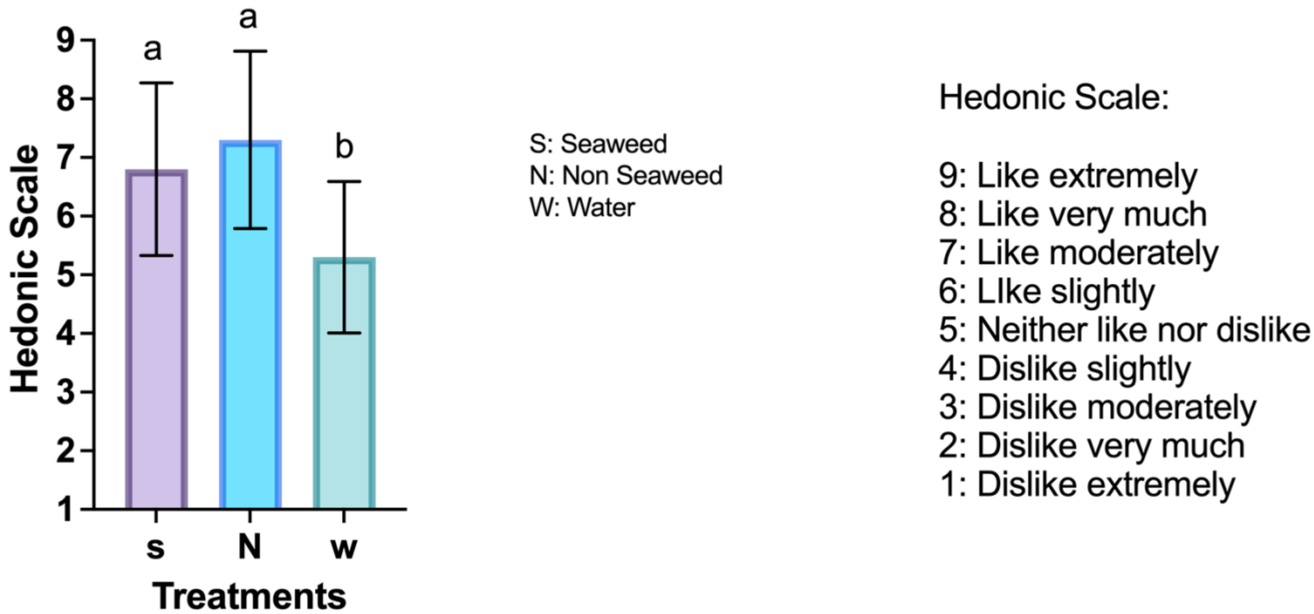

Figure 6.24: Treatment Flavour (9-point hedonic scale). Means ± SD, n=30. One-way ANOVA with a Tukey Kramer post-hoc test. Values with different superscript letters are significantly different (P≤0.05).

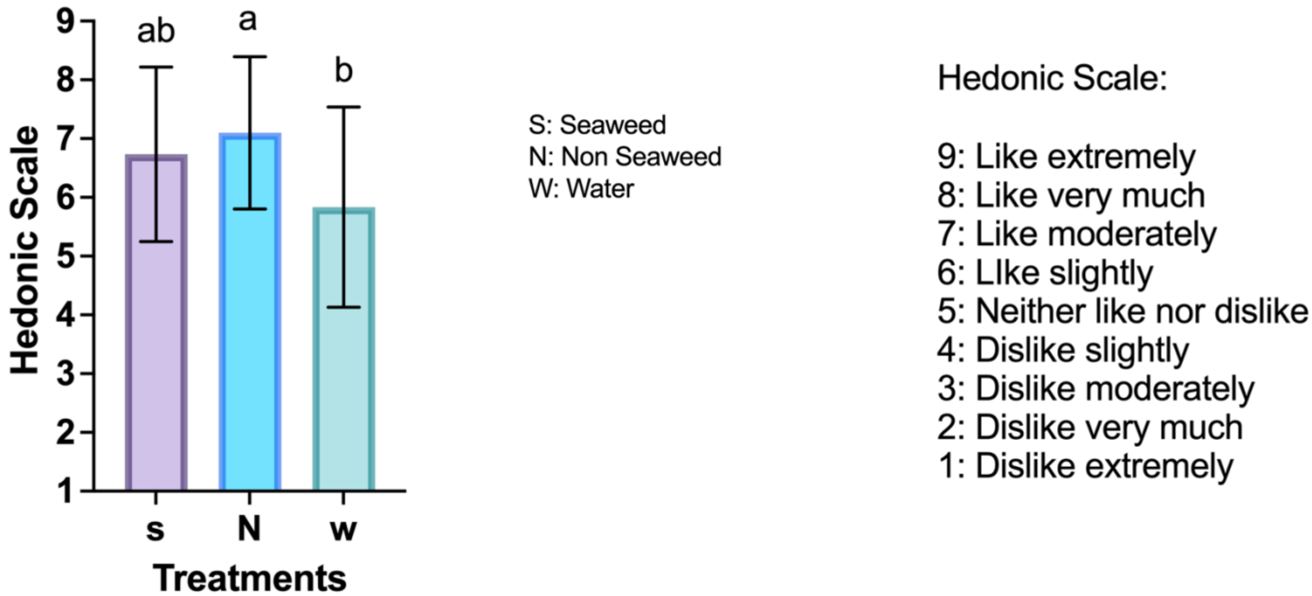

Figure 6.25: Treatment Mouthfeel (9-point hedonic scale). Means ± SD, n=30. One-way ANOVA with a Tukey Kramer post-hoc test. Values with different superscript letters are significantly different (P≤0.05).

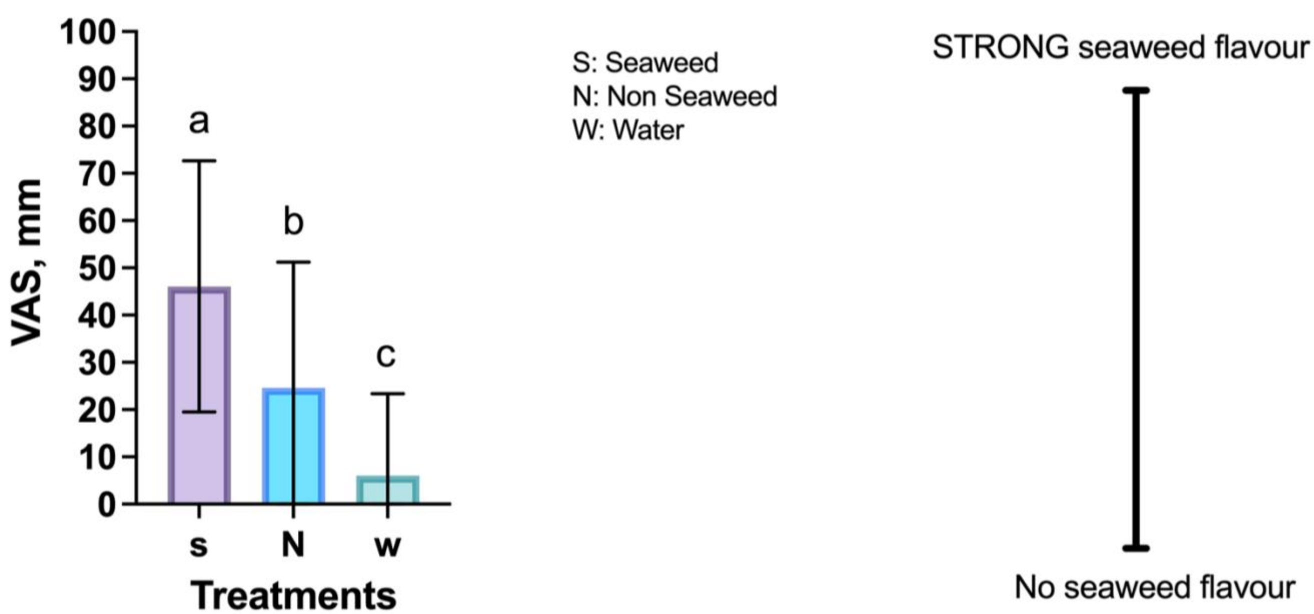

Figure 6.26: Treatment Seaweed Flavour (VAS). Means ± SD, n=30. One-way ANOVA with a Tukey Kramer post-hoc test. Values with different superscript letters are significantly different (P≤0.05).

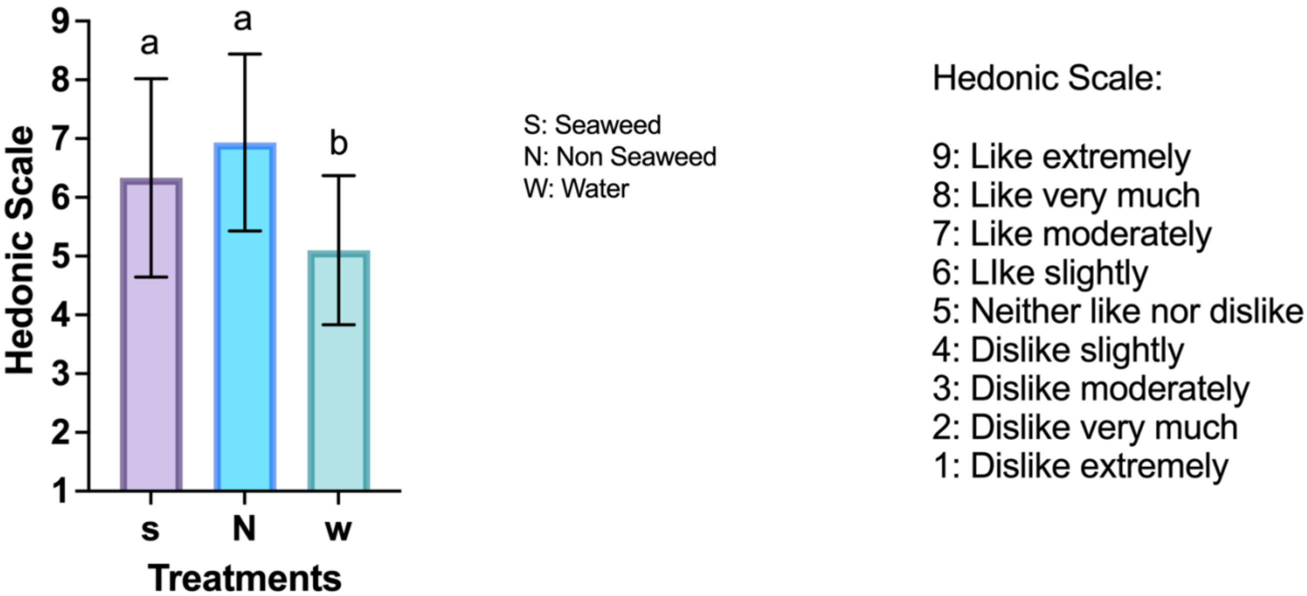

Figure 6.27: Treatment Aftertaste (9-point hedonic scale). Means ± SD, n=30. One-way ANOVA with a Tukey Kramer post-hoc test. Values with different superscript letters are significantly different (P≤0.05).

