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Ano 2018

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DESENVOLVIMENTO DE SOPAS ENRIQUECIDAS COM AS MACROALGAS *FUCUS VESICULOSUS* E *ULVA RIGIDA*

DEVELOPMENT OF FORTIFIED SOUPS WITH *FUCUS VESICULOSUS* AND *ULVA RIGIDA* SEAWEEDS

Raquel Ferreira Abreu

Desenvolvimento de produtos alimentares saudáveis: Incorporação de Macroalgas

Development of healthy food products: Incorporation of Seaweeds

Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biotecnologia Alimentar, realizada sob a orientação científica da Doutora Susana M. Cardoso, Investigadora Doutorada do Departamento de Química da Universidade de Aveiro e co-orientação científica do Doutor Artur Silva, Professor Catedrático do Departamento de Química da Universidade de Aveiro.

Trabalho efetuado no âmbito do projeto de investigação e desenvolvimento tecnológico em copromoção **POCI-01-0247-FEDER-003419**, SHARP - Seaweed for Healthier Traditional Products, co-financiado pelo Fundo Europeu de Desenvolvimento Regional (FEDER) através do Programa Operacional Temático Competitividade e Internacionalização, no âmbito do Programa "Portugal 2020"

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Agradecimentos

Primeiramente, quero agradecer à minha orientadora, Doutora Susana Maria de Almeida Cardoso, pela oportunidade que me deu em desenvolver este projeto, pela disponibilidade e motivação. A toda a equipa da *CentralRest Lda* pelos conselhos, pela ajuda sempre prestada e, principalmente, pelo conhecimento que me transmitiram.

Um agradecimento especial aos meus pais e irmão, pelo apoio incondicional, pelos valores que me transmitiram, confiança, paciência, ajuda e conselhos que me deram ao longo de toda esta etapa. À restante família agradeço a compreensão da minha ausência em alguns momentos e a união.

Ao Nuno agradeço pela força e ânimo que sempre me deu em todos os momentos, pelo carinho e pela tolerância sempre demonstrada. Por fim, mas não menos importante, agradeço a todos os meus amigos mais chegados, que sempre estiveram presentes mesmo quando eu não tinha tempo para eles, pela enorme amizade.

Palavras-chave Sopas enriquecidas, Macroalgas, *Fucus vesiculosus*, *Ulva rigida*, Composição nutricional, Idosos

Resumo

As macroalgas são uma excelente fonte de nutrientes e fitoquímicos bioativos associados à prevenção de diversas doenças, contribuindo para a promoção de dietas saudáveis e equilibradas. Apesar do seu consumo a nível Europeu ser reduzido comparativamente aos países asiáticos, estas são cada vez mais utilizadas como um ingrediente funcional para enriquecer nutricionalmente diversos alimentos e, simultaneamente, induzir novos hábitos alimentares.

O trabalho desenvolvido tem como objetivo incorporar a alga castanha *Fucus vesiculosus* e a alga verde *Ulva rigida*, em sopas de valor acrescentado. Releva-se ainda que este objetivo pretendia que as sopas formuladas tivessem composição nutricional suficiente para substituir uma refeição, particularmente para os idosos, e deveriam também ser organolepticamente semelhante a uma sopa tradicional portuguesa.

Para tal, desenvolveram-se duas sopas diferentes, nomeadamente uma contendo como base os ingredientes batata-doce, cenoura, abóbora, courgette, alho francês, grão-de-bico, cebola, azeite, água, sal e extrato hidrossolúvel de soja e sendo enriquecida com *Fucus vesiculosus* na base seca, e outra contendo batata-doce, cenoura, abóbora, courgette, alho francês, grão-de-bico, brócolos, azeite, água, sal e *Ulva rigida* na base seca, sem a presença de extrato hidrossolúvel de soja. Para cada uma das sopas foi concebido um controlo, que consistiu na sopa com todos os ingredientes exceto a alga. De forma a perceber possíveis benefícios nutricionais e funcionais, as sopas controlo e enriquecidas com algas foram analisadas quanto a propriedades físico-químicas como a cor, pH, acidez titulável e nutricionais como a humidade relativa, conteúdo proteico, fibra dietética, açúcares totais e redutores, conteúdo de cinzas e composição elemental (minerais). Além disso, realizaram-se análises fitoquímicas como a avaliação dos composto fenólicos totais e da atividade antioxidante, bem como a quantificação de pigmentos e carotenóides.

A incorporação das algas nas sopas foi notória ao nível da composição elemental das mesmas. É importante salientar o aumento de Fe, Mg e Ca em ambas, bem como a diminuição de Na, o que é positivo porque hoje em dia as dietas quotidianas são extremamente ricas em Na. Além disso, concluiu-se que as sopas tinham os valores nutricionais que se enquadram nos valores necessários de uma refeição para uma pessoa idosa.

Keywords Fortified Soups, Seaweeds, *Fucus vesiculosus*, *Ulva rigida*, Nutrition composition, Elderly

Abstract

Seaweeds are an excellent source of bioactive nutrients and phytochemicals associated with the prevention of various diseases, contributing to the promotion of healthy and balanced diets. Although their consumption at European level is low compared to Asian countries, they are increasingly being used as a functional ingredient to nutritionally enrich various foods while inducing new eating habits.

The objective of this work is to incorporate the brown seaweed *Fucus vesiculosus* and the green seaweed *Ulva rigida*, in soups with added value. It is further noted that this objective intended that the formulated soups had sufficient nutritional composition to replace a meal, particularly for the elderly, and should also be organoleptically similar to a traditional Portuguese soup.

For this, two different soups were developed, namely one with sweet potato, carrot, pumpkin, zucchini, leek, chickpeas, onion, olive oil, water, salt and a water-soluble soybean extract and being fortified with *Fucus vesiculosus* in dry basis, and another one containing sweet potato, carrot, pumpkin, zucchini, leek, chickpeas, broccoli, olive oil, water, salt and *Ulva rigida* in dry base, without the presence of water-soluble extract of soybean. For each one of the soups was designed a control, which consisted of a soup with all ingredients except seaweed. In order to understand possible nutritional and functional benefits, the seaweed-fortified and control soups were analyzed for physical and chemical properties such as color, pH, titratable acidity and nutrients such as relative humidity, protein content, dietary fiber, total and reducing sugars, ash content and elemental composition (minerals). In addition, phytochemical analyzes such as the evaluation of total phenolic compounds and antioxidant activity, as well as the quantification of pigments and carotenoids were carried out.

The incorporation of the seaweeds in the soups was notorious at the level of the elemental composition of them. It is important to emphasize the increase of Fe, Mg and Ca in both, as well as the decrease of Na, which is positive because nowadays daily diets are extremely rich in Na. In addition, it was concluded that the soups had the nutritional values that fit the required values of a meal for an elderly person.

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ABBREVIATIONS

SFFS – Soybean and *Fucus vesiculosus* Fortified Soup

UFS – *Ulva rigida* Fortified Soup

UK – United Kingdom

USA – United States of America

SPs – Sulfated Polysaccharides

LDL – Low-density Lipoprotein

FAO – Food and Agriculture Organization

WHO – World Health Organization

PUFAs – Polyunsaturated Fatty Acids

DHA – Docosahexaenoic Acid

EPA – Eicosapentaenoic Acid

DNA – Deoxyribonucleic Acid

PBPs - Phycobiliproteins

PBSs - Phycobilisomes

DPPH – 2,2 – Diphenyl-1-picrylhydrazyl

GAE – Gallic Acid Equivalents

EPA – Ascorbic Acid Equivalents

Phl - Phloroglucinol

IC_{50} – Extract concentration necessary to inhibit 50% of the sample

FRAP – Ferric-Reducing Antioxidant Power

PGE – Phloroglucinol Equivalents

TP – Traditional Pasteurized

EC50 – Half maximal effective concentration

AE – Antiradical efficiency

PortFIR - Plataforma Portuguesa de Informação Alimentar

CieLAB – Color space defined by International Commission on Illumination

AOAC – Association of Official Agriculture Chemicals

MES-TRIS – Buffer of 2-(N-morpholino)ethanesulfonic acid and 2-Amino-2-hydroxymethyl-propane-1,3-diol

MA – Massachussetts

BHT – Butylated hydroxytoluene

ABTS – 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)

UHPLC-DAD-ESI-MSn – Ultra-high performance liquid chromatography with a diode detector coupled to electrospray ionization mass spectrometry

UV/Vis – Ultraviolet/Visible

ESI – Electrospray

DRIs – Dietary Reference Intakes

RDD – Recommended Daily Dose

RDA – Recommended Dietary Allowances

AIs – Adequate Intake

EAR – Estimated Average Requirement

TPC – Total Phenolic Compounds

AA – Antioxidant Activity

TE – Trolox Equivalents

RPM - Revolutions per minute

1. LITERATURE REVIEW

1.1 Seaweeds

Seaweeds are multicellular photosynthetic organisms that due to not having vascular roots, leaves or tissues (phloem and xylem) are considered to be different from the so-called upper plants [1]. Seaweeds are usually found in marine habitats and play an importante role in the maintenance and structuring of ecosystems due to using sunlight, carbon dioxide and water to produce organic material [2, 3].

Seaweeds are classified based on their pigment composition and although this classification system has been changed over time, it is considered that the green, red and brown seaweed belong to the Filo *Clorophyta*, *Rhodophyta* and *Heterokontophyta*, respectively [4]. Despite the fact that seaweed have diverse pigments, they all have one in common, chlorophyll. Green seaweed contains lutein and zeaxanthin and is characterized by the chlorophyll composition "a" and "b", this same composition is also responsible for their typical green colour. In regards to brown seaweed, they get their brown colour from the pigment fucoxanthin, have a significant morphological diversity and are characterized by the primary photosynthetic pigments: chlorophyll "a" and "c1" and "c2" and other pigments such neofucoxanthin and β-carotene [5]. At last, red seaweeds are mainly characterized by the chlorophyll "a" and the phycobilins phycoerythrin and phycocyanin. Depending on the proportion of phycoerythrin and phycocyanin their colour can vary and in addition to these pigments, these seaweeds also have α- and β-carotene, zeaxanthin and lutein [1].

It is important to know that compared to green seaweeds, brown and red seaweeds have higher morphological and anatomical differentiation. In addition, brown seaweeds exhibit all three modes of reproduction: vegetative, asexual, and sexual [1].

In recent years, there has been a growing interest in so-called functional food groups, amongst which seaweeds seem to be able to play an important role as they have been claimed to provide physiological benefits, additional to nutritional as, for instance, anti-hypertensive, anti-oxidant or anti-inflammatory [6, 7]. A functional food can be defined as a food that produces a beneficial effect in one or more physiological functions,

increases the welfare and/or decreases the risk of suffering from the onset or development of a particular disease [7]. The functionalities are far more preventative than curative. Frequently, functional foods are obtained from traditional foods enriched in an ingredient which is able to provide or promote a beneficial action for human health. These are the so-called functional ingredients. According to Madhusudan et al., many biologically active compounds are present in seaweeds, which can be used as therapeutic agents in dietary supplements [6, 7]. Furthermore, new types of products, derived from natural products (including foods and medicinal plants), often referred to as nutraceuticals have recently been developed and extensively marketed. These products are usually employed as food supplements, rather than whole foods, and are marketed as tablets and pills and claimed to provide important health benefits [7].

Seaweeds have been consumed for centuries by some populations. Indeed, food remains containing residues of brown algae, belonging to the genus *Sargassum* or *Eisenia*, shellfish, and fish have been found [8]. In Korea, fragments of brown seaweeds have been found in fossilized meals dating back 10 000 year. Concerning to Europe, the introduction of seaweeds into human food began in the 15th century.

Currently, the use of seaweeds in human food is broadly spread throughout the world, especially where the algae are exploited as a resource for the production of food, food additives, and nutritional supplements in Asia, Europe, North and South America, Africa, and Pacific Islands nations [9]. There is a thriving seaweed industry whose products are used as food, fodder, fertilisers and cosmetics and for a wide range of industrial purposes [10]. Around the globe there are 221 species of seaweeds that are used in diverse industries. From those 221 species, 125 are red seaweed, 64 are brown seaweed and 32 are green seaweed. Around 145 of these are intended for direct food use, while the rest are used in industries such as traditional medicine, ficocoloid, agriculture and animal feeding. [11]. The world seaweed production reached in 2000 around 10 million tons including wild and maricultured. The top 12 main producing countries are China, France, UK, Japan, Chile, Philippines, Korea, Indonesia, Norway, USA, Canada and Ireland. The wild seaweed harvesting did not change much for the last 12 years but aquaculture (including integrated mariculture) is increasing incessantly [12, 13].

1.1.1 Composition and nutritional value

Having many characteristics relevant for health maintenance such as being practically fat-free, having a low caloric content and being very rich in essential minerals, vitamins, proteins and other phytochemicals makes seaweeds an organism with interesting nutritional properties [14]. Indeed, the frequent consumption of seaweeds have been associated to the prevention of various diseases [15].

For example, urolithiasis affects approximately 10% of the world population and is strongly associated with calcium oxalate (C_aO_x) crystals. Currently, there is no efficient compound that can be used to prevent this disease. However, seaweeds's sulfated polysaccharides (SPs) have the ability to change the C_aO_x crystals surface's charge and thus modify the crystallization dynamics, due to the interaction of the negative charges of these polymers with the crystal surface during their synthesis. It was observed that the SPs of *Caulerpa cupressoides* modify the morphology, size and surface charge of C_aO crystals. Thus, these crystals are similar to those found in healthy persons [16]. In addition, it has been suggested that the increase in the incidence of adult diseases in Japan, such as diabetes, hypertension and hyperlipidemia, was caused by the decrease in dietary intake of marine algae, fish and shellfish [15].