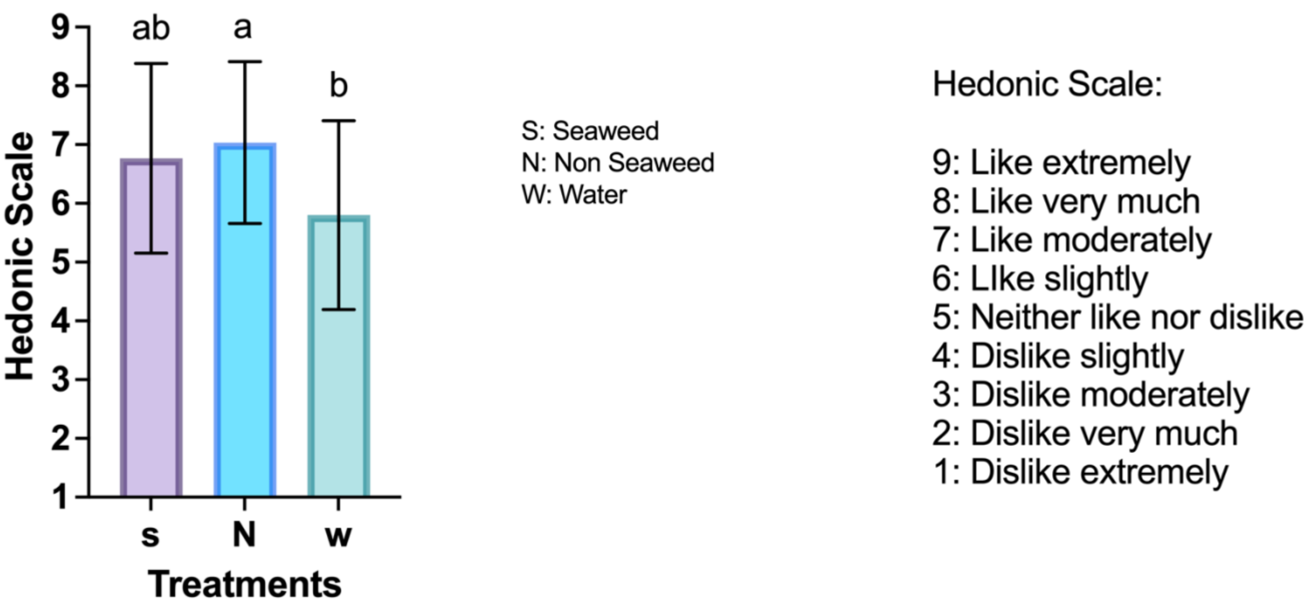

Figure 6.28: Treatment Appearance (9-point hedonic scale). Means ± SD, n=30. One-way ANOVA with a Tukey Kramer post-hoc test. Values with different superscript letters are significantly different (P≤0.05).

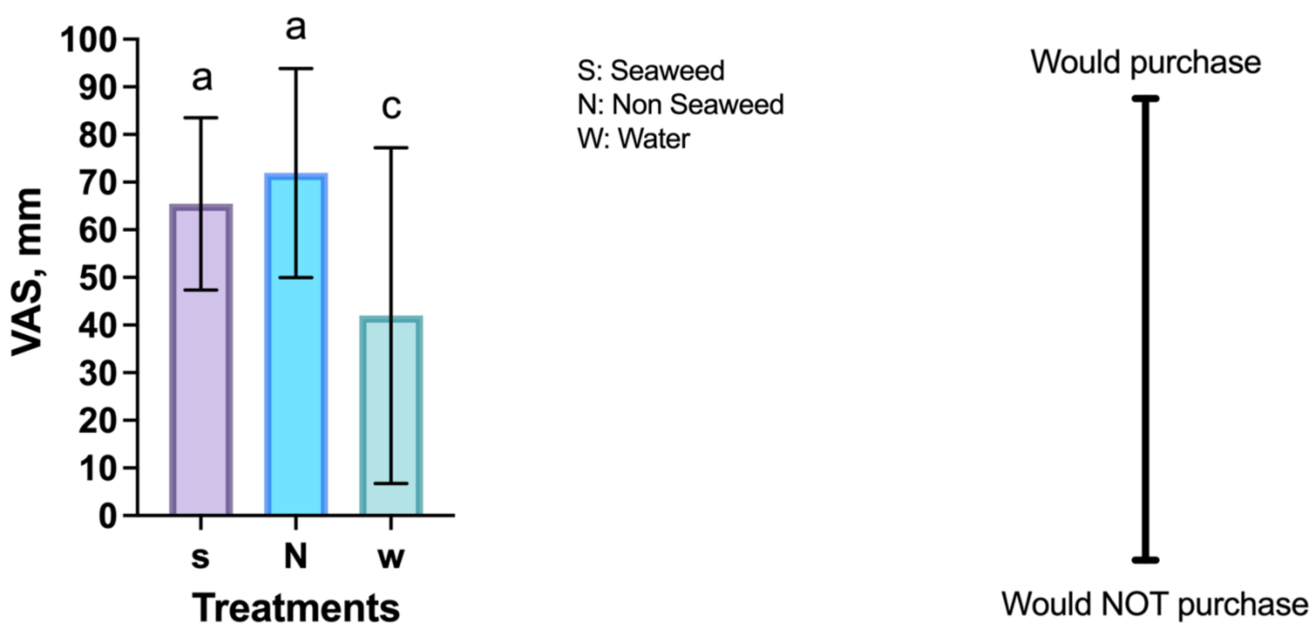

Figure 6.29: Treatment Prospective Purchasing (VAS). Means ± SD, n=30. One-way ANOVA with a Tukey Kramer post-hoc test. Values with different superscript letters are significantly different ($P \leq 0.05$).

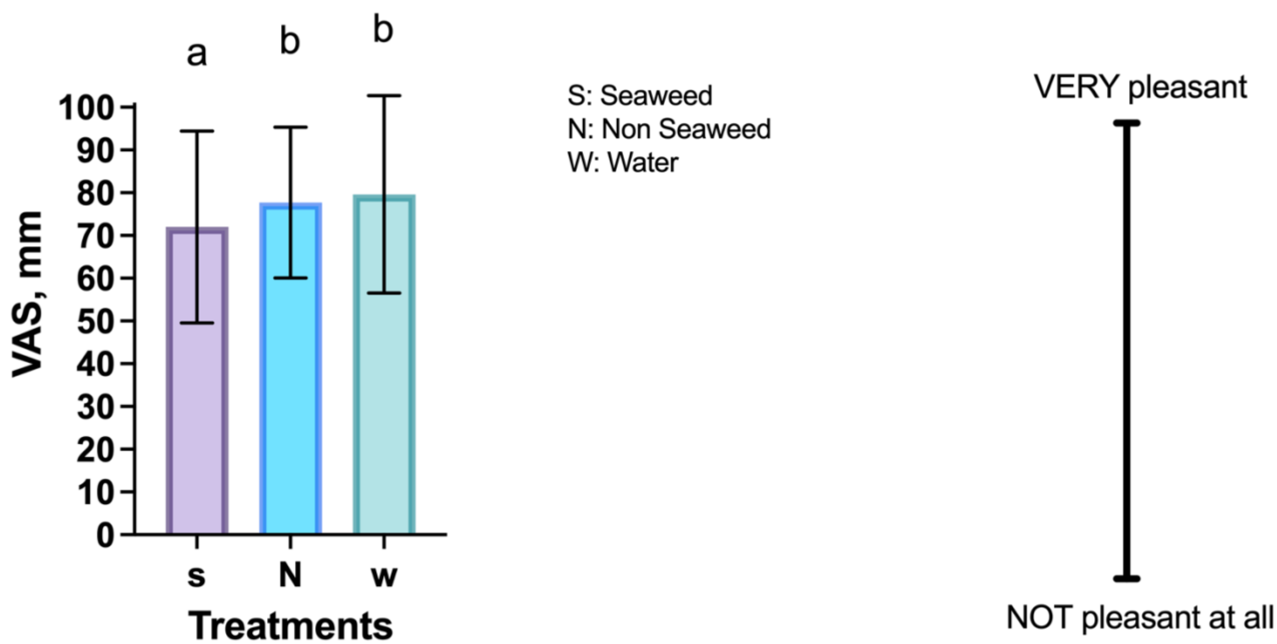

Figure 6.30: Pizza Meal Pleasantness (VAS). Means ± SD, n=30. One-way ANOVA with a Tukey Kramer post-hoc test. Values with different superscript letters are significantly different ($P \leq 0.05$).

Chapter 7: Discussion

This is the first research to investigate the satiating properties of whole *Chondrus crispus* seaweed. Including 5g of *Chondrus crispus* seaweed in a mixed meal lowered *ad libitum* FI at 120 minutes (-115 kcal, P=0.05) compared to the omelette without additional seaweed. Notably, adding 5g of dry seaweed to the omelette raised the treatment's caloric content from 309 to 318 kcal (**Table 4.3**). Consequently, the cumulative FI over two hours tended to be 106 kcal lower after S than N (P=0.08). Cumulative FI after N increased by 186 kcal compared to cumulative FI after water control (P=0.0008). However, the cumulative FI after S was not significantly higher than after water control (P=0.2, **Table 6.2**). The caloric compensation after S was approximately twice higher than the caloric compensation after N (P=0.02), which can explain the observed difference between caloric treatments on cumulative FI.

A Previous study conducted with 12 overweight but otherwise healthy males who consumed a 214 kcal seaweed-enriched bread (approximately 4g of added brown seaweed *Ascophyllum nodosum*) demonstrated a 16.4% reduction in FI four hours after seaweed-enrich bread consumption compared to the 214 kcal treatment with bread without added seaweed [238]. Similar amounts of seaweed were employed in the present study (5g) and *ad libitum* pizza meal was served in two hours. A reduction of 12.3% in FI was observed after S compared with N. While, there was no effect on *ad libitum* FI observed at 200 minutes in a different study with 20 healthy male and female participants, where 5g of whole brown seaweeds *Laminaria digitata* or *Undaria pinnatifida* or 5g of pea protein were served with starchy drink (30g of starch, 22 g sugar-free lemonade powder, 150 ml, 120-128 kcal) and 500 ml of water [235]. The primary difference in the present investigation was the nature of the test meals and the timing of *ad libitum* FI. Consequently, the omelette with added seaweeds represents a solid food while the starchy drink served with brown seaweed was a liquid meal [235]. It has been shown that a liquid food has a lower satiating efficiency than a solid food, mainly owing to their rapid consumption rate (>200g/min for liquid, and <100g/min for solid food) and oral processing [280]. Another variable that might have affected *ad libitum* FI in the study with 5g of *L. digitata* and *U. pinnatifida* was that the seaweeds were provided with the liquid meal but not as part of it [235]. It has been shown that adding water to the preload increased fullness and reduced hunger and subsequent energy intake at lunch while the same amount of water served as a beverage with food had no effect on satiety [281]. Finally, the time of *ad* *libitum* meals is also an essential aspect. In the present study, *ad libitum* meal was served at 120 min, and the average appetite score did not return to its initial level (**Figure 6.4**). However, in the study with *L. digitata* and *U. pinnatifida,* FI was assessed at 200 min while the satiety and fullness scores returned to the baseline level after 90-130 min, which might explain the absence of the effect of seaweed treatments on FI [235]. The observed differences in *ad libitum* FI from the current study between S and N may be explained by the increased perceived chewiness of S compared to N, and a reduced perceived pleasantness of pizza meal after S compared to N. The present study investigated both the hedonic and sensory intensity of the treatments in relations to their texture. Thus, there was a comparable hedonic perception of food texture, mouthfeel and chewiness between S and N ($P>0.05$); however, the intensity of chewiness was approximately 40% higher after S compared to N ($P=0.0003$). The relationship between oral processing and satiety was revealed in the research examining the impact of varying amounts of wheat bran added to extrusion-cooked cassava-soy porridge [282]. The intake of porridge containing a higher dosage of bran was related to a greater number of bites and a slower eating rate, which led to a longer oro-sensory exposure and a greater decrease of subjective hunger compared to products containing a lower dose of bran [282]. A systematic review and meta-analysis indicates that food viscosity and textural complexity impact subjective appetite and FI [283]. Due to their high viscosity and gelling capabilities, seaweed polysaccharides such as alginate, agar, and carrageenan alter the textural characteristics of food [166]. *Chondrus crispus* is a rich source of carrageenan, which can bind water to form gels [169]. It has been demonstrated that foods/beverages that were harder, chunkier, thicker and more solid resulted in less energy consumed [284]. As shown above in the study with porridge and added wheat bran [282], the hard, chewy, crunchy and less moist/lubricated foods need more chewing and are kept longer in the oral than liquids or soft foods. These differences in oral processing behaviour profoundly impact food and energy intake [285]. In a meta-analysis of the impact of chewing, 10 of 16 investigations revealed that increased chewing reduced subsequent food intake [286]. While the current study results reflect the effect of whole dried *Chondrus crispus* used as an ingredient in a mixed meal, it may not reflect the effect of other forms of seaweeds with various degrees of their particle size or rehydration as their oro-sensory characteristics would differ from those of dried seaweeds.

The findings supported the hypothesis of the current study that consuming a meal formulated with seaweed would reduce subjective appetite over two hours. Compared to W, both S and N significantly reduced desire to eat, hunger, prospective food consumption and increased fullness, resulting in lower average appetite scores compared to W. Moreover, the 2-hour tAUC for all appetite parameters was significantly greater after W than after S and N. No significant difference between S and N was detected, indicating that both caloric treatments suppressed appetite over two hours. As discussed above, the subjective appetite remained suppressed at 120 min and did not return to the baseline level suggesting that it would be essential to examine the effect of the treatments on subjective appetite beyond two hours. In the present study, *ad libitum* FI was associated with DTE, hunger and PFC, and inversely associated with fullness over 2 hours (**Table 6.7**). Similarly, *ad libitum* FI was inversely associated with SQ for DTE, hunger and PFC, and positively associated with fullness (**Table 6.7**). There was no difference in SQ for appetite sensations between N and S (**Table 6.4**). Adding *Chondrus crispus* seaweed to the omelette meal did not cause any adverse effects on the perceived level of physical comfort, including energy, fatigue, diarrhea, flatulence, stomach pain and nausea (**Table 6.6**).

This was the first study to examine a potential relationship between sensory and satiating properties of *Chondrus crispus* seaweed included in a mixed meal. In the present research, the amount of seaweed (5g, dry) added to the omelette was 2.6% per serving. The results of the current study's sensory evaluation were comparable to the previous study that examined the consumer acceptability of whole-wheat bread containing 2%, 4%, 6%, and 8% *Chondrus crispus* seaweed powder, where *Chondrus crispus* bread was found to be most acceptable at 2% level [144]. Increasing the level of seaweed in the food formulation was related to the decrease in perceived taste, texture, appearance, aroma, and overall acceptability of the bread. It was suggested that seaweeds should not exceed 20% of the dry weight of the meal, since higher amounts/concentrations either "overwhelmed" traditional recipes or altered the taste which led to the dish being rejected by the consumers [287]. The current study indicated that a meal containing 5g of whole *Chondrus crispus* seaweed is acceptable to consumers. All hedonic attribute mean scores fell between "like slightly" and "like moderately" for the meal with added seaweeds. A mean acceptability score of seven or above on the 9-pt HS is considered significant quality [288]. Therefore, the perception of taste and pleasantness for the meal with added seaweed was 7.2 and 7.1 pts, respectively (**Table 6.8**).

The other hedonic attributes such as perceived texture, chewiness, flavour, mouthfeel and appearance were between 6.5 and 7.0 pts, whereas the hedonic perception of saltiness and aftertaste were between 6.0 and 6.5 pts (**Table 6.8**). On the 9-pt hedonic scale, it was assumed 1-pt difference between the treatments was a meaningful indicator of sensory acceptance. To detect 0.5 pt difference (α=0.05, β=0.8), a sample size of 93 participants would be needed, which is consistent with the other findings suggesting a sample size of 40 participants was required to detect 0.6 pt difference on 9-pt HS [289].

The decision to use an omelette as a vehicle for seaweeds was based on its popularity in Canada and its easiness of preparation. Thus, Dietitians of Canada developed the resources such as Healthy Eating Guidelines for eating out [290], or for people with chewing disorders [291], or the examples of Mediterranean diet menus [292], or the menus for gluten-free eating [293] using the omelette as one of the foods that could be prepared and modified (e.g., by adding vegetables) by consumers without specific cooking skills required. The pleasantness of omelette meal with added seaweed assessed with 100mm VAS was 72.8±15.5 mm and was close to the mean palatability of commercial pizza of 76.4±19.3 mm (**Table 6.8**). This fact indicates a high level of acceptability for the meal with added seaweeds given that pizza is one of the most popular and staple foods in Canada chosen by 81% of consumers at least once a month [294]. The hedonic perception of both omelette meals with and without added seaweeds fell within the same range reaching the difference of 0.1-0.6 on 9-pt HS (P>0.05, **Table 6.8**). Although the treatments were blinded to participants, the perception of seaweed flavour in the treatment with S was almost twice higher than in the treatment with N (P=0.0005). Interestingly, the participants perceived the seaweed flavour in the omelette without added seaweed, but barely sensed the seaweed flavour in the water control (**Table 6.8**). The increased sense of seaweed flavour did not affect the hedonic perception of flavour, which was 6.8±1.5 and 7.3±1.5 pts for S and N, respectively. Surprisingly, the addition of seaweeds which contain 220 mg of sodium to the omelette did not result in increased perception of saltiness. This could be potentially explained by the delayed release of sodium from the food matrix during oral processing [295]. The addition of seaweeds to the omelette did not increase bitterness, and while it required more mastication, as measured by the VAS for chewiness intensity, the hedonic perception of chewiness was comparable between the meals with and without additional seaweeds (**Table**

6.8). Similarly to the omelette without additional seaweeds, the omelette with 5g of dried seaweed had a high likelihood of being purchased, demonstrating its favourable sensory perception (**Table 6.8**).

There was a tendency in the relationship between perceived pizza pleasantness and *ad libitum* FI (*r*=0.19, P=0.07). Unexpectedly, a difference was seen between treatments in the palatability of pizza delivered 2 hours after treatment. Previous studies using a similar preload design and the assessment of *ad libitum* FI at 120 min did not detect the effect of a treatment on the pleasantness of the pizza meal at 120 min [262,296,297]. However, in the present study, *ad libitum* FI of pizza meal at 120 min after S showed lower palatability compared to N (-5.7mm, P=0.02) and W (-7.6mm, P=0.04). Although it is known that the palatability of the test food affects satiation but not satiety [298], both caloric treatments used in the present study had similar pleasantness (>7 pt on 9-pt HS, **Table 6.8**). It is unclear whether the intake of a meal with added seaweed could program the lower perception of pleasantness of a subsequent meal two hours post-ingestion via the gut-brain axis. A study with mice demonstrated the involvement of vasoactive intestinal peptide–expressing interneurons in the infralimbic and prelimbic divisions of the medial prefrontal cortex in reducing the intake of high-calorie palatable food but not for low-calorie rodent chow [299]. However, it is unclear whether the ingestion of seaweed can trigger such or similar mechanisms of neural control of FI.

In general, seaweed is an excellent source of iodine [300]. Five grams of *Chondrus crispus* used in the current study contained 660mcg of iodine (**Table 4.3**). Canadian Health Measures Survey reported a median urinary iodine concentration (UIC) of 1.06 µmol/L (adequate intake: UIC between 0.79 and 1.57 µmol/L) with about 22% and 7% of Canadians aged 3 to 79 at risk for mild and moderate iodine deficiency, respectively [301]. In Canada, the recommended dietary allowance (RDA) for iodine intake for adults is 150 µg/day and the tolerable upper level (UL) is 1100 µg/day [302] while in Japan, where seaweed is habitually eaten, the RDA is 130 µg/day, the UL 3000 µg/day [303], and the average daily intake of iodine is estimated to be 1000–3000 µg [11]. The total iodine content in the omelette meal formulated with added 5g of *Chondrus crispus* and iodized table salt was below the Canadian tolerable UL for iodine.