1.1.1.1 Polysaccharides and dietary fiber

The total polysaccharide concentrations in the seaweed species range from 4% to 76% of dry weight. Species like *Ascophyllum*, *Porphyra* and *Palmaria* have the highest contents of polysaccharides (42-70%, 50-76% and 38-74% respectively) [17]. Nevertheless green seaweed species such as *Ulva sp.* also have a high content, up to 65% of dry weight [17]. Marine algae are characterized by the presence of polysaccharides, such as storage polysaccharides, mucopolysaccharides and frame polysaccharides (Table 1). The frame polysaccharides of marine algae mainly consist of cellulose. While green algae contain sulfuric acid polysaccharides, brown algae, like *Fucoidan* and *Sargassan*, contain alginic acid and red algae contain agar-agar and porphyran as mucopolysaccharides located in the intercellular space. Starch and laminarin are present in marine algae as storage

polysaccharides [15]. There is a predominance of alginate in brown algae, relating to cell wall polysaccharides, while in red seaweeds agar and carrageenan prevail. To a lesser extent, polysaccharides containing sulfated fucose (brown seaweeds), xylans (red and green seaweeds) and cellulose (generally found in all genera of higher plants) are found. At the level of the reserve polysaccharides, green seaweeds have starch present, brown seaweeds have laminarin (β-1,3 glucan), while red seaweeds have floridean amide (amylopectin linked to glucan) (Table 1) [15].

Table 1 - Polysaccharides in marine algae [15].

	Frame polysaccharides	**Mucopolysaccharides**	**Storage polysaccharides**
Green algae	Cellulose	Sulfated glucoronoxylorhamnan	Amylose
	β -1,3-Xylan	Sulfated xyloarabinogalactan	Amylopectin
	β --1,4-Mannan	Sulfated glucuronoxylorhamn ogalactan	
Brown algae	Cellulose	Alginic acid	Laminarin
	Hemicellulose	Fucoidan	
Red algae	Cellulose	Agar-agar	Starch
	Hemicellulose	Carrageenan	
	β -1,3-Xylan	Porphyran	
	β -1,4-Mannan		

Many of the aforementioned polysaccharides are not digested by the secretions of the human gastrointestinal tract, so they are considered dietary fiber. They are very different in chemical structure and composition as well as in their physicochemical properties, their capacity to be fermented by the colonic flora, and their biological effects on animal and human cells [18]. In general, dietary fiber is classified into two types according to its solubility in water: insoluble fiber and water-soluble fiber. Examples of insoluble fiber are cellulose, xylan and mannan, and examples of water-soluble fiber are alginic acid, agar-agar, furonan, porphyrane and laminarin [15, 17].

When compared to higher plants, seaweeds have a higher amount of fiber that range between 33-62% dry matter [19]. It is important to consider that the values of total, insoluble and soluble dietary fiber may diverge depending on the seasonal variations, geographical origin and even the processing (drying, milling) after harvest [20, 21]. As we

can see in Table 2, the total fiber values of diverse seaweeds can differ from 33.4% to 38.1% for *Enteromorpha sp.* and *Ulva lactuta*, respectively. Concerning to the insoluble fiber values, they range from 5.3% (for *Undaria sp.*) to 16.8% (for *Ulva lactuta* and *Porphyra sp.*). On the other hand, the soluble fiber values are higher than the last ones as they range from 17.2% to 30% (*Enteromorpha sp. and Undaria sp.*, respectively).

Table 2 - Dietary fiber content of some seaweeds compared to whole foods (vegetables, fruits and cereals) expressed in % of dry weight (Adapted from [17, 22]).

	Insoluble Fiber	**Soluble Fiber**	**Total Fiber**
Porphyra sp.	16.8	17.9	34.7
Undaria sp.	5.3	30	35.3
Ulva lactuta	16.8	21.3	38.1
Enteromorpha sp.	16.2	17.2	33.4
Onions	13.32	3.59	16.89
Potatoes	4.85	2.14	6.99
Pears	39.53	27.3	66.83
Whole corn	87.47	0.40	87.87

The typical algae carbohydrates are not digestible by the human gastrointestinal tract and, therefore, they are dietary fibers [19]. The dietary fiber, which constitutes 25-75% of the dry weight of marine algae and represents their major component, is primarily soluble fiber [22]. The content of total dietary fiber ranges from 33–50g/100g d.w. Accordingly, the fiber content of seaweed varieties is usually higher than those found in most fruits and vegetables. The nutritional compositions of 34 edible seaweed products of the *Laminaria sp*., *Undaria pinnatifida*, *Hizikia fusiforme* and *Porphyra sp*. varieties were analyzed. According to C. Dawczynski et al., in general, the marine seaweed varieties tested proved to be a rich source of dietary fiber (46.2±8.0 g/100 g s.w) [19]. In fact, the consumption of this dietary fiber has been related to multiple health promoting effects. Its consumption promotes the growth and protection of the beneficial intestinal flora and greatly increases stool and reduces the risk of colon cancer. It is also important to note that in combination with high glycemic load foods, its consumption, reduces the overall glycemic response, seaweed fiber acts as a hypoglycemic [19]. So, they are powerful as an anticoagulant and reduce low-density lipid (LDL)-cholesterols in rats

(hypercholesterolemia), they prevent obesity and diabetes and they also have antiviral activities [2, 15, 23, 24].

In addition, seaweed dietary fibers have associated valuable substances and nutrients, which leads to a deal of interest in seaweed meal, functional foods and nutraceuticals for human consumption because polysaccharides show anti-tumor and anti-herpetitic bioactivity [2, 10, 15, 23, 24].

1.1.1.2 Proteins and amino acids

The protein content of seaweeds is usually high, although it differs according to species. Higher protein contents are recorded especially for green and red seaweeds, generally in the range of 9-26% to 47% dry weight, respectively [17, 25]. Concerning to brown seaweeds protein content, it is usually lower, representing between 3-15% of the dry weight [25, 26]. For example, *Undaria* has the maximal content of 24% dry weight, followed by several species (*Sargassum*, *Fucus* and *Laminaria*/*Saccharina*) with maximal content around 17-21% and with *Ascophyllum* with the lowest content (maximum 10% of dry weight) [17]. The protein content of marine algae also depends on several factors such as seasonal period, environmental conditions and life cycle. Indeed, protein content seems to be subject to large variations during year, with the maximum concentration during winter and the beginning of spring, and the minimum concentration during summer and early autumn period [27]. For example, the protein levels of *Palmaria palmata* (Dulse) collected on the French Atlantic are higher during the end of the winter and spring (around 25%), while only reach 9% in summer months [25, 27].

Concerning to the amino acid composition of seaweeds, it has been regularly studied and compared to that of other foods such as eggs or soybean and it is considered high. For example, histidine, which is an indispensable amino acid for children, is present at a parallel level to leguminous and egg proteins (Table 3) [25]. Mostly, seaweeds are full of all essential amino acids close to the values recommended by the Food and Agriculture Organization/World Health Organization (FAO/WHO) [17, 28]. For the majority seaweeds, aspartic and glutamic acids constitute together a large part of the amino acid fraction [17, 25]. Brown seaweeds have high amounts of glutamic acid and aspartic acid,

representing 22 and 44% of the total amino acids in *Fucus sp*. In green seaweeds, the level of glutamic and aspartic acid can represent up to 26 and 32% of the total amino acids of the species *Ulva rigida* and *Ulva rotundata*, respectively. However, these amino acids levels seem to be lower in red seaweed species such as *Palmaria palmata* and *Porphyra tenera* (14 and 19% of the total amino acids, respectively). Besides, red seaweeds have isoleucine and threonine levels very similar to those found in leguminous proteins.

Table 3 - Comparison of amino acid content among seaweeds, leguminous plants, egg protein and recommended values by FAO/WHO (Adapted from [29, 30, 31]).

Amino acids (aa) g aa/g protein	***Porphyra sp.***	***Ulva pinnatifida***	***Laminarina sp.***	**Leguminous plants**	**Ovalbumin**	**FAO/WHO**
Histidine	2.4-2.6	0.5-2.5	2.2	3.8-4.0	4.1	-
Isoleucine	3.1-3.3	2.8-4.1	2.7	3.6	4.8	2.8
Leucine	5.9-7.1	7.4-8.4	4.9	7.3	6.2	6.6
Lysine	4.9-5.2	3.6-5.6	3.9	6.4-6.5	7.7	5.8
Metionine	1.7	1.7-2.0	0.9	1.2-1.4	3.1	2.5
Phenylalanine	33-3.5	3.6-4.7	3.2	2.4	4.1	-
Threonine	5.2	4.4-5.4	3.5	4.0	3.0	3.4
Tryptophan	0.7	0.7-1.1	0.5	1.6-1.9	1.0	1.1
Valine	4.5-5.2	5.2-6.88	3.8	4.5	5.4	3.5
Alanine	2.2-6.2	4.4-4.7	5.7	-	6.7	-
Arginine	5.9	3.0-5.2	3.3	13.0-14.0	11.7	-
Aspartic acid	8.5	5.9-8.7	12.5	4.7-5.4	6.2	-
Glutamic acid	9.3	6.5-14.5	23.8	6.4-6.7	9.9	-
Cysteine	1.2	0.9	1.2	1.2-1.3	-	-
Glycine	4.2-5.1	3.6-5.1	4.0	-	3.4	-
Proline	3.5	3.0-3.6	3.1	-	2.8	-
Serine	4.0-4.9	2.5-4.0	3.3	-	6.8	-
Tyrosine	3.4	1.6-2.9	1.7	2.3-2.6	1.8	6.3

1.1.1.3 Lipids and fatty acids

Lipids are a broad group of naturally occurring molecules which includes fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E and K), mono-, di- and triacylglycerols, diglycerides, phospholipids, and others. Lipids represent up to 4.5% of the seaweed on a dry weight basis, and this content is lower than that of other marine organisms [17]. Although seaweeds are not a conventional source of energy (their total lipids content is very low, about 0.7-1.8% dry weight), their polyunsaturated fatty acids contents can be as high as those of terrestrial vegetables [32]. Despite its low percentage, the lipidic fraction of seaweeds is very interesting from the nutritional point of view, since it is mainly composed of PUFA [32]. PUFAs are the important component of all cell membranes and precursors of eicosanoids that are essential bioregulators of many cellular processes [33]. PUFAs effectively reduce the risk of cardiovascular diseases, cancer, ostheoporosis, and diabetes. Because of the frequent usage of seaweeds could contribute to the improvement of a low level of omega-3 PUFAs, particularly in the Western diet. The major commercial sources of ω-3 PUFAs are fish, but their wide usage as food additives is limited for the typical fishy smell, unpleasant taste, and oxidative nonstability. Nevertheless, growing requirements of healthy functional food have led to produce PUFAs as nutraceuticals in controlled batch culture of marine microalgae, specially *Thraustochytrium* and *Schizochtrium* strains [33]. Given the incapacity of the human body to synthesize PUFAs with the double bond at carbon 3 or 6 (considered to be essential fatty acids), they must be obtained from the diet. These acids are present important heath benefits, given their potential to reduce the risk of a lot of diseases such as cardiovascular diseases, osteoporosis and diabetes [33]. The fatty acids of seaweed in general have linear chains, an even number of carbon atoms, and one or more double bonds [32]. In particular, seaweeds can be a source of essential fatty acids such as eicosapentaenoic acid, C20:5ω3; ω3 fatty acids such as C20:5ω3 are thought to reduce the risk of heart disease, thrombosis and atherosclerosis. Thus, these acids are beneficial for health, because of their potential to reduce the risk of cardiovascular diseases, diabetes and osteoporosis [33]. It has also been reported that the fatty acids of certain seaweeds have antiviral activity [32]. Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are obtained from the omega 3 (ω-3) series of PUFAs. DHA is present at high levels in brain tissue and human

retina and it plays a key role as a structural component of cell membranes. Arachidonic acid is obtained from the omega 6 (ω-6) series of PUFAs and works as a precursor of eicosanoids, synthesized from linoleic acid [33].

It is relevant to refer that the ratio of ω-6 and ω-3 essential fatty acids should be close to 4:5-5:1 and should not exceed 10:1, since a low ratio is associated with a low occurrence of cardiovascular diseases [34, 35]. However, nowadays Western diets end up being deficient in ω-3 and have excessive amounts of ω-6 given that they contain about 15:1 [35]. In seaweeds, the ratio of ω-6 is low, with ω-6/ω-3 ratio being about 0.8:1 in brown seaweeds and 1.1:1 in red seaweeds [19]. Generally, the fatty acids of red and brown seaweeds have 20 carbon atoms, such as eicosapentaenoic acid (20:5 ω-3) and arachidonic acid (20:4 ω-6). Brown seaweeds have quite high amounts of palmitic (16:0) and oleic (18:1 ω-9) acids, while green macroalgae have high palmitic and oleic acid and hexadecatetraenoic acid [36]. Besides fatty acids, the unsaponifiable fraction of seaweed contains carotenoids, such as β-carotene, lutein and violaxanthin in red and green algae and fucoxanthin in brown algae, as well as tocopherols and sterols [17]. Like other biochemical components, the fatty acid content varies with the season and other environmental factors. It is generally known that algae can accumulate polyunsaturated fatty acids (PUFAs) when there is a decrease in the environmental temperature. Aquatic species that live in colder water generally contain larger quantities of PUFAs, and the maximum content of lipids in the fond of *Saccharina*, *Laminaria* species and *A. esculenta* was generally found in winter. However, the total lipids of *Fucus sp*. were most abundant in summer, with highest levels recorded in August. A attached specimen of *Gracilaria* accumulated twice as much lipid compared to the unattached form, with no difference in fatty acid composition [17].

1.1.1.4 Minerals

The mineral content of seaweeds is very important and is likely to clarify many of their positive effects on health. Seaweeds are a good supplied of the 56 minerals and trace minerals necessaries for the body's physiological functions [14]. These have a high mineral content (8-40% dry weight) [31] This can be explained by the ability of seaweeds to retain inorganic substances by the characteristics polysaccharides on the cell surface [8, 9]. However, this content may differ depending on the phylum which they belong, the

environmental, geographic and seasonal conditions [9] and also depends on the type of seaweed processing [37] and on the mineralization methods used [8, 38].