Excessive consumption of seaweed presents certain risks related to the content of iodine, sodium and heavy metals, such as arsenic, cadmium, lead, and mercury [194]. Although, adding 5g of *Chondrus* *crispus* seaweeds to a meal increased the sodium content by 220 mg (**Table 4.3**), it was estimated that the replacing up to 10% of food with seaweed-derived products would not have any adverse effects such as elevated sodium intake or exposure to heavy metals [304]. Similarly, another report indicates no meaningful contribution to dietary intake of sodium when considering a daily portion of 5 g (dry weight) of 17 brown seaweed and 17 red seaweed food products sourced from China, Japan, and South Korea [305].

There are several limitations that need to be addressed. The study was conducted with males only to eliminate a possible effect of sex and gender on sensory perception, satiety and food intake. While the study was employing the preload design using a fixed amount of the treatment, the effect of an *ad libitum* consumption of a mixed meal formulated with *Chondrus crispus* on satiation and satiety beyond 2-hour time frame is yet to be determined. Finally, the investigation of the effect of seaweed on gastric emptying rate may help to mechanistically explain the effect of seaweed on short-term food intake.

Chapter 8: Summary and Implications for Future Research

The current study, to the researcher's best knowledge, is the first attempt to investigate the effects of the addition of *Chondrus crispus* seaweed to food on satiety and short-term food intake. The addition of 5g of *Chondrus crispus* seaweed into omelette led to reduced *ad libitum* FI with pizza meal at 120 min compared to the omelette without added seaweed. Such difference possibly could be due to increased mastication of the food with added seaweed, and a lower sensory acceptance of a subsequent meal. Additionally, the results supported that consuming a meal formulated with or without seaweed would reduce subjective appetite over two hours. The results of this study will help to better understand the sensory perception of foods formulated with seaweed and their physiological effect related to satiety and physical comfort. The contribution of this research will help in the development of new food products with domestically produced seaweed with positive hedonic perceptions.

The study was conducted with males only; however, a carefully designed study with females taken into the consideration with the factors (e.g., menstrual cycle phase) affecting study outcomes need to be conducted. While the study was focused on sensory perception, satiety and food intake, the metabolic measurements, including blood glucose response over two hours were not performed. A new study designed to measure blood glucose biomarkers as well as gastric emptying rate may help to mechanistically explain the effect of seaweed on short-term food intake. In addition, precise chemical composition measurements of seaweed are necessary to thoroughly investigate the health-promoting properties of *Chondrus crispus* seaweed or seaweed in general.

Generally, seaweed is considered as a novel food ingredient by many. Although previous studies revealed that seaweed, a new food additive, showed great acceptability among customers, the sensory evaluation of food products formulated with *Chondrus crispus* seaweed remains under-investigated. More studies assessing the palatability of food formulated with seaweeds are needed to help gather a conclusive understanding of how seaweeds are liked by Canadian consumers.

Conclusion

Adding 5g of dried whole *Chondrus crispus* to a meal results in acceptable sensory characteristics, high physical comfort, reduced subjective appetite and food intake suppression possibly due to increased mastication of the food with added seaweed, and a lower sensory acceptance of a subsequent meal.

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Appendix 1a: Recruitment Letter for Participants

Dear Participant,

A team of researchers from Mount Saint Vincent University are investigating how meals made with seaweed influence appetite and food intake. We are asking for male participants 19-35-year-old to take part in a research study on three separate days with one week apart between research sessions. Each session will take around two and a half hours of your time and if you will participate in the study you will be asked to come to our laboratory at the same time for each of three research sessions.

If you are interested to participate in this study please contact us through the e-mail or telephone and we will contact you and ask some questions to determine if you are eligible to participate in this study. You will be also invited to attend the Information and Screening session to learn more about the study and complete the questionnaires about your food and eating habits. We will also measure your weight, height and body composition using an analyzer similar to electronic body weight scale. This will not hurt you and will not cause any pain or discomfort. Once we determine that you are eligible to participate in this study, we will schedule you to attend three research sessions with one week apart between the sessions.
The study will take place in food research laboratory in the Centre of Applied Research, Department of Applied Human Nutrition (47 College Rd) MSVU.

There are criteria for participation that you need to be aware of, eligible participants must not be:

- Regular Breakfast skipper
- Having disordered eating patterns (e.g., focusing too much on body weight, body imaging, dieting, self-limiting the choice of foods, performing specific food rituals, feel anxious or guilty when eating or after eating, having bulimia, anorexia or binge eating disorder).
- Smoker (including e-cigarettes) / cannabis consumer
- Be underweight, overweight or obese
- Having chronic diseases including diabetes
- Taking certain medications that may affect central and peripheral mechanisms of food intake regulation, cognitive performance and sedative medications (e.g., Ranitidine, Amphetamine, Diazepam or others. The list of medications to be checked is included below)
- Having difficulty to comprehend the questions and fill out the study questionnaires
- Having food allergies or gastrointestinal disorders (e.g., irritable bowel syndrome, or others)
- Have no multiple food restrictions or strong aversion towards seaweeds (a strong dislike towards many foods and seaweeds)

- Uncomfortable having their face recorded while eating

We will review these conditions with you over the telephone and will measure your body weight and height during the Information and Screening session.

To thank you for your participation you will receive $30 for each session with a total of $90 for completed study. At the end of each research session, you will be served with a pizza and then with chocolate milk or fruit juice and cookies after pizza meal.
The ethical components of this research study have been reviewed by the University Research Ethics Board and found to be in compliance with Mount Saint Vincent University's Research Ethics Policy.
If you would like to participate, or to get more information about this study, please contact Appetite.Study@msvu.ca or leave a message at 902-457-6568 and we will contact you.
Thank you for your support in this research.
Sincerely,
Dr. Bohdan Luhovyy

Appendix 1b: Recruitment Poster for Participants

MOUNT SAINT VINCENT UNIVERSITY

Department of Applied Human Nutrition

MALE PARTICIPANTS AGED 19-35 NEEDED

Would you like to participate in a research at MSVU Appetite Lab

The purpose of the study is to learn more about products formulated with seaweed and their effect on appetite

You are eligible if you are:

-19-35 years- old healthy male, non-smoker, non-cannabis consumer, not a breakfast skipper
-not taking certain medications (eg: Ranitidine, Amphetamine, Diazepam or others etc)
-have no known food allergies or aversion to seaweeds

Study involves:

-Information session
-3 food sessions over 3 weeks
-3 hours per each food session
- Eating a meal made with seaweed ingredients and a meal of pizza at food sessions

As a thank you You will receive $30 for your participation for each session ($90 for the study)

Interested?

Please contact us at Appetite.Study@msvu.ca or leave a message at 902-457-6568 and we will contact you

Appetite.Study@msvu.ca 902-457-6568 @msvufoodstudy

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Appetite.Study@msvu.ca 902-457-6568 @msvufoodstudy

Appendix 2a: Telephone Screening Questionnaire

ID: __________________ Date (DD/MMM/YYYY):____________________

Telephone Screening Questionnaire (part 1)

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

TO BE KEPT SEPARATELY FROM DATA FORMS

Name:
____

Month and year of birth ______/_______ (______ y: calculated by recruiter)

What is your ethnic background?
____

Will you be willing to eat a food product prepared with seaweeds? Yes ____ No____

Are you vegetarian? Yes ____ No____, vegan? Yes ____ No____, following a special diet? Yes ____ No___

Notes:
____

To be completed by Staff: Eligible to participate: Yes ____ No____

If not eligible, this concludes the conversation and the form is to be shredded.

If eligible, continue with the remaining parts 1 and 2.

Address:

____

____

____

Cell phone: ______________________________ Can we send text messages? ______ Yes ______ No

Home phone: (______) ____________ What time would be convenient to call you? ___________________

E-mail: ____________________@______________________________

Participant ID assigned: ______________________

ID: ___________________ Date (DD/MMM/YYYY):____________________

Telephone Screening Questionnaire (part 2)

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Weight (circle the correct unit):_____ lbs kg Height:_____cm (Calculated by recruiter)
BMI:_____ kg/m^2

Do you regularly consume breakfast? Yes__ No___

Are you physically active? Yes ___ No___

Can you briefly recall your daily normal physical activity routine?

____

____

____

______

Do you consume cannabis? Yes___ No___ Do you smoke tobacco? Yes__ No__
Do you use e-cigarettes and similar products? Yes__ No__
Do you have any allergies to any foods? Yes __ No__
If yes, are you allergic to any of the following wheat, dairy, eggs, seaweed or other foods? Yes ___ No___
Other foods? ____________________
How many alcoholic beverages do you consume per day? _________ per week? _________

Do you have any major disease or medical conditions? Yes ____ No____ If yes, please specify:

____

__

Do you take medications? Yes ____ No____ If yes, please specify:

____

__

Can you eat the food with seaweeds? Yes ____ No____

Do you like pizza? Yes ____ No____

Are you comfortable with having your face recorded while eating? We will be using software that is able to accurately determine emotions to help us better understand people’s natural reaction to the food products.

Yes ____ No____

____

__

To be completed by Staff: Eligible to participate: Yes ____ No____

Screening scheduled at:

____

Appendix 3a: COVID−19 Risk Assessment for Research

University Research Ethics Board

COVID−19 RISK ASSESSMENT for RESEARCH

Date (YYYY/MMM/DD): ____________________

- The following risk assessment must be completed for every participant in face-to-face research studies conducted by members of the Mount Saint Vincent University research community.
- **Contact information** is required for **Contact Tracing** by the Nova Scotia Department of Health.
- The Risk Assessment is used to facilitate participant, student, staff and researcher safety.
- It is the responsibility of the Principle Researcher to ensure that this assessment is completed.
- Contact tracing documents must be sent daily to ethics@msvu.ca
- Documents must be labeled as Ethics File **XXXX-XXX ResearcherLastName MMDDYYYY**

Name	
Address	
Email	
Best telephone # for contact	

If the answer to **any** of the boxes below is yes, researchers **must** reschedule the meeting.

Do you currently have any of the following symptoms? If the answer to **any** of the boxes below is yes, researchers **must** reschedule the meeting.
☐ An unexplained fever greater than 38°C, or fever-like symptoms?
☐ New or worsening cough

Do you currently have any of the following **new or worsening** symptoms?			
☐ Sore throat	☐ Runny nose	☐ Headache	☐ Shortness of breath
☐ No Symptoms			
Two or more of the above symptoms **will** require the meeting to be rescheduled.			

Non-Symptomatic Risk Factors. If the answer is yes to any of the questions below, the meeting must be rescheduled.