Certainly, seaweeds contain 10 to 20 times the minerals of edible land plants and are plenty of vitamins and other elements essential to metabolism, as can be seen in Table 4 [11, 39]. It is important to highlight that seaweeds levels of magnesium (Mg), sodium (Na), potassium (K), zinc (Zn), iodine (I) and iron (Fe) are really high, in particular in some species. For example, as we can see in Table 4, *Fucus vesiculosus* can reach a content of Na like 5469 mg/100 g of dry weight, which is around 40 times the content of Na in spinaches (140 mg/100 g of dry weight). Concerning to K, a banana have thousand times less K than the same seaweed over mentioned. The Na/K ratio of the seaweeds is low (in spite of the high Na and K content), being an important detail in order to balance the Western diets that are rich in NaCl [8]. Also note that minerals play an important role in the proper performance of human metabolism. For example, the magnesium needs in calcium absorption; calcium in the activity of many enzymes; iodine related to thyroid; chromium in the regulation of blood sugar and iron, which is very important in the transport of oxygen, synthesis of deoxyribonucleic acid (DNA) and transport of electrons [40, 41, 42].

Table 4 - Mineral content of seaweeds compared to whole foods, expressed in mg/100 g of dry weight (Adapted from [11, 39]).

	Na	**K**	**P**	**Ca**	**Mg**	**Fe**	**Zn**	**Mn**	**Cu**	**I**
Fucus vesiculosus	2450 - 5469	2500 - 4322	315	725 - 938	670 - 994	4 - 11	3.71	5.50	< 0.5	14.5
Gracilaria spp.	5465	3417	-	402	565	3.65	4.35	-	-	-
Ulva rigida	1595	1561	210	524	2094	283	0.6	1.6	0.5	-
Spinach	140	500	-	170	54	2.1	0.7	-	0	2
Bananas	1	400	-	6	34	0.3	0.2	-	0.1	8
Peanuts	2	670	-	60	210	2.5	3.5	-	1	20

1.1.1.5 Vitamins

Seaweeds are an excellent source of vitamins and have a higher content of vitamins A, C, D, E, B1, B12 [1, 14], although the bioavailability of algal vitamin B12 in humans is

contradictory [19, 43]. The source of the vitamin B12 in seaweeds is proposed to be bacteria living on the surface or in the adjacent waters. Contrasting to many plants, some seaweeds such as *Porphyra* and *Ulva* have a high amount of vitamin B12, thus becoming a relevant algae for the vegetarians, since this vitamin is usually found in products of animal origin [1, 44]. Seaweeds provide one of very few plant sources of vitamin B12, a 100 g portion of *Ulva lactuca* can provide up to 35% of the recommended daily intake [45]. The vitamin B12 is particularly recommended to mitigate the effects of aging and anemia [1].

Seaweeds are also a good source of riboflavin, niacin, pantothenic acid, and folic acid. Brown seaweeds have higher levels of vitamin E when compared to the green and red ones, containing the α, β and γ tocopherol forms, whereas green and red seaweeds have only the presence of α-tocopherol [1]. As generally accepted, vitamin E is very important. α-tocopherol, the most biologically active form of vitamin E, is the second-most common form of vitamin E in the diet. This variant can be found most in abundance in wheat germ oil, sunflower, and safflower oils [46, 47]. As a fat-soluble antioxidant, it interrupts the propagation of reactive oxygen species that spread through biological membranes or through a fat when its lipid content undergoes oxidation by reacting with more-reactive lipid radicals to form more stable products [47, 48, 49]. Regular consumption of more than 1,000 mg (1,500 IU) of tocopherols per day [49] may be expected to cause hypervitaminosis E, with an associated risk of vitamin K deficiency and consequently of bleeding problems. The antioxidant capacity of vitamin E allows the inhibition of, for example, the oxidation of low-density lipoprotein, giving to this vitamin an important role in human nutrition [50].

Concerning to vitamin C, red seaweeds generally have lower levels than brown and green ones. This is an important vitamin in intestinal iron absorption and regeneration of vitamin E [45]. The daily intake of seaweeds as a source of vitamin C can strengthen the immune defense system, activate the intestinal absorption of Fe, trap free vitamin E inhibiting the oxidation of low-density lipoproteins, and is present in higher amounts in brown algae as compared to red and green algae. The gamma and alpha tocopherols increase the production of nitric oxide and nitric oxide synthetase activity and also play an important role in the prevention of cardiovascular diseases [1].

1.1.1.6 Phytochemicals

It is important to highlight that the habitat where most marine seaweeds live in offer extreme conditions, leading to the formation of free radicals. However, they have intrinsic chemical defense mechanisms, being able to produce secondary metabolites able to act against oxidative stress [51]. For example, in a complex mixture obtained from the brown seaweeds extract, was possible to find phytochemical compounds such as pigments and phenolic compounds [52]. Fucoxanthin is a xanthophyll, a characteristic carotenoid found in brown seaweeds that has an alenic linkage and two epoxide groups. This carotenoid is a major pigment present in the chloroplasts of brown seaweeds and it has been the focus of much attention, due to its claimed biological activities, which include anti-obesity and anti-diabetic effects, and others, including excellent antioxidant properties and the enhancement of docosahexaenoic acid synthesis in the mouse liver [53].

The levels of fucoxanthin in brown seaweeds vary according to the life cycle and the season, but as a rule, they present higher values in the winter than in the summer [54]. This content in brown seaweed is relatively high as compared to other carotenoids in natural products. Thus, brown seaweed will be good source of the functional allenic carotenoid, fucoxanthin [53]. Carotenoids constitute a significant share of low molecular metabolites in the algae genus *Sargassum* and fucoxanthin is the main component of carotenoids. In *Sargassum sp*. of the Sea of Japan amounts of fucoxanthin varied from 3.4 up to 5.9% of total lipids. In *S. pallidum*, it changed from March to December in the range of 6.0% - 9.3% with maximum in summer months and early autumn [54].

In red seaweeds, the main pigment found is R-phycoerythrin, a phycobiliprotein. Phycobiliproteins (PBPs) are antennae-protein pigments involved in light harvesting in cyanobacteria (blue-green algae, procaryotic), rhodophytes (red algae, eukaryotic), cryptomonads (biflagellate unicellular eukaryotic algae) and cyanelles (endosymbiotic plastid-like organelles). In red seaweeds, the phycobiliproteins are organized in phycobilisomes (PBSs) - supramolecular complexes - which are assembled in regular arrays on the outer surface of the thylakoid membranes and their levels are particularly high in winter [55] . Currently, these proteins are used as a natural protein dye in the food industry (C-phycocyanin) and in the cosmetic industry (C-phycocyanin and R-phycoerythrin) [56]. In addition, they have been associated with several biological

activities. In particular, phycoerythrin has been shown to exhibit antioxidant, anti-inflammatory and neuroprotective effects [57].

Phenolic compounds are chemically characterized by an aromatic ring with one or more substituent hydroxyl groups, ranging from monomeric, oligomeric or polymeric compounds. Phlorotannins constitute an extremely heterogeneous group of molecules (structure and polymerisation degree heterogeneity) providing a wide range of potential biological activity. Highest contents are found in brown seaweeds, where phlorotannin range from 5 to 15 % of the dried weight [58]. Among these, phlorotannins are polymers of phloroglucinol (1,3,5-trihydroxybenzene), are phenolic exclusive compounds from brown seaweeds, also not being produced by land plants. These constituents are found in vesicles, specifically in the fisoids of cells [59]. In this context, brown species such as *Laminaria japonica, Undaria pinnatifida, Ecklonia kurome* are considered as good sources of phlorotannins [60]. Some phlorotannin oligomers have been isolated, namely fucophlorethol A (a trimer), tetrafucol A (a tetramer), and trifucodiphlorethol A (a hexamer) from *Fucus vesiculosus* [61]. These compounds have a high antioxidant, anti-inflammatory and antibacterial capacity, proved by studies carried out by several authors [62, 63, 64]. Phenolic compounds and antioxidant properties of seaweeds are positively correlated. Algae polyphenols also called phlorotannins, are different from terrestrial plant polyphenols. Algae polyphenols are derived from phloroglucinol units (1,3,5-trihydroxybenzene), whereas in plants polyphenols are derived from gallic and ellagic acids. Brown seaweeds contain the highest phlorotannin concentrations, imparting them with a wide range of potential biological activity [1].

1.2 Seaweed-fortified products

Nowadays, society's lifestyle is characterized by too much stress and lack of time, leading to the consumption of processed food and to a reduce of physical activity. These kind of fast-food diets are reflected in unhealthy eating habits, which are closely related to the main civilization health problems, such as diabetes, obesity, reduced defenses and lack of nutrients [65]. Following this trend, the appearance of new products with seaweeds, occurs mostly in the Asian market. However, in the European Union there is a demand for

products containing seaweeds in every case, leading to an increase in the launch of new products on European markets. In Europe, between 2011 and 2013, France, Germany, Spain and the UK were the countries with the highest number of new seaweed-containing products. Seaweeds were used in different forms, either whole or in flakes, powders or extracts. The concern to demonstrate the bioactive potential of foods with the seaweed has been reflected in an increasing of scientific works in this research area.

Table 5 synthesizes some studies that used powdered seaweed and the main physical-chemical and sensorial effects in the food in which they were incorporated. A study by Jiménez-Colmenero et al. [66] intended to verify the effect of the incorporation of 3.3% of *Himanthalia elongata* seaweed powder on the physical-chemical characteristics (emulsion stability, loss of cooking, color, texture and microstructures) and sensorial characteristics of low-fat "frankfurters" prepared with the "konjac" gel as a substitute for pork fat and reduced salt content. This procedure showed that the incorporation of the seaweed/“konjac” gel together with the decrease of the added salt content resulted in an increase in cooking loss and reduction of emulsion stability in the gel/emulsion system. Comparing with the other samples, relative to color, the authors found that the incorporation of seaweed/”konjac” gel led to a decrease in brightness (*L**) and redness (*a**), and an increase in yellowness (*b**). Regarding to texture, the effects of seaweed addition on low salt sausages varied depending on the proportion of the “konjac” gel used in the formulation, as verified by the difference in the microstructure. Thus, the authors concluded that there is some interference in the properties of the meat matrix by incorporation of seaweed/gel.

Choi et al. [67], in a similar study, intended to observe the effect of seaweed on the physical-chemical and sensorial properties of "frankfurters" sausages with reduced salt content. To do this purpose, they used 1% of the powder of the seaweeds *Laminaria japonica*, *Undaria pinnatifida*, *Hizikia fusiforme* and the plant *Salicornia herbacea L.* independently in different tests, in order to understand the effects on the proximal composition, salinity, cooking loss, stability emulsion, pH, color, texture, apparent viscosity and sensory characteristics of reduced-salt (NaCl) frankfurters and meat batter. The results showed that the combination of low-salt and seaweed (powder of *L. japonica* and *U. pinnatifida*) in the formulation, provided a good improvement in the loss by cooking and the stability of the emulsion in the reduced-salt frankfurters, improving

sensory characteristics to levels similar to the regular salt control. The combination of seaweed and low salt content in sausages with reduced salt content showed better results with seaweeds *L. japonica* and *U. pinnatifida*.

Cox et al. [68] conducted a similar study to analyse the result of the addition of 10-40% of the *Himathalia elongata* seaweed as a source of dietary fiber and antioxidant on physical, chemical, microbial and sensory traits of cooked beef patties throughout chilled storage. The beef patties with seaweed showed reduced cooking losses and were about 50% more tender when compared to the control. Regarding microbiology and lipid oxidation, the values were lower in the beef patties with incorporated seaweed, relative to the control. The incorporation of the seaweed led to a significantly increase in the dietary fiber content, total phenolic content and the DPPH radical scavenging activity when compared to the control beef patties. This way, sensory analysis indicated that the seaweed patties were accepted by consumers in terms of aroma, appearance, texture and taste. In terms of acceptability, the addition of seaweed led to an improvement in texture and mouthfeel. Thus, the addition of seaweed in beef patties leads to the improvement of the nutritional quality with an acceptable sensory quality.

Senthil et al. [69] studied the influence of the powder on the quality of fish cutlet. *Eucheuma* powder was used at different levels, namely 5, 7.5, 10, 12.5 and 15% and physicochemical characteristics of fish cutlet were studied. It was concluded that the incorporation of the seaweed played a significant influence in the texture of the fish cutlet and that it is possible to incorporate the powder up to 10%, without affecting its acceptability, appearance and texture.

Chang et al. [70] aimed to understand how the introduction of the powder of the green seaweed *Monostroma nitidum* into the Chinese Fresh Egg Noodles formulation influenced the texture and quality properties. For this, green seaweed powder was incorporated in proportions of 4, 6 and 8%, with or without additional eggs. They found that the addition of seaweed powder increased the fiber content, consequently leading to an increase in water absorption during cooking by the fibers and the polysaccharides present in the seaweed. Higher water absorption by the seaweed led to softer and spongier textural intensities in the noodles. In conclusion, the results showed that additional seaweed powder can significantly affect the quality of fresh Chinese noodles either with or without the addition of eggs.

Chang et al. [71] carried a similar study with the purpose of understanding the changes caused by the addition of powdered seaweed and cuttlefish pasta in the texture of fresh egg noodles. The seaweed *Monostroma nitidum* was incorporated in proportions of 0, 3 and 6% in noodles and the liquid eggs replaced by cuttlefish pasta in 0, 1/3, 2/3 and full replacement. The highest cooking yields were obtained with the incorporation of 6% seaweed due to the absorption of water during cooking by the seaweed's fibers and polysaccharides in fresh noodles prepared with seaweed. As in the previously mentioned study, the high absorption of water by the seaweed resulted in a softer and spongier textural intensities in the noodles. The texture parameters were influenced by cuttlefish paste replacement and additional seaweed and by cooking properties, in which yields were higher with the incorporated seaweed.