Have you or anyone in your household travelled outside of Nova Scotia in the last 14 days? If yes, where? ______________________________	☐ Yes	☐ No
In the last 14 days have you been in close contact with someone who has COVID-19 or is suspected to have COVID-19?	☐ Yes	☐ No
In the last 14 days have you been to any location identified by NS Public Health and instructed to self-isolate and be tested for COVID-19?	☐ Yes	☐ No
Do you live or work within a known community or facility cluster as identified as a COVID−19 Hub?	☐ Yes	☐ No

☐ **IDENTIFIED COVID−19 RISK**	☐ **NO IDENTIFIED COVID−19 RISK**
Confirmed case OR symptomatic (Two or more of: new or worsening cough, fever, sore throat, headache, runny nose, new or worsening shortness of breath) / other acute respiratory illness consistent with infection OR travel outside of Atlantic Canada OR contact with known or suspected COVID−19 case OR residence in a geographic location with known community / facility cluster OR symptoms / exposure cannot be determined due to physical and / or mental status	Asymptomatic and no known contact with confirmed or suspected case or residence in a facility or community cluster with COVID−19 cases

Verified by (print) ________________________ Signature: _________________________________

This document has been adapted from the COVID_19 Risk Assessment for Patients by the Nova Scotia Health Authority: E:\Program Files (x86)\Access\Intelligent Form\CommonProject\nshealth-logo-black.eps (retrieved April 30, 2021)

Halifax Nova Scotia B3M 2J6 Canada *Tel 902 457 6350 • msvu.ca/ethics

Appendix 3b: Information Letter: COVID-19 Risks and Contact Tracing for In-Person Research Studies

University Research Ethics Board

Information Letter:
COVID-19 Risks and Contact Tracing for In-Person Research Studies

With the global COVID-19 pandemic, Canadian public health authorities have strongly recommended that everyone (especially individuals at high risk or in contact with individuals at high risk) avoid visiting certain businesses and workplaces, and take additional precautions including those outlined in this letter. The Government of Nova Scotia declared a province-wide state of emergency under *The Emergency Management Act* on March 22, 2020 to protect the health and safety of all Nova Scotians and to reduce the spread of the novel coronavirus (or **COVID-19**). COVID-19 is easily spread by contact by people who have the virus. Please read the following statements carefully and feel free to contact the research team with any questions.

Mount Saint Vincent University University (MSVU), which is under the jurisdiction of Nova Scotia Public Health, has put in place measures to reduce the spread of COVID-19, however MSVU cannot guarantee that any individual attending the MSVU campus, using MSVU facilities, or participating in activities organized by MSVU, whether on-campus or off-campus will not become infected with COVID-19. Further, attending the MSVU campus and participating in MSVU activities, could increase the risk of contracting COVID-19. We ask that you follow relevant local public health directives for the safety of participants, researchers, and the broader community. COVID-19 can result in severe illness, medical expenses, loss of income and in severe cases, death. If you are feeling unwell or experiencing **any** potential COVID-19 symptoms, please stay home and notify a member of the research team that you cannot attend.

Self-Screening

COVID-19 infection can result in severe illness (present and future), medical expenses, loss of income and death. Potential COVID-19 symptoms can vary but may include include, but are not limited to: cough, fever, shortness of breath or difficulty breathing, runny nose, stuffy nose, sore throat, painful swallowing, headache, chills, muscle or joint aches, feeling unwell in general, new fatigue or severe exhaustion, gastrointestinal symptoms (e.g., nausea, vomiting, diarrhea, or unexplained loss of appetite), loss of sense of smell or taste, or pink eye. The list of symptoms noted above is constantly evolving, and research participants should monitor the most current information from the Government of Nova Scotia at the following link: https://novascotia.ca/coronavirus/when-to-seek-help/#symptoms . The government of Canada provides information on COVID-19 risks and prevention and on taking care of your mental health during the COVID-19 pandemic.

Safety Procedures for Participants

We ask that you follow Nova Scotia Public Health directives, as well, for the safety of participants and researchers. Because you are coming to the MSVU campus, and the researcher may need to be closer to you than the recommended 2 metre distance, the following safety protocols must be followed:

- The day before your visit, a member of the research team will contact you to answer questions for a required COVID-19 screening assessment.
- On the day of your visit, a researcher will conduct the screening again at the building entrance, before escorting you to the study location.
- Please wash or sanitize your hands upon arrival. Hand sanitizer will be provided.
- Please wear a mask or face covering. Masks will be provided if you lack one.
- Avoid touching your face with unwashed hands.
- Avoid physical contact with other individuals to the extent possible.
- Advise a researcher as soon as possible if you believe a safety measure is not being taken, or that your safety is at risk.
- If taking public transit for this visit, please follow all guidelines from the transit service and public health, such as face masking/covering and hand sanitizing.
- Consider using the washroom before leaving home. Notify the researcher of any washroom needs; washroom capacity is limited to allow physical distancing.
- Provide your personal contact information for contact-tracing purposes.

- Attend the study visit alone, or, if needed, bring only one support person/parent. The support person must also follow the protocols outlined above.

We will be collecting your personal contact information, which we must keep for one year, and will use this information only to follow up with you or support contact tracing if you (or your support person) may have been exposed to COVID-19 at the research site. Contact information will be stored securely and separately from research data.

What MSVU is Doing to Reduce Risk
To reduce the possibility of COVID-19 exposure, especially if study procedures cannot maintain 2-metre distancing, we have implemented the following safety procedures recommended by our Safety Office and public health:

- regular handwashing and use of gloves by all research team members,
- availability and use of hand sanitizer for study participants and researchers,
- sanitizing of high-touch surfaces and shared equipment,
- increased cleaning of washrooms and other shared spaces,
- scheduling and limits to reduce occupancy in buildings and laboratories,
- floor markings and signage to encourage physical distancing in shared spaces,
- wearing of face masks/face coverings, and
- where necessary, use of face shields, lab coats, goggles, and plexiglass barriers.

Please do everything you can to follow these health-related procedures and directives, to protect yourself and others.

If you feel that you are unable to wear a mask or are from a vulnerable group with respect to COVID-19 (e.g., an older adult; underlying medical conditions such as heart disease, hypertension, diabetes, chronic respiratory diseases, cancer, etc.; or a compromised immune system), please discuss your participation with the research team before consenting. You are under no obligation to participate and there are no consequences to you if you change your mind about participating in the research.

Confirmation Statement:
You are invited to attend in-person research study visit(s) on a voluntary basis. You are under no obligation to attend, and at any time you can change your mind, stop participating or withdraw from the study by notifying the researcher. If you have any questions about the study, please contact the research team.

By submitting this form, you confirm that you understand and acknowledge the information above about risks of COVID-19 exposure and related safety measures and that you are willing to come to the research site for in-person research study visit(s). You are not waiving your rights or releasing the investigator(s) or any institution(s) from their legal and professional responsibilities.

You will be asked to reconfirm this information and provide your contact information for contact tracing purposes each time you attend an in-person research study visit.

Please save and keep a copy of this information for your own files. You will be asked to reconfirm this information and provide your contact information for contact tracing purposes each time you attend an in-person research study visit.

Print name of participant/support person: ___________________________________

Signature of participant/support person: ___________________________________

Date: ___________________________________

REB.INFO.002
Info Letter – COVID-19

MSVU Halifax Nova Scotia B3M 2J6 Canada
Tel 902 457 6350 www.msvu.ca/ethics

Page 2 of 3

Information for Contact Tracing Participants:

MSVU has taken a comprehensive approach to assist Public Health in their contact tracing in the event of an identified positive case of COVID-19 on our campus or at an MSVU-sanctioned activity in an off-campus location. Safeguarding the health and safety of MSVU students, faculty, and staff as well as the broader community is a top priority.

MSVU's contact tracing role is about ensuring that we have ready access to contact information in the event that it is required for release to Public Health in relation to communicable disease investigation.

In order to assist a public health unit in the event that an individual who has an identified positive case of COVID-19 has been on MSVU's campus or at an MSVU event and was in close proximity to other students, staff, faculty, or community members, MSVU will work with the public health unit to provide them with contact information regarding those who may have been exposed. This will only be provided to a public health unit in the form of the individual's name, phone number, and email address. This could mean providing a list of students in a class, those working in a research lab, students in residence, participants in a research study, or staff/faculty engaged in recreational activities who may have been exposed to the virus. The information will be given to the contact tracer at a public health unit through a secure method. The public health unit will then contact the individuals who may have been exposed as per Nova Scotia Public Health.

Print Name (**required**):	
Telephone Number (**required**): *Include area code.*	
Email Address (optional):	
Date:	
Location of Research Study Visit	

*Please note that the information that you provide in this document:
- will not be stored with the research team or with study data;
- will be sent directly to, and securely stored, with the MSVU Research Office, accessible by the Associate Vice-President Research and the Research Ethics Coordinator;
- will be used only if required by Public Health to provide this personal information for COVID-19 contact tracing purposes, and;
- will be held for a period of one year, after which the information will be securely destroyed.

Acknowledgement The MSVU University Research Ethics Board has adapted this information letter from several Canadian university examples and wishes to extend its appreciation to the Research Ethics Boards at Brock University and the University of Waterloo.

Appendix 4a: Sleep Habits and Stress Factors Questionnaire

ID: __________ DATE (DD/MMM/YYYY):__________________

SESSION: __________ TREATMENT (Code): ______________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

1. Did you have a normal night's sleep last night? Yes______ No______

2. How many hours of sleep did you have? ___________

3. What time did you go to bed last night? ___________

4. What time did you wake up this morning? ___________

5. Recount your activities since waking:

Time Activity

________ ____

________ ____

________ ____

________ ____

6. Are you experiencing any feelings of illness or discomfort, other than those from hunger?
Today: Yes ____ No_____ Past 24 hours: Yes ____ No_____
If yes, please describe briefly:

____

____

7. Are you under any unusual stress? (Exams/reports/work deadlines, personal, etc.)
Today: Yes ____ No_____ Past 24 hours: Yes ____ No_____
If yes, please describe briefly:

____

____

8. Have you been involved in any physical activity within the past 24 hours that is unusual to your normal routine? Yes______ No______
If yes, please describe briefly:

____

____

Appendix 4b: Recent Food Intake and Activity Questionnaire

ID: __________ DATE (DD/MMM/YYYY):__________________

SESSION: __________ TREATMENT (Code): ______________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

At what time did you have dinner? _____________________

Please describe your dinner last night (list all food and drink and give an estimate of the portion size):
____
____
____

The following three questions relate to your food intake, activity and stress over the last 24 hours. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings.

How would you describe your **food intake** over the past 24 hours?

Much LESS than usual ____ Much MORE than usual

How would you describe your **level of activity** over the last 24 hours?