Mamat et al. [72] aimed to analyse the effect of seaweed flour on the texture of bread and dough with the propose of improving dough handling properties, increasing the quality of fresh bread, and extending the shelf life of stored bread. Thus, the authors added the powder of the red seaweed *Kappaphycus alvarezii* in a ratio of 2-8% in the wheat flour used to produce the bread. The results of the study indicated that the addition of seaweed powder increased the water absorption of the dough, decreased stickiness properties, and showed higher values of firmness. The authors suggested that seaweed powder could be used to replace wheat flour (up to 8%), while maintaining the final quality characteristics of the product.

Prabhasankar et al. [73] intended to use the powder of the seaweed *Undaria pinnatifida*, which is a seaweed rich in fucozanthin, as an ingredient of a pasta and to evaluate how this incorporation influenced the chemical, functional and structural behavior. With the purpose of evaluating the antioxidant properties, total phenolic content, fatty acid composition, fucoxanthin and fucosterol contents, as well as its acceptance, pasta with different levels of the seaweed powder, between 10-30% were prepared. With this study, it was possible to conclude that the pasta with 10% seaweed was acceptable sensorially. Total phenolic content ranged from 0.10 to 0.94 mg gallic acid equivalents (GAE)/g, while total antioxidant activity ranged from 0.16 to 2.14 mg ascorbic acid equivalents (EPA)/g between different samples. The ratio of ω-3 and ω-6 fatty acids in the control was 1:15.2 and in the bulk with the incorporated seaweed it was 1:3.4. Moreover, in the sensorially accepted pasta, the activity of DPPH and superoxide radical scavenging

activities in the samples containing the seaweed powder was 7.71% and 4.56%, respectively. The heat processes involved in pasta preparation did not destroyed fucoxanthin. Therefore, the incorporation of seaweed in the pasta, besides improving the profile of amino acids and fatty acids, increased the nutritional value due to the presence of biofunctional components such as fucoxanthin and fucosterol. As far as microstructures is concerned, it was possible to observe that the pasta containing up to 20% seaweed allowed an increase in the interaction between the starch granules and the protein matrix and consequently increased the pasta quality, thus it was not sensorially acceptable. A similar study carried out by Prabhasankar et al. [74] aimed to develop pasta with Indian brown seaweed, *Sargassum marginatum*, as an ingredient to improve the quality, biofunctionality and microstructure of the pasta. Different levels of seaweed (1.0, 2.5 and 5.0%) were incorporated in the pasta, whereas, the pasta without seaweed was used as control. The free radical scavenging activity (DPPH) was higher in the cooked pasta with the incorporated seaweed than in the raw pasta. The cooking loss was lower in the samples with the seaweed incorporated up to 2.5%. These authors concluded that, incorporating seaweed up to 2.5% enhances gluten network of pasta and allowed to obtain a pasta with better quality and biofunctionality.

Table 5 - Effect of incorporation of seaweed powder on food properties.

Seaweed Type	Quantity Added	Effect	References
Himanthalia elongata	3.3%	Incorporation of the seaweed/"konjac" gel: increase in the loss by cooking and decrease the stability of the emulsion in the gel/emulsion system; interference in the properties of the meat matrix.	[66]
Laminaria japonica, Undaria pinnatifida, Hizikia fusiforme	1%	Addition of *L. japonica* or *U. pinnatifida*: improved emulsion stability and loss by cooking; the seaweed/low salt combination was more effective; sensory properties were more similar to the control.	[67]
Himathalia elongata	10 - 40%	Addition of the seaweed in beef patties reduced the loss in cooking; the meat became more tender and increased dietary fiber content, phenolic compounds and DPPH activity.	[68]
Eucheuma	5, 7.5, 10, 12.5 and 15%	Addition of seaweed influenced the texture of the fish cutlet; possibility of incorporating up to 10% without affecting texture and acceptability.	[69]
Monostroma nitidum	4, 6 and 8%	Noodles with a softer and fluffy texture; increase in fiber content.	[70]
Monostroma nitidum	3 and 6%	Noodles with 6% have higher cooking yields. High absorption of water by seaweed leads to the formation of noodles with a softer and spongy texture.	[71]
Kappaphycus alvarezii	2 - 8%	Addition of the seaweed in dough and bread, caused an increase in water absorption and showed higher values of firmness.	[72]
Undaria pinnatifida	10 - 30%	The pasta with 10% seaweed had a greater sensorial acceptance. Heat processes in the pasta preparation did not destroy fucoxanthin. The incorporation of seaweed improved the profile of amino acids, fatty acids and nutritional value.	[73]
Sargassum marginatum	1, 2.5 and 5%	DPPH capturing activity was higher in the cooked pasta with the incorporated seaweed than in the raw pasta. The incorporation of the seaweed up to 2.5% improved the quality of the pasta.	[74]

In addition to the incorporation of seaweed powder, there are several studies in the literature that describe the incorporation of seaweed extracts and their effects, presented in summary in Table 6. The study by Barbieri et al. [75] had the objective of reducing the NaCl in steamed ham by modifying the heating parameters and adding the water-soluble extract of the seaweed *Palmaria palmata* as flavoring. The changing allowed the authors to obtain a cooked ham containing a lower percentage of salt (1.2 and 1%) instead of 1.8% (that is the usual content), thus reducing sodium intake by 25% and 35%, respectively. After a 6-month refrigeration period, seaweed extract did not have an effect on

technological or sensory parameters. Nevertheless, a certain dryness due to cooking and a small decrease in yield (approximately 5%) and were observed.

A study by Moroney et al. [76] intended to understand the effect of adding the extract of the brown seaweed *Laminaria digitata* containing laminarin and fucoidan, on the quality and shelf life of fresh and cooked chopped pork patties. For this, the macroalgae extract containing 9.3% laminarin and 7.8% fucoidan at different levels (0.01, 0.1 and 0.5%) (m/m) was added directly to the minced pork. Fresh and cooked minced pork patties were stored for 14 days at 4ºC in modified atmosphere packs in modified atmosphere packages containing 80% O2:20% CO2 and 70% N2:30% CO2, respectively. This allowed the authors to confirm that the addition of the laminarin and fucoidan-rich extract did not lead to an increase in quality parameters in fresh minced pork patties. On the other hand, it was possible to observe that the addition of 0.5% extract revealed ability to decrease lipid oxidation in cooked pork patties, although its acceptance was lower than control. In the laminarin/fucoidan extract of 0.01%, no effect on color, lipid oxidation, texture or sensory acceptance was observed when incorporated into pork patties. A study by Ortiz et al. [77], aimed to evaluate the effect on lipid and sensory quality parameters of canned Atlantic salmon (*Salmo salar*) muscle, using different seaweed extracts as covering liquids. It was used aqueous extracts of seaweed *Durvillaea antarctica*, *Ulva lactuca* and *Pyropia columbina*. The authors found that all types of fish sample had an acceptable oxidized odor as well as characteristic flavor scores, after a 140 day storage period at 40ºC. The use of seaweed extracts as part of the covering liquid, allowed to reduce the secondary peroxidation in canned salmon when compared to the control sample (without the addition of the seaweed extract). Besides, low values of oxidized odors were observed. Concerning to the sensory parameters, these were not significantly different between salmon canned with different cover liquids, being always within acceptable limits.

O'Sullivan et al. [78] have studied the possibility of introducing seaweed extract as a functional ingredient in milk. To this purpose, aqueous extracts were prepared from 80% (v:v) ethanol of *Ascophyllum nodosum* and 60% (v:v) of *Fucus vesiculosus*. The introduction of ethanolic extracts of *A. nodosum* and *F. vesiculosus* in the ratio of 0.25% to 0.50% showed an increase in greenness and yellowness of the fortified milk samples. The 60% ethanol extract from *F. vesiculosus* revealed to be similar to the phloroglucinol (Phl) standard as far as the antioxidant capacity in the milk is concerned. Concerning to

microbiological parameters, no effects were verified neither by the type nor by the concentration of extract. Sensorial analysis, showed that the water-prepared extracts were the most acceptable as a functional ingredient in milk.

Table 6 - Effect of incorporation of seaweed extracts on food properties.

Seaweed Type	Quantity Added	Effect	References
Palmaria palmata	0.11%	Reduction of the salt content of the ham, adding the extract and modifying the heating parameters.	[75]
Laminaria digitata	Containing laminarin (9.3%) and fucoidan (7.8%) at different levels (0.01, 0.1 and 0.5%)	The laminarin/fucoidan extract did not improve the quality of the fresh pork patties. In the extract of 0.01% did not affect the color, texture, lipid oxidation or sensory acceptance. The 0.5% extract revealed the best ability to decrease the lipid oxidation of cooked pork patties.	[76]
Durvillaea antarctica, Ulva lactuca, Pyropia columbina	30 mL of the covering liquid prepared from 500g of each seaweed in 2L of distilled water	Extracts as part of the covering liquid, allowed to decrease the lipid peroxidation of the canned salmon. It presented low values of oxidized odors.	[77]
Ascophyllum nodosum* e *Fucus vesiculosus	0.25 and 0.50%	Extracts prepared with water were more acceptable than ethanolic when incorporated into milk.	[78]

Consumers are becoming more and more aware of the food they buy, giving an increasing importance to the nutritional values and the benefits that the consumption of specific food can give them, namely those of botanical origin which are identified as strong allies for a longevity and healthy aging [79]. In addition to the aforementioned studies, where several altered physical-chemical and/or nutritional parameters are identified in foods containing the seaweed/extracts, there are still several studies that analyse their potential for the valorization of food products in functional terms. In this context, in particular, we will mention some studies that assess the capacity of seaweed in disorders such as diabetes and obesity and also the seaweeds antioxidant capacity. α-glucosidase and α-amylase enzymes are an important therapeutic target given their involvement in the digestive process stage of complex dietary carbohydrates. α-amylase hydrolyzes α-1,4-glycosidic bonds of various oligosaccharides, whereas α-glucosidase, metabolizes simpler disaccharides for intestinal absorption. Thus, inhibition of these enzymes, in addition to being able to retard the digestion of oligosaccharides and disaccharides, may also delay the absorption of glucose by the small intestine and reduce plasma glucose levels [60]. Table 7

shows some summarized studies demonstrating the ability of seaweed components to inhibit these enzymes.

Nwosu et al. [80], aimed to study the phenolic rich extracts of four edible marine seaweed, namely *Palmaria palmata*, *Ascophyllum nodosum*, *Alaria esculenta* and *Ulva lactuca*, and their respective biological effects towards cultured colon cancer cells and ability to inhibit digestive enzymes, in order to obtain potentials anti-diabetic effects. Of the four seaweed selected, the authors excluded *Ulva lactuca*, due to the low recovery of phenolics. *Ascophyllum nodosum* showed the highest phenolic content (4547.8 μg/g dry weight), with an IC_{50} (extract concentration necessary to inhibit 50%) about 0.1 μg/mL GAE (gallic acid equivalents), in the enzyme α-amylase. It should be noticed that this value is lower than that of acarbose (approximately 0.8 μg/ml) in an α-glucosidase and α-amylase inhibitor. Also the *A. nodosum* extract was shown to be effective in inhibiting the α-glucosidase enzyme, with an IC_{50} value of about 20 μg/mL GAE. Thus, in conclusion, this paper describe potential biological activities of polyphenols from some seaweeds and, specifically, the authors concluded that the phlorotannin components present in A. nodosum appear to have potential anti-diabetic effects, inhibiting these two key enzymes of sugar metabolism, α-amylase and α-glucosidase. A similar study by Apostolidis et al. [81] aimed to identify a seaweed with high phenolic content, to determine optimal extraction conditions in terms of seaweeds/water and temperature for later use for type 2 diabetes management as an inhibitor of α-glucosidase and α-amylase enzymes. Among the studied seaweed, *Ascophyllum nodosum*, *Ceramium virgatum*, *Ulva lactuca* and *Saccharina latíssima*, *A. nodosum* was chosen because its highest phenolic content. By varying the temperature between 20ºC and 80ºC, it was concluded that the extraction at 80ºC was the one that resulted in a highest total phenolic content (4.2 mg/g wet weight). The extracts presented similar levels of antioxidant activity in the range of 60% to 70%, against the DPPH free radical scavenging radical. The extract at 80ºC revealed the highest inhibitory activity of α-glucosidase and α-amylase with an IC_{50} value of 0.24 and 1.34 μg phenolcs, respectively, compared to the reference inhibitor (IC_{50} of acarbose), being 0.37 and 0.68 μg. These results show that *A. nodosum* has a strong inhibitory activity on α-glucosidase and a mild α-amylase inhibitory activities, being correlated with phenolic content. This study reveals a nutraceutical potential of *A. nodosum* based on phytochemical antioxidant and antihyperglycemia activities.

Another study by Hwang et al. [82] aimed to study the inhibitory activity of α-amylase and α-glucosidase by the *Sargassum hemiphyllum* seaweed and the enhances of insulin release in vitro throught the presence of this seaweed. To do this, aqueous extract (WES), 95% ethanol (EES) and 70% acetone (AES) were prepared. The AES revealed a higher polyphenolic content (36.66±2.01 mg/g), followed by ESS (22.35±1.41 mg/g) and WES (17.35±0.93 mg/g). Fucoxanthin was only detected in EES extract (7.89±0.03 mg/g) and AES (15.12±0.09 mg/g). Applying these extracts as inhibitors of α-amylase and α-glucosidase (sucrase and maltase), the authors found that the IC_{50} values of AES extract for α-amylase, sucrase and maltase were 0.35±0.05, 1.89±0.03 and 0.09±0.01 mg/mL, respectively. The EES extract inhibited sucrase and maltase alone with an IC_{50} of 3.47±0.10 and 2.88±0.09 mg/ml and the WES extract had no inhibitory effect. The results suggested that extracts of *S. hemiphyllum* could be used as pharmaceuticals and functional foods to minimize dosages of synthetic diabetes drugs and also that the inhibitory activity of the enzymes is correlated with the concentration of polyphenols and fucoxanthin present in extracts of *S. hemiphyllum*.