Much LESS than usual ____ Much MORE than usual

How would you describe your **level of stress** over the last 24 hours?

Much LESS than usual ____ Much MORE than usual

To be completed by staff only:

Arrived to the lab at: __________ Baseline blood glucose ______________ (mmol/L)

Treatment started at: ___________

Comments/Notes:

____

Appendix 5: Visual Analogue Scales Motivation to Eat, Energy and Fatigue & Physical Comfort

ID: __________ DATE (DD/MMM/YYYY):_________________

SESSION: __________ TREATMENT (Code): ______________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Motivation to Eat

Time point: 0 min (immediately before the treatment)

These questions relate to your "motivation to eat" at this time. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings.

1. How strong is your desire to eat?

Very WEAK ____ Very STRONG

2. How hungry do you feel?

Not hungry at all ____ As hungry as I have ever felt

3. How full do you feel?

Not full at all ____ Very full

4. How much food do you think you could eat?

NOTHING at all ____ A LARGE amount

5. How thirsty do you feel?

NOT thirsty at all ____ As thirsty as I have ever felt

ID: __________ DATE (DD/MMM/YYYY):__________________

SESSION: __________ TREATMENT (Code): ______________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Energy and Fatigue

Time point: 0 min (immediately before the treatment)

These questions relate to your energy level and fatigue at this time. Please rate yourself by placing a small "**x**" across the horizontal line at the point which best reflects your present feelings.

1. **How energetic do you feel right now?**

NOT ____ VERY
at all energetic

2. **How tired do you feel right now?**

NOT ____ VERY
at all

tired

ID: __________ DATE (DD/MMM/YYYY):__________________

SESSION: __________ TREATMENT (Code): ______________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Physical Comfort

Time point: 0 min (immediately before the treatment)

These questions relate to your "physical comfort" at this time. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings

1. **Do you feel nauseous?**

NOT at all ____ VERY much

2. **Does your stomach hurt?**

NOT at all ____ VERY much

3. **How well do you feel?**

NOT well at all ____ VERY well

4. **Do you feel like you have gas?**

NOT at all ____ VERY much

5. **Do you feel like you have diarrhea?**

NOT at all ____ VERY much

ID: __________ DATE (DD/MMM/YYYY):_________________

SESSION: __________ TREATMENT (Code): _____________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Motivation to Eat

Time point: 15 min

These questions relate to your "motivation to eat" at this time. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings.

1. How strong is your desire to eat?

Very WEAK ____ Very STRONG

2. How hungry do you feel?

Not hungry at all ____ As hungry as I have ever felt

3. How full do you feel?

Not full at all ____ Very full

4. How much food do you think you could eat?

NOTHING at all ____ A LARGE amount

5. How thirsty do you feel?

NOT thirsty at all ____ As thirsty as I have ever felt

ID: __________ DATE (DD/MMM/YYYY):_________________

SESSION: __________ TREATMENT (Code): ______________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Energy and Fatigue

Time point: 15 min

These questions relate to your energy level and fatigue at this time. Please rate yourself by placing a small "**x**" across the horizontal line at the point which best reflects your present feelings.

1. How energetic do you feel right now?

NOT at all ____ VERY energetic

2. How tired do you feel right now?

NOT at all tired ____ VERY tired

ID: __________ DATE (DD/MMM/YYYY):__________________

SESSION: __________ TREATMENT (Code): ______________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Physical Comfort

Time point: 15 min

These questions relate to your "physical comfort" at this time. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings

1. **Do you feel nauseous?**

   NOT at all ____ VERY much

2. **Does your stomach hurt?**

   NOT at all ____ VERY much

3. **How well do you feel?**

   NOT well at all ____ VERY well

4. **Do you feel like you have gas?**

   NOT at all ____ VERY much

5. **Do you feel like you have diarrhea?**

   NOT at all ____ VERY much

ID: __________ DATE (DD/MMM/YYYY):_________________

SESSION: __________ TREATMENT (Code): _____________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Motivation to Eat

Time point: 30 min

These questions relate to your "motivation to eat" at this time. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings.

1. **How strong is your desire to eat?**

Very WEAK ____ Very STRONG

2. **How hungry do you feel?**

Not hungry at all ____ As hungry as I have ever felt

3. **How full do you feel?**

Not full at all ____ Very full

4. **How much food do you think you could eat?**

NOTHING at all ____ A LARGE amount

5. **How thirsty do you feel?**

NOT thirsty at all ____ As thirsty as I have ever felt

ID: __________ DATE (DD/MMM/YYYY):__________________

SESSION: __________ TREATMENT (Code): ______________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Energy and Fatigue

Time point: 30 min

These questions relate to your energy level and fatigue at this time. Please rate yourself by placing a small "**x**" across the horizontal line at the point which best reflects your present feelings.

1. How energetic do you feel right now?

NOT at all ____ VERY energetic

2. How tired do you feel right now?

NOT at all ____ VERY tired

ID: __________ DATE (DD/MMM/YYYY):__________________

SESSION: __________ TREATMENT (Code): ______________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Physical Comfort

Time point: 30 min

These questions relate to your "physical comfort" at this time. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings

1. Do you feel nauseous?

NOT at all ____ VERY much

2. Does your stomach hurt?

NOT at all ____ VERY much

3. How well do you feel?

NOT well at all ____ VERY well

4. Do you feel like you have gas?

NOT at all ____ VERY much

5. Do you feel like you have diarrhea?

NOT at all ____ VERY much

ID: __________ DATE (DD/MMM/YYYY):_________________

SESSION: __________ TREATMENT (Code): _____________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Motivation to Eat

Time point: 45 min

These questions relate to your "motivation to eat" at this time. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings.

1. **How strong is your desire to eat?**

Very WEAK ____ Very STRONG

2. **How hungry do you feel?**

Not hungry at all ____ As hungry as I have ever felt

3. **How full do you feel?**

Not full at all ____ Very full

4. **How much food do you think you could eat?**

NOTHING at all ____ A LARGE amount

5. **How thirsty do you feel?**

NOT thirsty at all ____ As thirsty as I have ever felt

ID: __________ DATE (DD/MMM/YYYY):__________________

SESSION: __________ TREATMENT (Code): ______________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Energy and Fatigue

Time point: 45 min

These questions relate to your energy level and fatigue at this time. Please rate yourself by placing a small "**x**" across the horizontal line at the point which best reflects your present feelings.

1. **How energetic do you feel right now?**

NOT at all ____ VERY energetic

2. **How tired do you feel right now?**

NOT at all ____ VERY tired

ID: __________ DATE (DD/MMM/YYYY):_________________

SESSION: __________ TREATMENT (Code): _____________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Physical Comfort

Time point: 45 min

These questions relate to your "physical comfort" at this time. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings

1. **Do you feel nauseous?**

   NOT at all ____ VERY much

2. **Does your stomach hurt?**

   NOT at all ____ VERY much

3. **How well do you feel?**

   NOT well at all ____ VERY well

4. **Do you feel like you have gas?**

   NOT at all ____ VERY much

5. **Do you feel like you have diarrhea?**

   NOT at all ____ VERY much

ID: __________ DATE (DD/MMM/YYYY):_________________

SESSION: __________ TREATMENT (Code): ______________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Motivation to Eat

Time point: 60 min

These questions relate to your "motivation to eat" at this time. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings.

1. **How strong is your desire to eat?**

Very WEAK ____ Very STRONG

2. **How hungry do you feel?**

Not hungry at all ____ As hungry as I have ever felt

3. **How full do you feel?**

Not full at all ____ Very full

4. **How much food do you think you could eat?**

NOTHING at all ____ A LARGE amount

5. **How thirsty do you feel?**

NOT thirsty at all ____ As thirsty as I have ever felt

ID: __________ DATE (DD/MMM/YYYY):__________________

SESSION: __________ TREATMENT (Code): ______________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Energy and Fatigue

Time point: 60 min

These questions relate to your energy level and fatigue at this time. Please rate yourself by placing a small "**x**" across the horizontal line at the point which best reflects your present feelings.

1. **How energetic do you feel right now?**

NOT at all ____ VERY energetic

2. **How tired do you feel right now?**

NOT at all ____ VERY tired

ID: __________ DATE (DD/MMM/YYYY):__________________

SESSION: __________ TREATMENT (Code): ______________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Physical Comfort

Time point: 60 min

These questions relate to your "physical comfort" at this time. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings

1. **Do you feel nauseous?**

NOT at all ____ VERY much

2. **Does your stomach hurt?**

NOT at all ____ VERY much

3. **How well do you feel?**

NOT well at all ____ VERY well

4. **Do you feel like you have gas?**

NOT at all ____ VERY much

5. **Do you feel like you have diarrhea?**

NOT at all ____ VERY much

ID: __________ DATE (DD/MMM/YYYY):__________________

SESSION: __________ TREATMENT (Code): ______________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Motivation to Eat

Time point: 90 min

These questions relate to your "motivation to eat" at this time. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings.

1. **How strong is your desire to eat?**

Very WEAK	______________________________	Very STRONG

2. **How hungry do you feel?**

Not hungry at all	______________________________	As hungry as I have ever felt

3. **How full do you feel?**

Not full at all	______________________________	Very full

4. **How much food do you think you could eat?**

NOTHING at all	______________________________	A LARGE amount

5. **How thirsty do you feel?**

NOT thirsty at all	______________________________	As thirsty as I have ever felt

ID: __________ DATE (DD/MMM/YYYY):__________________

SESSION: __________ TREATMENT (Code): ______________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Energy and Fatigue

Time point: 60 min

These questions relate to your energy level and fatigue at this time. Please rate yourself by placing a small "**x**" across the horizontal line at the point which best reflects your present feelings.

1. **How energetic do you feel right now?**

NOT at all ____ VERY energetic

2. **How tired do you feel right now?**

NOT at all ____ VERY tired

ID: __________ DATE (DD/MMM/YYYY):_________________

SESSION: __________ TREATMENT (Code): _____________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Physical Comfort

Time point: 60 min

These questions relate to your "physical comfort" at this time. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings

1. **Do you feel nauseous?**

NOT at all ____ VERY much

2. **Does your stomach hurt?**

NOT at all ____ VERY much

3. **How well do you feel?**

NOT well at all ____ VERY well

4. **Do you feel like you have gas?**

NOT at all ____ VERY much

5. **Do you feel like you have diarrhea?**

NOT at all ____ VERY much

ID: __________ DATE (DD/MMM/YYYY):_________________

SESSION: __________ TREATMENT (Code): ______________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Motivation to Eat

Time point: 90 min

These questions relate to your "motivation to eat" at this time. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings.

1. **How strong is your desire to eat?**

Very WEAK ____ Very STRONG

2. **How hungry do you feel?**

Not hungry at all ____ As hungry as I have ever felt

3. **How full do you feel?**

Not full at all ____ Very full

4. **How much food do you think you could eat?**

NOTHING at all ____ A LARGE amount

5. **How thirsty do you feel?**

NOT thirsty at all ____ As thirsty as I have ever felt

ID: __________ DATE (DD/MMM/YYYY):__________________

SESSION: __________ TREATMENT (Code): ______________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Energy and Fatigue

Time point: 90 min

These questions relate to your energy level and fatigue at this time. Please rate yourself by placing a small "**x**" across the horizontal line at the point which best reflects your present feelings.

1. **How energetic do you feel right now?**

NOT at all ____ VERY energetic

2. **How tired do you feel right now?**

NOT at all ____ VERY tired

ID: __________ DATE (DD/MMM/YYYY):__________________

SESSION: __________ TREATMENT (Code): ______________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Physical Comfort

Time point: 90 min

These questions relate to your "physical comfort" at this time. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings

1. **Do you feel nauseous?**

NOT at all ____ VERY much

2. **Does your stomach hurt?**

NOT at all ____ VERY much

3. **How well do you feel?**

NOT well at all ____ VERY well

4. **Do you feel like you have gas?**

NOT at all ____ VERY much

5. **Do you feel like you have diarrhea?**

NOT at all ____ VERY much

ID: __________ DATE (DD/MMM/YYYY):_________________

SESSION: __________ TREATMENT (Code): ______________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Motivation to Eat

Time point: 120 min

These questions relate to your "motivation to eat" at this time. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings.

1. **How strong is your desire to eat?**

Very WEAK ____ Very STRONG

2. **How hungry do you feel?**

Not hungry at all ____ As hungry as I have ever felt

3. **How full do you feel?**

Not full at all ____ Very full

4. **How much food do you think you could eat?**

NOTHING at all ____ A LARGE amount

5. **How thirsty do you feel?**

NOT thirsty at all ____ As thirsty as I have ever felt

ID: __________ DATE (DD/MMM/YYYY):__________________

SESSION: __________ TREATMENT (Code): ______________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Energy and Fatigue

Time point: 120 min

These questions relate to your energy level and fatigue at this time. Please rate yourself by placing a small "**x**" across the horizontal line at the point which best reflects your present feelings.

1. **How energetic do you feel right now?**

NOT at all ____ VERY energetic

2. **How tired do you feel right now?**

NOT at all ____ VERY tired

ID: __________ DATE (DD/MMM/YYYY):__________________

SESSION: __________ TREATMENT (Code): ______________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Physical Comfort

Time point: 120 min

These questions relate to your "physical comfort" at this time. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings

1. **Do you feel nauseous?**

NOT at all ____ VERY much

2. **Does your stomach hurt?**

NOT at all ____ VERY much

3. **How well do you feel?**

NOT well at all ____ VERY well

4. **Do you feel like you have gas?**

NOT at all ____ VERY much

5. **Do you feel like you have diarrhea?**

NOT at all ____ VERY much

ID: __________ DATE (DD/MMM/YYYY):_________________

SESSION: __________ TREATMENT (Code): ______________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Motivation to Eat

Time point: 145 min

These questions relate to your “motivation to eat” at this time. Please rate yourself by placing a small “x” across the horizontal line at the point which best reflects your present feelings.

1. **How strong is your desire to eat?**

Very WEAK ____ Very STRONG

2. **How hungry do you feel?**

Not hungry at all ____ As hungry as I have ever felt

3. **How full do you feel?**

Not full at all ____ Very full

4. **How much food do you think you could eat?**

NOTHING at all ____ A LARGE amount

5. **How thirsty do you feel?**

NOT thirsty at all ____ As thirsty as I have ever felt

ID: __________ DATE (DD/MMM/YYYY):__________________

SESSION: __________ TREATMENT (Code): ______________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Energy and Fatigue

Time point: 120 min

These questions relate to your energy level and fatigue at this time. Please rate yourself by placing a small "**x**" across the horizontal line at the point which best reflects your present feelings.

1. **How energetic do you feel right now?**

NOT at all ____ VERY energetic

2. **How tired do you feel right now?**

NOT at all ____ VERY tired

ID: __________ DATE (DD/MMM/YYYY):__________________

SESSION: __________ TREATMENT (Code): ______________________

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale
Physical Comfort

Time point: 120 min

These questions relate to your "physical comfort" at this time. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings

1. **Do you feel nauseous?**

NOT at all ____ VERY much

2. **Does your stomach hurt?**

NOT at all ____ VERY much

3. **How well do you feel?**

NOT well at all ____ VERY well

4. **Do you feel like you have gas?**

NOT at all ____ VERY much

5. **Do you feel like you have diarrhea?**

NOT at all ____ VERY much

Appendix 6: Sensory forms (hedonic and visual analogue scales)

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

9-Point Hedonic Scale: Pleasantness

DATE: ________ Treatment ID ____

ID: ________________________ Actual time:________________

Please put a checkmark on the most appropriate response

How much did you enjoy the **Pleasantness** of the sample?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

9-Point Hedonic Scale: Taste

DATE: ________ Treatment ID ____

ID: ________________________ Actual time:________________

Please put a checkmark on the most appropriate response

How much did you enjoy the **Taste** of the sample?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

9-Point Hedonic Scale: Texture

DATE: ________ Treatment ID ____

ID: ________________________ Actual time:________________

Please put a checkmark on the most appropriate response

How much did you enjoy the **Texture** of the sample?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale: Saltiness

DATE: _________ Treatment ID ____

ID: ________________________ Actual time:________________

Please rate the **saltiness** of the food by placing a small "**x**" across the horizontal line at the point which best reflects your present feelings.

NOT salty at all ____ VERY salty

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

9-Point Hedonic Scale: Saltiness

DATE: ________ Treatment ID ____

ID: ________________________ Actual time:________________

Please put a checkmark on the most appropriate response

How much did you enjoy the **Saltiness** of the sample?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale: Chewiness

DATE: _________ Treatment ID ____

ID: ________________________ Actual time:________________

Please rate the **chewiness** of the food by placing a small "**x**" across the horizontal line at the point which best reflects your present feelings.

NOT chewy at all ____ VERY chewy

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

9-Point Hedonic Scale: Chewiness

DATE: ________ Treatment ID ____

ID: ________________________ Actual time:________________

Please put a checkmark on the most appropriate response

How much did you enjoy the **Chewiness** of the sample?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale: Bitterness

DATE: _________ Treatment ID ____

ID: ________________________ Actual time:________________

Please rate the **bitterness** of the food by placing a small “**x**” across the horizontal line at the point which best reflects your present feelings.

NOT bitter at all ____ VERY bitter

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale: Seaweed flavor

DATE: _________ Treatment ID ____

ID: ________________________ Actual time:________________

Please rate the **seaweed flavor** of the food by placing a small "**x**" across the horizontal line at the point which best reflects your present feelings.

NO seaweed flavor ____ STRONG seaweed flavor

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

9-Point Hedonic Scale: Mouthfeel

DATE: ________ Treatment ID ____

ID: ________________________ Actual time:________________

Please put a checkmark on the most appropriate response

How much did you enjoy the **Mouthfeel** of the sample?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

9-Point Hedonic Scale: Flavour

DATE: ________ Treatment ID ____

ID: ________________________ Actual time:________________

Please put a checkmark on the most appropriate response

How much did you enjoy the **Flavour** of the sample?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

9-Point Hedonic Scale: Aftertaste

DATE: ________ Treatment ID ____

ID: ________________________ Actual time:________________

Please put a checkmark on the most appropriate response

How much did you enjoy the **Aftertaste** of the sample?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

9-Point Hedonic Scale: Appearance

DATE: ________ Treatment ID ____

ID: ________________________ Actual time:________________

Please put a checkmark on the most appropriate response

How much did you enjoy the **Appearance** of the sample?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Visual Analogue Scale-Prospective purchasing

DATE: ____________________ Treatment ID ____

ID: ________________________ Actual time _____________

This question relates to the palatability of the food you just consumed. **How likely would you purchase this product?** Place a small "**x**" across the horizontal line at the point which best reflects your present feelings.

Would not purchase ____ Would purchase

ID: ___________________ Date (DD/MMM/YYYY):_____________________

Appendix 7: Food Acceptability

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Please indicate with a rating between 1 and 10 how much you enjoy the following foods **(1 = not at all, 10 = very much)** and how often you eat them **(never, daily, weekly, monthly).**

	Enjoyment?	How often?
1. Pasta	___________	___________
2. Rice	___________	___________
3. Potatoes (mashed, roasted)	___________	___________
4. Couscous	___________	___________
5. Pizza	___________	___________
6. Soy sauce	___________	___________
7. Crab meat	___________	___________
8. Fish	___________	___________
9. Seaweeds	___________	___________
10. Tofu or other soy-texturized products	___________	___________

At the end of each session, you will be provided with pizza. In order to provide you with a meal that you will enjoy, we ask that you rank the following pizzas according to your **personal preferences (i.e. 1st, 2nd choice)** in the space provided. If you **do NOT** like a particular type of pizza, then do not rank it but instead write **"I don't like"** in the space provided.

Pepperoni (cheese, pepperoni) ___________
Three-cheese (mozzarella, cheddar, parmesan) ___________

Appendix 8: Information Sheet and Consent Forms for participants recruited for seaweed meal study

Information Sheet and Consent Forms for participants recruited for seaweed meal study

Study title: The effect of a mixed meal formulated with Chondrus crispus seaweeds on satiety and food intake in young males

Investigators:

Dr. Bohdan Luhovyy, Principal Investigator
Dr. Priya Kathirvel, Co-Investigator
Department of Applied Human Nutrition, Mount Saint Vincent University
E-mails: bohdan.luhovyy@msvu.ca ; priya.kathirvel@msvu.ca

M.Sc. Students and Research Assistants:

Mr. Tongtong Li, MSc Student
E-mail: tongtong.li1@msvu.ca

Ms. Jade Farquharson, BSc Student
E-mail: Jade.Farquharson@msvu.ca

Mr. Erik Vandenboer, Research Assistant
E-mail: Erik.Vandenboer@msvu.ca

Ms. Gowshigga Thamotharampillai, Research Assistant
E-mail: gowshigga.thamotharampillai@msvu.ca

Ms. Yu Gao, MAHN Student
E-mail: Yu.Gao5@msvu.ca

Introduction:

You are invited to participate in the research study listed above. This form provides you with information about the study so you can make an informed decision about if you would like to participate. It will enable you to understand the purpose of the study, the risks and benefits of participating, and what you will be asked to do should you choose to participate. We will keep you informed of any new information that may influence your willingness to continue to participate in the study. A member of the research team will be available to answer any questions you may have. You may decide not to participate and you may withdraw from the study at any time. Your participation in the study is entirely voluntary.