Garcimartín et al. [83] took a different approach from previous studies as they sought to understand the effects of aqueous extracts supplementation on pork processed with 5g/100g of *Porphyra umbilicalis*, *Undaria pinnatifida* or *Himanthalia elongata* (used separately), in the activity of α-glucosidase and glucose diffusion were in vitro. These authors concluded that the low amounts of seaweed incorporated in processed pork matrix could mimic the properties found in seaweeds. The pork processed with the *H. elongata* aqueous extract was effective in inhibiting this enzyme (19.8%) as compared to the non-fortified product (control).

In addition to α-amylase and α-glucosidase enzymes, pancreatic lipase is also a therapeutic target in the treatment of obesity. In order to obtain inhibitors from natural sources, there are some studies that present seaweed as a promising source of anti-obesity agents. A study by Eom et al. [84] intended to study the phlorotannins isolated from *Eisenia bicyclis* in a methanolic extract as an inhibitor of pancreatic lipase. From the methanolic extract, they isolated and identified 6 florotanins: "eckol", "fucofuroeckol", "7-phloroeckol", "dioxindehydroeckol", "phlorofucofuroeckol A" and "dieckol". In conclusion, all the six phloroglucinol derivatives isolated from *E. bicyclis* showed pancreatic lipase showed inhibitory activity. Thus, among the isolated phloroglucinol

polymer, "fucofuroeckol" and "7-phloroeckol" were those that had potent inhibitory effects on pancreatic lipase with IC_{50} values in a range of 37.2±2.3 and 12.7±1.0 μM, respectively.

Another study by Wilcox et al. [85] aimed to study the modulation of pancreatic lipase activity by alginates. Thus, they found that the alginate obtained from the seaweed *Laminaria hyperborea*, with a high content of glucuronic acid, showed a greater capacity to inhibit pancreatic lipase than the alginate obtained from *Lessonia nigrescens*, with high content of manuronic acid. The study thus revealed that the inhibitory activity may vary depending on the source and chemical form of the compound.

Table 7 - *In vitro* effect of different seaweeds on enzymes associated with diabetes and obesity.

Disease	Seaweed	Effect	References
Diabetes	*Palmaria palmata*, *Ascophyllum nodosum*, *Alaria esculenta* e *Ulva lactuca*	Extract from *A. nodosum*: Higher phenolic content (4547.8 μg/g dry weight); IC_{50} value of 0.1 and 20 μg/mL (GAE) in the α-amylase and α-glucosidase enzymes, respectively.	[80]
	Ascophyllum nodosum, *Ceramium virgatum*, *Ulva lactuca* e *Saccharina latíssima*	Extract of *A. nodosum*: extract at 80 ºC gave a phenolic content of 4.2 mg/g wet weight; showed higher α-glucosidase and α-amylase inhibitory activity, with an IC_{50} value of 0.24 and 1.34 μg phenolics, respectively.	[81]
	Sargassum hemiphyllum	Acetone extract of *S. hemiphyllum*: higher polyphenolic content (36.66±2.01 mg/g); IC_{50} values of α-amylase, sucrase and maltase: 0.35±0.05, 1.89±0.03 and 0.09±0.01 mg/mL, respectively.	[82]
	Porphyra umbilicalis, *Undaria pinnatifida* e *Himanthalia elongata*	Low amount of extract incorporated in the meat, imitated the properties found only in seaweed. Meat with the aqueous extract of *H. elongata* inhibited α-glucosidase by about 19.8%.	[83]
Obesity	*Eisenia bicyclis*	"Fucofuroeckol" and "7-phloroeckol" had the highest inhibitory effect of lipase, with IC_{50} values in a range of 37.2±2.3 and 12.7±1.0 mM, respectively.	[84]
	Laminaria hyperborea e Lessonia nigrescens	Higher ability to inhibit lipase with the high glucuronic acid-containing alginate obtained from *Laminaria hyperborea* than the high mannuronic acid alginate obtained from *Lessonia nigrescens*.	[85]

Regarding to the seaweeds antioxidant activity, the most interesting phytochemical seaweeds compounds are those with antioxidant activity, such as carotenoids, pigments and phenolic compounds [86]. In plant foods, phytochemicals may be linked to cell wall molecules, inserted into organelles or free into the cytoplasm. Processing techniques that degrade cell structure cause compounds bound to the cell wall to become lost in the plant matrix, and this is beneficial when the extraction is of interest [86, 87, 88].

Jiménez et al. [89] determined the antioxidant activity of fresh and processed edible seaweeds, throught aqueous/organic extracts of them using three different methods: free radical (DPPH·) scavenging, ferric-reducing antioxidant power (FRAP) and inhibition of

copper-catalysed *in vitro* human low-density lipoprotein (LDL) oxidation. Scavenging activity correlated well (r =0.73) with the corresponding total polyphenolic content measured by the Folin–Ciocalteu procedure and expressed as phloroglucinol equivalents (PGE). *Fucus* seaweed showed the highest antioxidant activity in two of the test methods used (1g dry matter had a DPPH· activity and a FRAP value equivalent to those 0.18 and 0.07 mmol of Trolox, respectively) and the highest total polyphenolic content (41.4 g PGE/kg dry matter). Regarding to the antioxidant activity and to the total phenolic compounds content, they decreased with processing and storage in the seaweeds tested. Furthermore, *Fucus* showed good efficiency in the in vitro inhibition of LDL oxidation. The autors concluded that commercial seaweeds showed lower antioxidant capacity than fresh seaweeds, suggesting that processing and storage could decrease this antioxidant capacity.

Another study by Kelman et al. [90], aimed to study the antioxidant activity of Hawaiiane marine algae. In this respect, relatively little is known about the bioactivity of Hawaiian algae that could be a potential natural source of such antioxidants. It was determined the total antioxidant activity of organic extracts of 37 algae samples, comprising of 30 species of Hawaiian algae from 27 different genera by employing the FRAP assays. The extract of *Turbinaria ornata* was found to be the most active and his bioassay-guided fractionation led to the isolation of a variety of different carotenoids as the active principles. Carotenoid fucoxanthin was the major bioactive antioxidant compoundidentified. These results showedthat numerous Hawaiian algae exhibit important antioxidant activity, a property that could lead to their application in one of many useful healthcare or related products as well as in chemoprevention of a variety of diseases including cancer.

Another study was performed about hydrophilic and lipophilic antioxidant capacities of commercial Mediterranean vegetable soups (Gazpachos) by Pinilla et al. [91]. Antioxidant activities of commercial gazpachos, processed by different technologies (traditional pasteurized, slight pasteurized, and frozen), were assessed. Both lipophilic and hydrophilic DPPH• radical scavenging capacities (L-RSC and H-RSC, respectively) were determined in terms of EC_{50} and antiradical efficiency (AE). AE parameter takes into account not only antioxidant concentration but also antioxidant kinetic. The results

obtained in this work clarify the role,in terms of concentration and kinetic, of the hydrophilic and lipophilic compounds of the commercial Mediterranean vegetable soups in an *in vitro* RSC model. This study provides evidence of the significant role of the 3 classes of antioxidants, vitamin C, carotenoids, and polyphenols, in antioxidant capacity. The autors concluded that the commercial gazpachos analyzed in this study have potential antioxidant properties, with independence of the technology employed to manufacture them.

1.3 Study Objects

1.3.1 Seaweeds in focus: *Fucus vesiculosus* and *Ulva rigida*

Brown seaweeds are the most affected in terms of development and morphology, due to environmental conditions, presenting different flora characteristics in different geographic regions. The brown seaweed *Fucus* contain higher levels of vitamin E than green and red seaweed [1]. *Fucus vesiculosus* (Figure 1) is commonly found in temperate waters. Members of the order *Fucales* generally contain air bladders to freely float on the water surface and are examples of a diplontic life cycle where only sperm and egg represent the haploid condition. *Fucus* has an average height between 30 and 50 cm [1]. Structurally it has a thallus which is much branched and supported by a short narrow stalk that is attached to a discoid holdfast. The branching is dichotomous, with each flattened segment having a prominent central midrib surrounded on both sides by a narrower wing. The wings usually bear scattered cryptoblasts, which are basically sterile conceptacles with large numbers of hairs, that facilitate the uptake of nutrients from the seawater [2]. This seaweed contains some compounds with nutraceutical value and potential health benefits, namely omega 3 and 6 and fatty acids for the prevention of cardiovascular diseases and diabetes. Fucoxanthin is also present with an anti-inflammatory and anti-obesity activity as well phlorotannins with an antioxidant activity and fucoidan with a hypolipidemic effect [11].

Figure 1 - *Fucus vesiculosus* [92][92]**.**

Ulva rigida is an edible green seaweed (Figure 2) that belongs to the phylum *Chlorophyta*. By the structure similar to a garden lettuce, the *Ulva rigida*, is also known as sea lettuce. It consists of a light green stem with two cell membranes, flat and membranous, of variable shape and size. This seaweed is commonly used for confectionery of soups and salads [93]. It normally grows on rocks in the middle to low intertidal zone, although the fronds are not situated at the same level throughout the year. During the colder months the plants grow mainly in the middle intertidal zone, covering wide vertical areas. In the warmer months the *Ulva* is lower in the intertidal zone and in a narrower band. Here the fronds are less exposed and subjected to less desiccation, which is more damaging to the plants in the high summer temperatures [2, 4].

Figure 2 - *Ulva rigida* [93][4].

The sea lettuces comprise the genus *Ulva*, which are widely distributed along the coasts of the world's oceans. There are more than 125 species of *Ulva* currently accepted

taxonomically worldwide. Marked seasonal variations can be seen in the morphology of *Ulva*, for example, young plants are dark green in color and soft to the touch, whereas older thalli become light green and their surface becomes slimy. The thalli are distromatic (composed of two layers of cells) in which each cell contains a cup-shaped chloroplast. Cell walls contains an economically important polysaccharides – ulvans, which has a variety of industrial applications including disease control [94, 95]. Fast growth of *Ulva* makes it opportunistic seaweed, which can germinate immediately and form populations in favorable conditions. This occurs primarily because of a rapid growth rate and the ability to take up and store nutrients available in pulsed supply [2]. The biochemical composition of *Ulva rigida* can also vary throughout the year and depending on the location. But, in general, this alga contains high amounts of protein, carbohydrates and minerals (Table 8).

Table 8 – Chemical composition of *Ulva rigida* [96, 97, 98]

Compounds	Medium Values
Carbohydrates [a]	426.0
Protein [a]	6.1
Lipids [a]	9.0
Reducing Sugars [a]	106.0
Chlorophyll a [b]	55.8
Chlorophyll b [b]	26.1
Carotenoids [b]	17.5
Mineral [a]	286
Dietary Fiber [a]	119.0

a) Expressed in g/kg; b) Expressed in mg/m^2

Regarding to the ulvans, they are mostly composed of rhamnose, xyloses, iduronic acid and glucuronic acid, sulphated or partially sulfated in rhamnose and xylose residues. Together with cellulose and, in smaller fractions, xyloglucans and glucuranes, ulvans constitute a large part of the cell walls of green algae belonging to the order Ulvales, namely of the genus *Ulva* and *Enteromorpha* [94]. The interest in the medicinal and pharmaceutical field of green algae (including *Ulva rigida*) is essentially due to the bioactive activity of ulvans, since these polysaccharides are generally accepted as having anti-tumor, immuno-modulating, antihyperlipidemic, anticoagulant activity , antioxidant, among others [99, 100]. Specific examples of ulvans bioactivity have been demonstrated in the polysaccharide extracted from *Ulva lactuca*, which revealed anti-tumor activity in

different human cancer cell lines [101]. Already Mezghani et al. (2013) found promising antioxidant activity of *Ulva rigida* extracted from a cellular model of oxidative stress [102].

1.3.2 Seaweed soups for the elderly

In modern western societies, older people represent the fastest growing age group. In Europe, the proportion of the population aged 65 years and over is projected to increase from 13.7% in 1990 to 22.4% by 2025 [103]. This demographic trend creates a major public health concern, because with increasing age there is a higher risk of the development of age-related pathologies [104, 105]. There is increasing evidence that good nutrition is an important lifestyle factor essential to the health, independence and elderly's quality of life and one of the major determinants of successful aging [106, 107]. Impaired nutritional status is a frequent problem in the elderly. Individuals in long-term care facilities in particular are prone to deficits in nutritional status. Prevalence rates of protein-energy malnutrition are high and range from 30% to 60%. Causes of reduced food intake are various: disease, physical impairment, age-related physiologic changes, and psychological and psychosocial issues. Alone or in combination they can result in a reduced nutritional status, which is associated with an impaired functional status and higher morbidity and mortality [108]. The functional consequences of malnutrition very often lead to an increasing isolation and a greater dependency that ultimately impair quality of life [109]. A sufficient food supply according to the needs of this special population is therefore highly important [108]. So, the development of a fortified seaweed soup could contribute to overcome elderly nutrition problems.

Nowadays, companies are betting more and more on product innovation through the use of knowledge, as a strategic tool in order to develop new products and new formulations. Interest in innovative traditional food products seems to be related to the possibility of obtaining healthier products, even though with loss of original taste associated risk [110].

There are just a few reports focusing seaweed soups, however some can be found about fortified soups in general. The nutritional evaluation of a healthy vegetable soup powder supplemented with soy flour, mushroom, and moringa leaf has been described by

T. Farzana et al. In this study, the authors concluded that the developed fortified soup powder is nutritionally superior to locally available soup powders and sufficient to meet day-to-day nutritional requirements as a supplement [111].