Funding Source:
Funding for this project is provided by the NSERC Engage Grant and Acadian Sea Plants Limited.

Background and Purpose of Research:
Seaweeds are a rich source of nutrients including proteins, lipids, minerals, polysaccharides, soluble and insoluble fibers and trace elements and they are traditionally consumed in Asian countries as sea vegetables. Apart from these nutrients, seaweeds contain other bioactive compounds and previous studies have shown the benefits of some of these compounds on body weight control and satiety. However, the consumption of seaweeds in Canada is very low. It is possible that dietary incorporation of seaweed might provide satiating effects. This study is designed to examine the effect of meals formulated with seaweed on the appetite and food intake in young healthy adult males. The results from this study may help in the development of new or reformulated food products with seaweed. This study will not cost you anything. We anticipate having about 30 male participants enrolled in this study. This study is funded by NSERC (Natural Sciences and Engineering Research Council of Canada) and Acadian Sea Plants Limited. There are no conflicts of interest between the investigators and the sponsor. The Primary Investigator of the study receives no financial compensation or non-financial benefits from the industry partner.

Invitation to Participate:
You are being invited to take part in this study. If you chose to take part and meet eligibility criteria, you will be asked to visit our laboratory for three sessions with one week apart between sessions. At each session, your appetite, physical comfort, energy level and fatigue will be measured using simple paper scales before and after eating the treatment (meal or water) and after eating a pizza meal. Each session will take up to three hours of your time.

Eligibility:
To participate in this study, you must be considered overall healthy, and not being underweight, overweight or obese, and not having any diseases. You must be a male between the ages of 19 and 35. You must be a non-smoker (including e-cigarettes) and you cannot be taking certain medications that may affect central and peripheral mechanisms of food intake regulation, cognitive performance and sedative medications and consume cannabis products. Please tell us if you are taking any medication and we will determine if you are eligible to participate in this study. You will not be able to participate if you have allergies to any food, aversion to seaweeds or if you usually skip breakfast, have disordered eating patterns (e.g., focusing too much on body weight, body imaging, dieting, self-limiting the choice of foods, performing specific food rituals, feel anxious or guilty when eating or after eating, having bulimia, anorexia or binge eating disorder), or unable to comprehend the questions and fill out the questionnaires used in the study (we will show you the samples of the questionnaires used in this study). To find out if you can take part in this study, you will be asked to fill out questionnaires, which ask questions about your age, if you smoke, exercise, your health, if you are on any medications and your eating habits. Your height and weight will be measured. The study will take place in the Department of Applied Human Nutrition, Room 211 in the Centre of Applied Research, Mount Saint Vincent University, 47 College Rd, Halifax, NS.

Procedure:
You will be asked to fast for at least 10 hours overnight before each session and arrive to our laboratory between 8 am and 10 am in the morning. However, you may drink water until one hour before arriving to the laboratory. Please note that you will have to arrive exactly the same time at each of the three sessions. Upon arrival, we will measure your fasting blood

glucose level with a portable glucose meter in a drop of blood through the finger prick test and then we will ask you about your recent food intake, stress level, sleep and physical activity. If your blood glucose will be higher than normal fasting level (higher than 6 mmol/L), we will repeat the test within 30 min and if it still be high then we will need to reschedule the session. Similarly, if you had experienced stress, unusual food intake, alcohol consumption, did not sleep enough or had extra physical activity in the day before your session, we will need to reschedule your session as well. If your fasting blood glucose level is normal and you did not experience any unusual stress, sleep deprivation, extra physical activity, excessive food intake and alcohol consumption in previous day, the session will commence. We will ask you to complete the questionnaire related to your appetite and physical comfort, and then will take you to the feeding cubicles and will serve you with a meal with a glass of water (two sessions) or just the glass of water (in one session). You will have up to 12 minutes to consume your food and water and evaluate their taste. You will be asked to consume the whole amount of food and water provided. Then you will be taken back to the Appetite Lab and you can read, study or use your computer during the next 112 minutes. Please note that you may not browse any content related to food or eating. Our research assistants will ask you to complete the same questionnaires related to your appetite and physical comfort every 15 min in the first hour, and every 30 min during the second hour. At 120 min, we will ask you to proceed to the feeding cubicles and will serve you with a pizza meal. You will be asked to eat until you feel comfortable full. The new tray with sliced pizza and new bottle with spring water will be provided every 8 minutes and the previous tray and water bottle with the left over (if any) will be taken back by our research assistant. Once you completed your meal, we will ask you to fill out the questionnaire rating your appetite and physical comfort. Then you will be offered to drink a glass of chocolate milk or fruit juice of your choice and you are free to go.

An Example of a Potential Time and Activity Schedule for Each Session:

Time	Activity
8:45	Arrive at the laboratory
8:50	Fill in Sleep, Stress, and VAS questionnaires and take fingerpick blood sample
9:00 - 9:12	Eat the treatment (0 min)
9:15 - 11:00	Fill out VAS questionnaires at 15, 30, 45, 60, 90 and 120 min
11:00 – 11:20	Eat the lunch meal until you feel comfortably full
11:20	The session is completed. You will receive a glass of chocolate milk or fruit juice

VAS= Visual analogue scale

Voluntary Participation and Early Withdrawal:

Participation in this study is voluntary. You may choose to stop being in the study at any time without any negative consequences.

Risks:

There is a very little risk of food poisoning. All of the foods that you will be asked to consume will be prepared using practices to ensure food safety. Therefore, your risk of developing a food borne illness from participation in this study is very minimal. Pricking the finger to obtain a drop of blood may be associated with a momentary slight discomfort. The risk of infection related to blood sampling is minimal as you will be using a disposable lancing device. We will explain you how to sanitize your finger with the alcohol swabs. After the overnight fast you may feel faint or dizzy, however the risk of this is minimal. If this happens and you feel it will be unsafe to travel the session will be rescheduled.

Benefits:

You will not benefit directly from taking part in this study. However, the study results will advance nutritional science and may lead to practical dietetic recommendations.

Confidentiality and Privacy:

Confidentiality will be respected and no information that shows your identity will be released or published without your permission unless required by law. Your name, personal information and signed consent form will be kept in a locked filing cabinet in the investigator's office. Your results will not be kept in the same place as your name. Your results will be recorded on data sheets and in computer records that have an ID number for identification, but will not include your name. Your results, identified only by an ID number, will be made available to the study sponsor if requested. Only study investigators will have access to your individual results. If you withdraw from the study, your consent form and any other paperwork associated with your ID number will be destroyed.

Publication of Results:

Although the results of this study are intended for our industry partner, it is possible that the results of this study may be published in scientific literature or presented at a conference or seminar. Confidentiality will be upheld, and no names or identifying information about you will be used in any publication or presentation.

New Findings:

If anything is found during the course of this research which may change your decision to continue, you will be told about it.

Compensation:

You will be paid $30 per experimental session with a total of $90 for completed study. Payment will be in the form of cash and can be paid either after each session or after the end of the study. Please note that in order to process your compensation the collection of your personal information including your full name, current mailing address and signature is required by the university's financial services department. This information is collected, stored and accessible to the financial services department alone other than the initial collection and is not linked to the current study in any way.

Injury Statement:

If you begin to feel sick following participation in the study, please seek medical advice as soon as possible. We will provide your medical specialist with information about the food you have consumed during the session. You can send the email to Dr. Luhovyy (Bohdan.Luhovyy@msvu.ca) and ask your question and leave your contact information and we will contact you back at the earliest convenience.

Rights of Participants:

Before agreeing to take part in this research study, it is important that you read and understand your role as described here in this study information sheet and consent form. You waive no legal rights by taking part in this study. If you have questions about how this study is being conducted and wish to speak with someone not involved in the study, you may contact the Chair of the University Research Ethics Board (UREB) c/o MSVU Research Office, at 457-6350 or via e-mail at research@msvu.ca

The ethical components of this research study have been reviewed by the University Research Ethics Board and found to be in compliance with Mount Saint Vincent University's Research Ethics Policy.

Voluntary Participation and Early Withdrawal:
Participation in this study is voluntary. You may choose to stop being in the study at any time without any negative consequences. If you wish to withdraw simply inform a member of the research team and you are free to leave.

Dissemination of findings:
A summary of results will be made available for you to pick up in one year after the study is done.

Copy of informed consent for participant:
You will be given a copy of this informed consent to keep for your own records.

Consent form
TO BE KEPT SEPARATELY FROM DATA FORMS

Study titleThe effect of a mixed meal formulated with *Chondrus crispus* seaweeds on satiety and food intake in young males
Participant ID assigned: ______________________

PARTICIPANT AUTHORIZATION:

I have read or had read to me this information and authorization form and have had the chance to ask questions which have been answered to my satisfaction before signing my name. I understand the nature of the study and I understand the potential risks. I understand that I have the right to withdraw from the study at any time without any problems, and to do so simply need to inform a member of the research team. I have received a copy of the Information and Authorization Form for future reference. I understand that to receive compensation I will need to provide personal information including full name, current mailing address and a signature on the compensation form which will be returned to the university's financial services department. I freely agree to participate in this research study.

Would you like to receive a summary of the results when they are available? Yes ___ No ____.
Would you like to be contacted for future research? Yes ___ No____.

Name of Participant: (Print) ____

Date: ______________ Time: _________________ Participant ID: ______________________________

Sex _____ M _____ F, Age ____ (y). Month and year of birth (mm/yyyy) _____________

If you would like to receive the summary of the results and/or be contacted for future research, please print your address below:

____

Participant Signature: _____________________

Date: ______________ Time: _________________

STATEMENT BY PERSON PROVIDING INFORMATION ON STUDY AND OBTAINING CONSENT

I have explained the nature and demands of the research study and judge that the participant named above understands the nature and demands of the study. I have explained the nature of the consent process to the participant and judge that they understand that participation is voluntary and that they may withdraw at any time from participating.

Name: (Print) _____________________ Signature: _____________ Position: ______________________

Date: ______________________ Time: ______________________