2. OBJECTIVE

Nowadays, with an aging population and an increasing number of diseases such as diabetes and obesity, it is important to ensure that there is a consumption of healthy foods with a high nutritional value. Thus, with the current state of the art description, it is attractive to develop a work in which it is intended to use seaweed as a functional ingredient in the formulation of a food product.

The objective of this work is to incorporate the seaweed *Fucus vesiculosus* and/or *Ulva lactuca* in a nutritionally enhanced soup and realize how this incorporation could be beneficial in nutritional and/or functional terms for the consumer. This soup should have enough nutritional information to replace a meal, particularly with the elderly, and should also be organoleptically similar to a Portuguese traditional soup. It is now known that seaweed contains numerous bioactive substances shown to lower cholesterol, reduce blood pressure, promote healthy digestion and tackle free radicals. Add to this the fact that it is virtually fat and calorie free and it is easy to see why seaweed is building a reputation as the new “superfood”. In conclusion, seaweeds bring all the ideal and desired benefits to incorporate into a soup of this nature and to combat existing weaknesses in the health of the elderly today.

3. MATERIALS AND METHODS

3.1 Reagents

Water (H_2O); Acetone (C_3H_6O); Etanol (C_2H_5OH); Sodium hydroxide (NaOH); Hydrochloric acid (HCl); Metanol (CH_3OH); Acetic acid (CH_3COOH); BHT - Butylated

hydroxytoluene ($C_{15}H_{24}O$); Folin-Ciocalteu reagent; Sodium carbonate (Na_2CO_3); Gallic acid ($C_7H_6O_5$); ABTS - 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ($C_{18}H_{18}N_4O_6S_4$); Ascorbic acid ($C_6H_8O_6$); Megazyme's total dietary fiber assay kit – amyloglucosidase enzyme, protease, α-amilase; MES buffer - 2-(N-morpholino)ethanesulfonic acid ($C_6H_{13}NO_4S$); TRIS buffer - 2-Amino-2-hydroxymethyl-propane-1,3-diol ($C_4H_{11}NO_3$); Luff-Schoorl reagent; Carrez I solution; Carrez II solution; Potassium iodine (KI); Sulfuric acid (H_2SO_4); Sodium thiosulfate ($Na_2S_2O_3$); Nitric acid (HNO_3); Milli-Q water.

3.2 Production and development of Seaweed soups

The production and development of this new product was carried out at the company *Centralrest, Lda*, headquartered at Gafanha de Encarnação, in Aveiro. As stated above, the aim of the practical work was to achieve a sufficiently nutrient rich soup base to constitute a meal for the elderly population while being tasty at the same time. It is important that the final product has a traditional taste and appearance, taking into account the target audience.

Two different soups were produced, SFFS and UFS. Both of the samples (SFFS and UFS) are supposed to be marketed in a package of 300g to support a full nutritional rich meal for the elderly. The soups produced were pasteurized and homogenized with a hand blender. They were stored in the refrigerator at -20ºC until the physicochemical analyzes were performed.

For food formulation, the Stop & Learn system will be adopted which consists in a series of iterative trials, evaluations and appreciations using different algae/extract concentrations until achieving a final validated formulation. This validation will be achieved by assessing the possible organoleptic modifications of the successive test formulations through a sensory analysis consisting of triangular and/or preference tests (conventional versus innovative formula) using a panel of untrained members.

3.3 Physicochemical analysis of Seaweed soups

3.3.1 Colour determination

The colour analysis was performed using the CIELab color space at 25°C, by determination of the parameters *a** (red/green colour), *b** (yellow/blue colour) and *L** (luminosity). A Konica Minolta CM 2300d spectrophotometer (Minolta Konica, Tokyo, Japan) was used and the CIELab parameters were determined using the original SpectraMagic™ NX Software, Konica Minolta, USA, according to regulations of the International Commission on Illumination. The standard illuminant D65, a standard observer 10º and an aperture of 8 mm were used. The total colour difference (*ΔE**) was calculated by Equation 1 [113]:

$$\Delta E^* = [(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2]^{1/2} \qquad (1)$$

3.3.2 Determination of Titratable acidity and pH

The determination of the titratable acidity was carried out according to the method described in the Association of Official Agricultural Chemicals (AOAC) (1998). For this, 5 g of each sample were homogenized in 45 mL of boiled distilled water using an Ultra-Turrax at 12.000 rpm for 1 minute. The mixture was then centrifuged at 6000 rpm for 10 minutes at 20°C and the supernatant was filtered through G4 porous plate funnel. The pH reading was performed on the obtained filtrate. After the pH was read, the filtrate was titrated with 0.1M NaOH standard solution in the presence of a few drops of phenolphthalein (1%) as indicator. The titratable acidity results were expressed as a percentage of lactic acidity or mg of lactic acid/100g of soup, calculated by the formula represented in Equation 2:

$$\text{Lactic acidity (\%)} = \frac{\text{mL titrated base x base normality x acidity eq}}{\text{sample volume (mL)}} \text{ x } 100 \qquad (2)$$

3.3.3 Nutritional analysis

3.3.3.1 Moisture content

To determine the moisture content, the previously dried crucibles were weighed in the oven (2 hours at 105°C). Thereafter, about 2 g of sample was placed in each of the crucibles and brought to the oven at a temperature of 105°C overnight (about 10-12 hours). After cooling the desiccator crucibles, the respective weighing was carried out, allowing calculating the percentage (%) relative humidity of the sample by Equation 4:

$$\text{Relative Moisture (\%)} = \frac{\text{water mass (g)}}{\text{sample mass (g)}} \text{ x } 100 \qquad (4)$$

Where, water mass (g) = initial sample mass (g) – dry mass (g).

3.3.3.2 Protein content

The protein content was determined by elemental analysis of % nitrogen (N), through thermal conductivity using a *Truspec* 630-200-200 analyzer. The protein content was calculated using the 6.25 conversion factor by the Equation 5:

$$\text{Protein (\%)} = \frac{\frac{\text{\%N}}{\text{sample mass (g)}} \text{ x } 6.25}{\text{sample mass (g)}} \text{ x } 100 \qquad (5)$$

3.3.3.3 Dietary fiber content

Megazyme's "total dietary fiber assay kit" was used to determine the soluble and insoluble fiber content. 1 g of each extract was weighed into an erlenmeyer flask and then 40 ml of MES-TRIS buffer solution (pH 8.2), 50 µl of α-amylase enzyme and a magnetic stirrer were added. The erlenmeyer was placed in a boiling water bath for a period of 30 minutes (counted from the time the water began to boil). The erlenmeyers were then removed from the bath and cooled to a temperature of 60°C. Then, 10 mL of distilled water was added and after stabilization of the temperature at 60°C, 100 µL of the protease enzyme was added, allowed to incubate for a period of 30 minutes. After that, 5 mL of

0.56N HCl, 6 drops of 5% HCl and 200 μL of the amyloglucosidase enzyme were added. The enzyme was incubated for a period of 30 minutes (counted from the time the bath temperature stabilized at 60°C). For the determination of insoluble and soluble dietary fiber, the crucibles with about 1 g of celite previously dried in the oven overnight at 105°C and tared were used. For the determination of insoluble dietary fiber, a vacuum filtration was performed with the crucible containing the celite, coupled to a kitasate. About 5 mL of distilled water was added in order to redistribute the celite through the crucible. After this procedure, the sample was filtered, the crucible residue being washed twice with 10 mL of distilled water preheated to 70ºC. The crucibles containing the residue were then washed twice with 10 mL of 95% ethanol and 10 mL of acetone. For soluble dietary fiber determination, the recovered liquid (filtered and water washes) was placed in a new erlenmeyer where 95% ethanol was added at a temperature of 60ºC in a ratio of four times the volume of the recovered liquid, then allowed to precipitate at room temperature for one hour. After this, a vacuum filtration was performed with the crucible containing the celite, coupled to a kitasate. About 15 mL of 78% ethanol was added in order to redistribute the celite through the crucible. The sample was filtered and the crucibles with the residue were washed twice with about 15 mL with 78% ethanol, 95% ethanol and acetone. All crucibles containing soluble and insoluble fiber were placed overnight in the oven at a temperature of 105°C. The next day, they were removed to a desiccator and later weighed. After weighing, the contents of the crucibles (sample + celite) were homogenized in a ceramic mortar so as to remove about 2 mg for protein analysis. The remaining sample was placed back into the crucible, and then placed in the muffle at 525°C for 5 hours. After this period, the crucibles were removed to the desiccator and weighed. The percentage of soluble and insoluble dietary fiber was calculated by the formulas represented in Equations 6 and 7:

$$\text{Dietary Soluble Fiber (\%)} = \frac{\text{MS}-\text{PS}-\text{CS}}{\text{MA}} \text{ x } 100 \qquad (6)$$

$$\text{Dietary Insoluble Fiber (\%)} = \frac{\text{MI}-\text{PI}-\text{CI}}{\text{MA}} \text{ x } 100 \qquad (7)$$

Where, M_A represents the sample mass, M_S represents the mass of the soluble residue, P_S is the soluble fiber protein mass, and C_S is the soluble ash mass, M_I represents the insoluble residue mass, P_I is the insoluble fiber protein mass, and C_I mass of insoluble ash.

3.3.3.4 Total and Reducing Sugars

For the determination of the reducing and total sugars, the standard NP-1420 of 1987 for fruits, vegetables and their derivatives was followed. In this standard the Luff-Schoorl technique is used. This method applies to food products containing low molecular weight sugars. Reducing sugars are oxidized, while Cu^{2+} (from Luff-Schoorl reagent) is reduced to Cu^{+}. The excess amount of Luff-Schoorl reagent, which does not react with the sugars, reacts with the KI, oxidizing the ion I^{-} to I_2. The I_2 will be titrated by sodium thiosulfate. Thus, the higher the amount of reducing sugars, the lower the Luff-Schoorl reagent, the lower the amount of iodine formed, and the lower the titrant volume spent. A modification was made to the experimental procedure of said standard, and the boiling time was changed from 8 to 30 minutes in order to allow the complete extraction of the sugars.

At first about 5 g (wet basis) of sample was accurately weighed and transferred into a 200 ml dilution flask, washed with 50 ml of distilled water. At this stage designated by defecation, 12.5 mL of Carrez I solution plus 12.5 mL of Carrez II solution were added to this flask, the volume of the flask was filled distilled water. The mixture was stirred again by filtration as soon as a white precipitate was formed. One part of the filtrate was subjected to inversion to determine the total sugars and another was used directly for the determination of the reducing sugars. The inversion step was performed only for determination of the total sugar content. In a 100 mL erlenmeyer, 50 mL of the filtrate obtained after defecation was added, and 3.5 mL of hydrochloric acid (1.19 g/cm^3) stirred and placed in a water bath at 69°C for 5 minutes (after the liquid contained therein had reached that temperature). It was immediately cooled and neutralized with a solution of sodium hydroxide in the presence of phenolphthalein until the change to a pink colour. A few drops of dilute hydrochloric acid were added until the medium was slightly acidified. The pH of the medium was monitored with the aid of the potentiometer, stirred and transferred to a 100 mL dilution flask and the flask volume was made up with distilled water.

To determine the reducing sugars, 25 mL of the LuffSchoorl solution was measured and 10 mL of the defecated solution was added to a 250 mL round bottom flask, distilled

water was added to make a total volume of 50 mL, and boiling regulators were used. The flask was fitted to a reflux condenser and, with the aid of a heating mantle, boiled after two minutes and boiled for 30 minutes. After the boiling time cooled immediately without stirring over a stream of cold water. After 2 minutes, 9 mL of potassium iodide solution (0.166 g/cm^3) was added, 20 mL of sulfuric acid solution (0.25 g/cm^3) was stirred until the effervescence ceased. The iodine formed was titrated with the sodium thiosulphate solution (0.1N), adding 2 mL of starch cooking (after showing a mustard coloring, which occurred after adding approximately 8 mL of sodium thiosulphate), to the color change from blue to white.

By the difference of volumes of sodium thiosulphate solution used in the blank test and in the sample test, the weight of the invert sugar expressed in milligrams may be determined from the table in the Appendix A. For the determination of the total sugars, as described above for the determination of the reducing sugars, only the volume of the defected solution was replaced by a volume of the solution obtained by the inversion. The calculation of the percentage of sugars was determined according to the Equations 8 and 9 presented in the standard used for the determination of sugars and two independent tests were carried out, each in duplicate.

$$\text{Reducing Sugars (\%)} = \frac{20 \text{ x mr}}{V' \text{x m}} \qquad (8)$$

$$\text{Reducing Sugars (\%)} = \frac{20 \text{ x mt}}{V' \text{x m}} \qquad (9)$$

Where m_r and m_t represent the mass (mg) of invert sugar, for the reducing and total sugars respectively, and V' the filtrate volume after defecation and inversion.

3.3.3.5 Ashes content

For ash determination, the sample previously used for moisture determination was taken to the muffle (*Select-Horn, JP Selecta*) at a temperature of 550°C for 6 hours. After cooling the crucibles in the desiccator, the mass of the obtained residue was registered and the percentage of ash in the sample was calculated according to Equation 10:

$$\text{Ashes Content (\%)} = \frac{\text{ashes mass (g)}}{\text{dry mass (g)}} \text{ x } 100 \quad (10)$$

3.3.3.6 Elemental composition: Minerals

The acid digestion was based on microwave-assisted digestion proposed by *Speedwave MW-3+* (Berghof, Germany) for dried plant samples with some modifications for determination of Na, K, Ca, Mg, Fe, Mn, Cu and Zn in samples of seaweed soup. A sample with up to 0.4 g of each soup was placed in the digestion vessel and 4 mL of concentrated nitric acid were added. The vessels were capped and placed in a microwave pressure digestor *Speedwave MWS-3+* (Berghof, Germany) and subjected to microwave radiation at 20 bar according to the following program: room temperature was raised first to 130°C at 22°C/min and 30% of irradiation power, then to 160°C at 6°C/min and 40% of irradiation power, for 5 min, and to 170 °C at 5 °C/min and 50% of irradiation power, for 5 min. The cooling process consisted of decreasing temperature first to 100°C for 4 min and then to room temperature. After cooling, acid digests were made up to 20 mL with Milli-Q water. Three replicates were performed for each sample as well as blanks. The content of each element is expressed as the mean plus standard deviation [114, 115].

Mineral content determination of Na, K, Ca, Mg, Fe, Mn, Cu and Zn was performed using an were quantified in a Perkin Elmer (Waltham, MA, USA) Analyst 100 flame atomic absorption spectrometer equipped with single hollow cathode lamps for each element and an air-acetylene burner [116].

For salt content determination the amount of salt was determined according to the Equation 11:

$$\text{Salt Content (g)} = \frac{[\text{Na}](\text{mg}/100\text{g})}{1000} \text{ x } 2.5 \qquad (11)$$

3.3.4 Phytochemical analysis

3.3.4.1 Obtaining of extracts

Two different types of extracts were prepared from different extractions: the first one for the quantification of phenolic compounds and antioxidant activity and the second one for the quantification of pigments and carotenoids.

The quantification of phenolic compounds and antioxidant activity was performed using a methanolic extract. The extract was made by adding 20 mL of MeOH with 1% acetic acid to 2 g of soup in a beaker for 1 hour and 30 minutes under stirring at 200 rpm. After this time the contents were placed for 1 hour in the freezer and then centrifuged at 6000 rpm at a temperature of 5ºC for 20 minutes. Subsequently, vacuum filtration was performed with a G4 porous plate funnel. The recovered filtrate was transferred to a flask and the extracts were then frozen and stored at 4°C until use.

For the quantification of pigments and carotenoids, the soup samples were subjected to an extraction procedure using 80% acetone in the presence of 0.1% butylated hydroxytoluene (BHT) for 14 hours at a ratio of 1:20 (mass:volume) at room temperature. It should be noted that acetone is a favorable solvent for extracting polar pigments of lipid character, such as chlorophylls and carotenoids, maintaining their stability [117, 118]. After extraction, all samples were centrifuged at 6000 rpm for 20 minutes at 5°C and filtered through a G4 porous plate funnel. After this filtration, the samples were again subjected to extraction, with solvent renovation for 4h. After this second extraction, all samples were again centrifuged at 6000 rpm for 20 minutes at 5°C and filtered through a G4 porous plate funnel. Removal of the non-aqueous solvent and/or concentration on the rotary evaporator at 30°C followed. The sample was then resuspended in 3 mL acetone with 0.1% BHT. The resulting extracts were filtered through 0.45 μm porosity nylon filter (Whatman^{TM}). The extracts were then frozen and stored at 4°C until use.

3.3.4.2 Evaluation of Phenolic compounds content and Antioxidant activity

The quantification of the phenolic compounds was carried out using the Folin-Ciocalteu method, while the antioxidant activity was determined by the $ABTS^{\bullet+}$ radical cation method.

Concerning the Folin-Ciocalteu method it was followed according to Singleton et al., with some adaptations [119]. In the 96-well microplate, 60 µL of distilled water, 15 µL of Folin-Ciocalteu reagent and 15 µL of sample or standard were added. After 5 minutes, 150 µl of 7% Na_2CO_3 solution (m/v) was added. The microplate was incubated in the oven at 30ºC for 60 minutes. After this time, the absorbance was read at a wavelength of 750 nm in a microplate reader (Biotek). Gallic acid was used as the standard for the calibration curve, over a concentration range of 0.0088-0.1388 mg/mL. The total amount of polyphenols was expressed in grams mGAE/mL of extract.

Regarding the antioxidant activity, it was determined by the $ABTS^{\bullet+}$ radical cation method. The $ABTS^{\bullet+}$ cation was generated by the ABTS reaction (7 mM) in a solution of $K_2O_8S_2$ (2.45 mM) maintained in the dark for about 12 hours. The $ABTS^{\bullet+}$ solution was then diluted in water (1:80 mL) and the absorbance at 734 nm adjusted between 0.750-0.800. The extract extracts (dissolved in MeOH with 1% acetic acid) were prepared at a concentration range of 28-38 mg/mL. Subsequently, various amounts of the extract solution (50-100 µL) were added in eppendorfs, the volume of 500 µL was filled with MeOH with 1% acetic acid. In a 96-well microplate, 50 µL of each of the dilutions of the standard extracts and 250 µL of $ABTS^{\bullet+}$ solution was placed. The microplate was left for 20 minutes in the dark at room temperature, and then read on the microplate reader (Biotek) at 734 nm. Ascorbic acid was used as the standard for the calibration curve over a concentration range of 0.00384 and 0.0512 mg/mL. Control was performed in the presence of all reagents and in the absence of the extract. The percent inhibition was determined for each concentration by the formula represented in Equation 12 [120, 121]:

$$\% \text{ Inhibition} = \frac{\text{AbsC} - \text{AbsE}}{\text{AbsC}} \text{ x } 100 \quad (12)$$

Abs_C: Absorbance of control

Abs_E: Absorbance of the extract after 30 minutes in the dark

3.3.4.3 Identification of Pigments and Carotenoids

The identification of pigments and carotenes and/or xanthophylls was made based on literature review and was performed by Ultra-High Performance Liquid Chromatography with a Diode Detector coupled to Electrospray Ionization Mass Spectrometry (UHPLC-DAD-ESI-MSn). For this purpose an Ultimate 3000 (Thermo SCIENTIFIC) equipped with a photodiode detector (3000 RS-DAD) was used, coupled to a Linear Ion Trap 2D XLT mass spectrometer.

The chromatographic system consisted of a quaternary pump, an automatic sampler, a photodiode detector and a thermostatic column compartment. The analysis was performed with a Hypersil GOLD column (100 mm long, 2.1 mm I.D. and 1.9 mm particle diameter) maintained at 30ºC, the injection volume being 2 mL. The mobile phase for the separation of seaweed extracts was 0.1% formic acid (v/v) (A) and acetronitrile: methanol (70/30) (B). The solvent gradient started with 85% B, remaining in isocratic mode for 3.9 minutes, then increasing to 100% B in 2.2 minutes and maintaining these conditions for up to 25 minutes, followed by the restoration of the Initial conditions. Prior to UHPLC analysis, each extract or fraction (5 mg) was filtered through a 0.2 mm pore nylon filter (Ge Healthcare Life Sciences, UK). The flow rate of the run was 200 mL/min. UV-Vis spectral data for all peaks were collected over a range of 219-450 nm, and the chromatographic profiles were recorded at 280 nm. The mass spectrophotometer used was an XLQ XL Linear Ion Trap 2D (Thermo Scientific) equipped with an electrospray (ESI) orthogonal ionization source. The analyses were carried out in negative mode with a voltage of 5.00 kV and ESI capillary temperature at 275ºC, applying a collision energy of 20-25 eV in the fragmentations.

4. RESULTS AND DISCUSSION

4.1 Production and Development of Seaweed Soups

The initial work comprised a bibliographical research on the nutritional constitution of vegetables, in which the information provided by the food information platform in

Portugal, PortFIR (Plataforma Portuguesa de Informação Alimentar) was used [112]. A research for the best and nutritionally richest ingredients for the soup base was made. Calculations were made to find the nutritional formulation closer to what was sought. Given the first steps, the theoretical results obtained were put into practice. The ingredients initially chosen for the soup base were as follows: sweet potato, carrot, pumpkin, zucchini, onion, leek, chickpeas, cabbage and a liquid ingredient, soybean water-soluble extract. All the tested soups were tested with the incorporation of the soybean water-soluble extract and without it, using water in its replacement. The *Fucus vesiculosus* seaweed was first introduced as powder (F1.0716.M). On the one hand, tests were carried out with the introduction of *Fucus vesiculosus* powder, keeping all the ingredients of the base and adding only *Fucus vesiculosus* powder (Figure 3). On the other hand, tests were done adding to the base formulation *Fucus vesiculosus* powder and still chopped cabbage. In the first one, where only *Fucus vesiculosus* powder was added, powder amounts ranging from 0.19% to 0.51% were tested. In the second, powdered *Fucus vesiculosus* amounts ranging from 0.18% to 0.27% were tested. In some of these samples, the percentage of water had to be increased because the texture was not as desired.

Concluding this first phase, some conclusions were withdrawn, such as the fact that the soup even without added salt, the soup already had an intense and appealing flavor conferred by seaweed. However, for the palate accustomed to eating salt foods, we realized that we should add a small amount of salt. Another conclusion we obtained was that even in samples in which we increased the percentage of water, the soup was too consistent and too thick.

After the tests with *Fucus vesiculosus* powder, we realized that the amount of powder added was very reduced and did not enrich nutritionally the soup as we intended but already it altered quite the flavor of the soup. Thus, we chose to experiment with the addition of dried *Fucus vesiculosus* seaweed (F1.4617.D) cut into small pieces after obtaining an infusion (boiling) of this seaweed. Subsequently this infusion was added to the soup. At this point the percentage of chopped *Fucus vesiculosus* incorporated was 5.19% of dry basis. It was found that the introduction of dry seaweed allowed to incorporate higher seaweed percentages than with the powder. However, at the time of incorporation of the infusion into the soup, a gelatinization of the soup base almost

immediately was observed. There was saturation of the base, the infusion was so overloaded with alginates released when the seaweed boiled that it was not possible to create a homogeneous mixture (Figure 4). A third experiment was also made with the same quantities of the previous one: using a hand blender, the seaweed that was in the soup was crushed into small pieces before the cabbage was added. It was found that the texture became more homogeneous, creamier and more velvety but the sea flavor intensified. New experiments were carried out in order to improve the texture, increasing the percentage of water and, consequently, decreasing the percentage of *Fucus vesiculosus.* Another *Fucus vesiculosus* test was performed, which percentage was 1.26% on a dry basis and the result of this one was more satisfactory in terms of texture and flavor than the previous ones.

Figure 3 - Soup with *Fucus vesiculosus* powder (0.51%).

Figure 4 - Soup with chopped *Fucus vesiculosus* (5.19%).

In addition to *Fucus vesiculosus* experiments, experiments were also performed with dried *Ulva rigida* (U1.4117.D). Given its texture and low density, we easily realized that we could not incorporate the same amount of *Ulva rigida* we had incorporated from *Fucus vesiculosus* (1.26%) because with *Ulva rigida*, the soup was too saturated with seaweed. Concerning to *Ulva rigida* test and based on the last *Fucus vesiculosus* test (1.26%), it was tested a soup with 0,63% dry base of *Ulva rigida* (half the percentage of *Fucus vesiculosus* for the density reasons mentioned above). In addition to the tests performed with dry *Ulva rigida*, *Ulva rigida* powder (U1.3817.M) tests were performed in the same percentages as those performed with *Fucus vesiculosus* powder and the colcusions were similar to the previous ones with *Fucus vesiculosus*.

After this, a first sensory analysis was performed and it was open to those who wanted to participate internally. All sensory analyzes were carried out at the company

Centralrest, Lda with a group of non-trained tasters, including company employees, students, teachers and non-teaching staff, with a varied range of ages. The tests were carried out soon after the confection of the soups. The soups tested were: coarsely chopped *Fucus vesiculosus* (1.26%) soup; soup with *Fucus vesiculosus* (1.26%) crushed with hand blender; soup with *Fucus vesiculosus* powder (0.51%); coarsely chopped *Ulva rigida* (0.63%) soup; *Ulva rigida* (0.63%) soup crushed with hand blender; soup with *Ulva rigida* (0.51%) powder. All the soups tested in this first sensory analyzes had the soybean water-soluble extract incorporated. The evaluation involved several parameters, namely: the general appearance, color, taste, texture and smell. The scale used for the classification of sensory tests was as follows: 1 - I greatly disliked; 2 - I really disliked; 3 - I disliked moderately; 4 - slightly disagree; 5 - I did not like or dislike it; 6 - I liked it slightly; 7 - liked moderately; 8 - I liked it a lot; 9 - I liked it very much.

As a conclusion of this first test, it was considered that the soybean water-soluble extract incorporated into the soup base conferred an unusual taste, not expected in a soup and not very advantageous in the case of non-soybean consumers. Despite being a balanced product and quite nutritionally rich, it had little of traditional. Considering this, it was considered interesting to test a soup base without incorporation of soybean water-soluble extract. It was intended to perceive the organoleptic differences and simultaneously the impact of this alteration in the nutritional composition of the soups produced from this new base. For this purpose, the formulation was altered in such a way that the loss in nutritional intake was the smallest possible. First, various concentrations of *Fucus vesiculosus* were tested in the soup. The objective was to understand the behavior of the seaweed in a soup base without soybean water-soluble extract, knowing that the soybean extract also has a great influence on the texture and final consistency of the soup. On the other hand, and considering also the influence of soybean extract in the final flavor of the product, it was important to make an analysis of the final flavors taking into account various concentrations of seaweed in this new soup base. At the end of several tests, provisional results were reached: the base was optimized for a better nutritional composition, and the most balanced concentration of seaweed was found. The behavior of both the seaweed used, *Fucus vesiculosus* and *Ulva rigida*, was predicted, in order to maximize their differences. With the new optimized base, tests with *Fucus vesiculosus* powder, *Ulva rigida* powder, dried *Fucus vesiculosus* and dried *Ulva rigida* were

performed. Related to the tests with the powder, for both of them the percentage of powder added was 0.37% in terms to be a tasty soup and not saturated by the seaweed. Concerning to the soups with dry *Fucus vesiculosus*, the percentages tested ranged between 0.83 and 1.53%. With dry *Ulva rigida*, the percentages were a little lower and ranged between 0.52% and 0.83%. After these last tests a new organoleptic test was performed to evaluate the new developments, based on the same principles mentioned previously for the first test. The tested soups were the optimized recipes without water-soluble soybean extract: coarsely chopped *Fucus vesiculosus* (1.53%) soup; soup with *Fucus vesiculosus* (1.53%) crushed with a hand blender; soup with *Fucus vesiculosus* (0.37%) powder; coarsely chopped *Ulva rigida* (0.83%) soup; *Ulva rigida* (0.83%) soup crushed with a hand blender; soup with *Ulva rigida* (0.37%) powder. The evaluation involved the same several parameters and scale as the first one.

In general, there was greater acceptance with the new formulation. In this test, the sample with *Ulva rigida* (0.83%) coarsely chopped, was the favorite. In terms of texture, the sample containing *Fucus vesiculosus* (1.53%) crushed was chosen. It was suggested to incorporate a cut vegetable, along with the seaweed, to give an even more appealing and familiar look and give a palate even closer to the traditional. In the following tests were incorporated red beans and carrot cubes in the different formulations. The incorporation of *Gracilaria sp. (G1.0817.M)* (Figure 5) and the *Fucus vesiculosus* and *Ulva rigida* junction were also tested in the same soup (Figure 6). Concerning to dry *Gracilaria* tests, the incorporation of dried *Gracilaria* was tested in the percentage of 6.42%. It was expected that *Gracilaria* would confer an intense flavor, but curiously, a bigger amount of this seaweed could be incorporated without the taste and odor becoming less acceptable. However, the incorporation of dry *Gracilaria* without being crushed proved to be more complex, making the final product less appealing and more difficult chewing given the texture and consistency of this seaweed. Bearing this in mind, this soup was crushed with a hand blender. The appearance has improved but the issue of chew has remained, is a seaweed that easily gets stuck in the teeth, which is not advantageous considering that the target audience are the elderly. After this, the incorporation of *Gracilaria* powder (G1.3815.M) in the percentage of 0.59% was tested. This conferred a nice taste and flavor to the soup (Figure 5). Regarding the *Fucus vesiculosus* and *Ulva rigida* junction tests, two ways of conjugating these seaweeds were tested. The first, with crushed *Fucus vesiculosus*

(0.63%) and chopped *Ulva rigida* (0.63%), obtaining a soup with 1.26% of dry seaweed (Figure 6). In the second, with both seaweeds chopped, it was added 0.38% of *Ulva rigida* and 0.64% of *Fucus vesiculosus*. Thus, a soup with 1.02% dry seaweed was obtained. These samples with *Fucus vesiculosus* and *Ulva rigida* presented an appealing aspect.

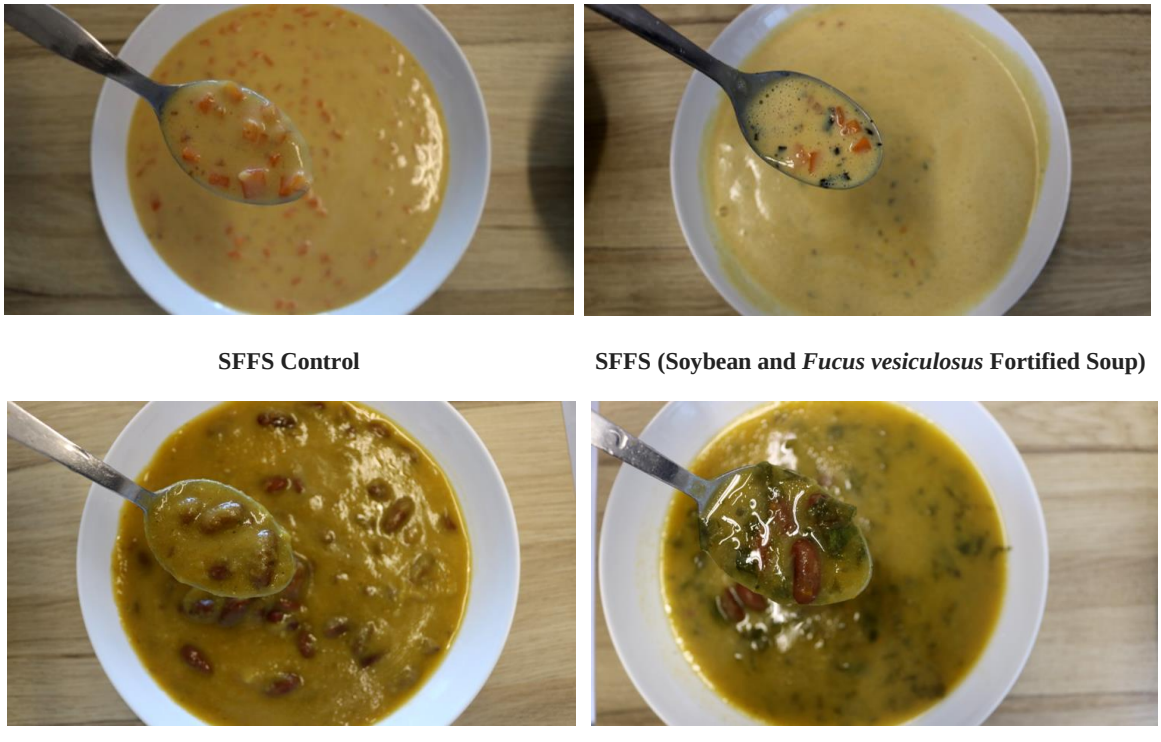
Figure 5 - Soup with crushed *Gracilaria* (6.42%) - below, and *Gracilaria* (0.59%) powder - above.

Figure 6 - Soup with crushed *Fucus vesiculosus* (0.63%) and chopped *Ulva rigida* (0.38%).

After these tests, a third and last sensorial analysis was carried out, in which the following formulations were prepared with water-soluble soybean extract: soup with chopped *Ulva rigida* and carrot cubes; soup with chopped *Ulva rigida* and chopped *Fucus vesiculosus* with carrots cubes; soup with chopped *Gracilaria* and carrot cubes; and formulations without soybean water soluble extract : soup with chopped *Ulva rigida* and carrot cubes; soup with chopped *Ulva rigida* and chopped *Fucus vesiculosus* with carrots cubes; soup with chopped *Gracilaria* and carrot cubes; soup with chopped *Ulva rigida* and red beans.

Considering the results of all the organoleptic tests, and considering that the company strategy is to have two references of soup with the different bases (a base with water soluble extract of soybean and another base without this extract) were chosen the following references: soup base without water soluble soybean extract with chopped *Ulva rigida* (0.32%) and red beans and soup base with water-soluble soybean extract with crushed *Fucus vesiculosus* (0.75%) and carrot cubes. Regarding the ingredients of the final soups, both of them contain sweet potatoes, carrots, pumpkin, zucchini, leek, chickpeas,

olive oil, salt and water at their bases, although in different amounts. The differentiating ingredients of the bases of the two soups are the broccoli present in the soup of *Ulva rigida* and the onion and water-soluble soybean extract, present in the soup of *Fucus vesiculosus*. The red beans were added to the base of the soup without water soluble extract of soybean with chopped *Ulva rigida*, and the carrot cubes were added to the base of the soup with water soluble soybean extract and crushed *Fucus vesiculosus*. For the laboratory analyzes, each of the above-mentioned soups was produced, as well as a control of each. The control consisted exactly of the soup in question but with no seaweed. At the end we had four different samples: crushed *Fucus vesiculosus* (0.75% dry base) soup with water-soluble soybean extract and carrot cubes (SFFS – Soybean and *Fucus vesiulosus* soup); control of Soybean and *Fucus vesiculosus* soup (SFFS Control); chopped *Ulva rigida* (0.32% dry base) soup without soluble soybean extract and with red beans (UFS – *Ulva rigida* Fortified Soup); control of *Ulva rigida* Fortified soup (UFS Control) (Figure 7). As it was mencioned before, both of the samples (SFFS and UFS) are supposed to be marketed in a package of 300g to support a full meal nutritional rich for the elderly.



SFFS Control **SFFS (Soybean and *Fucus vesiculosus* Fortified Soup)**

UFS Control **UFS (*Ulva rigida* Fortified Soup)**

Figure 7 - Final Seaweed Soups: SFFS Control; SFFS with 0.75% of dry *Fucus*

vesiculosus and 51.13% of Soybean water-soluble extract; UFS Control and UFS with 0.32% of dry *Ulva rigida*.

4.2 Physicochemical analysis of Seaweed soups

4.2.1 Color

The color and superficial appearance of food are the first quality parameters evaluated by consumers, and are thus significant factors for acceptance of the food item by the consumer. The color of this surface is the first sensation that the consumer perceives and uses as a tool to accept or reject food and the observation of color thus allows the detection of certain anomalies or defects that food items may present. Even if there are diverse color spaces, the most used of these in the measuring of color in food is the *L*, a*, b** color space due to the uniform distribution of colors, and because it is very close to human perception of color [122, 123]. The parameters *L**, *a** and *b** were recorded for the two soups and respective controls, and the results are shown in Table 9.

Table 9 - SFFS Control, SFFS, UFS Control and UFS redness (*a**), yellowness (*b**), lightness (*L**) and total color change variation (*⊿E**).

	SFFS Control	**SFFS**	**UFS Control**	**UFS**
L*	59.41±2.04	54.33±1.83	49.86±2.24	46.77±3.63
a*	10.08±0.72	8.54±0.63	5.86±0.42	3.53±0.46
b*	29.97±3.35	31.35±3.21	23.28±2.51	22.35±4.38
⊿E*	-	16.74±4.60	-	8.98±0.71

Comparing the values of the *L** (lightness) parameter of the two soups with the respective controls, we can conclude that the soup with the biggest variation of the *L** parameter was SFFS (from 59.41±2.04 to 54.33±1.83 with the introduction of *Fucus vesiculosus*). In the UFS, the difference found was lower (from 49.86±2.24 to 46.77±3.63

with the introduction of 0.32% *Ulva rigida*). It should be noted that the parameter *L** in SFFS is much higher when compared to the UFS. This is probably due to two factors: the UFS contains red beans, which eventually darken the soup, lowering its luminosity; the SFFS contains a water soluble soybean extract, contributing to a clearer and brighter final soup color, as can be seen in the Figure 7 shown above. The obtained values for this parameter are in agreement with the literature [124].

For the redness parameter (*a**), significant differences were observed comparing SFFS with UFS. For SFFS, *a** value is 8.54±0.63 and for UFS is 3.53±0.46. Comparing each soup with the respective control, it should be noted that for both controls, the values are higher than for the respective samples. Thus, we can conclude that the introduction of seaweeds led to a decrease in the *a** value, which was already expected. Concerning to *b** parameter (yellowness), as happened with parameter *a**, higher values were also recorded for FS than for UFS. For SFFS, *b** value is 31.35±3.21 and for UFS is 22.35±4.38. There were no significant differences between samples and respective controls for this parameter.

The *ΔE** parameter was calculated with the objective of comparison between samples and respective controls. A clear difference between food products global color state is considered perceptible by the consumer when they differ by a total color difference, *ΔE**, higher than 2.0–3.5 [125]. Note that the standard list defines that when *ΔE** ranges between 0-1 the observer does not perceive the color difference; between 1-2, the experienced observer may note the difference; between 2-3.5, experienced and inexperienced observers see the difference; between 3.5 and 5 reveals clear differences in color and, when above 5, observers see two different colors [126]. According to these standards, and given that the values of *ΔE** obtained are 16.74±4.60 and 8.98±0.71 for SFFS and UFS, respectively, we can conclude that they are values higher than 5. Thus, the observers clearly see two distinct colors, as expected because the soups composition is really different from one to another. Accordingly, it is possible to conclude that it would be possible to observe the difference between SFFS and UFS by experienced and inexperienced observers.

4.2.2 pH and Titratable acidity

Table 10 – pH and Titratable acidity (% Lactic acid) of SFFS Control, SFFS, UFS Control and UFS.

	SFFS Control	SFFS	UFS Control	UFS
pH	6.20±0.01	6.14±0.01	5.98±0.01	5.95±0.00
Acidity (% Lactic acid)	75.54±1.29	76.88±7.48	73.26±6.46	71.59±4.38

As shown in the Table 10, the pH of the seaweeds soups decreases comparing with the pH of the control soups. Soup's pH varied between 6.20±0.01 and 5.95±0.00 which is in agreement with the values reported in the literature that vary between 4 and 6, depending on the type of soup and its composition [127, 128, 129].

Soup's titratable acidity ranged between 71.59±4.38 and 76.88±7.48 mg lactic acid/100g soup which consistent with the literature [130]. It can be concluded that both SFFS and UFS have acidity values similar to the respective controls and therefore, the inclusion of seaweeds does not influence the percentage of lactic acid in the soups.

4.3 Nutritional analysis

4.3.1 Moisture content, Protein, Dietary Fiber, Reducing and Total Sugars

Table 11 – Relative moisture, protein, dietary fiber and sugars content of SFFS Control, SFFS, UFS Control and UFS (%).

		SFFS Control	SFFS	UFS Control	UFS
Relative Moisture (%)		88.20±0.03	88.79±0.02	86.59±0.10	87.44±0.24
Protein (%)		19.98±0.73	20.21±0.00	19.38±1.05	19.83±0.00
Dietary Fiber (%)		23.59±0.45	26.12±0.30	26.26±3.12	27.96±1.78
	Soluble Fiber (%)	8.06±0.86	12.98±1.20	8.17±0.35	8.17±1.36
	Insoluble Fiber (%)	15.54±0.40	13.14±0.90	18.57±2.97	21.05±3.27
Total Sugars (%)		4.66±0.26	4.68±0.19	4.74±0.12	4.60±0.15

Reducing Sugars (%)	4.66±0.26	4.68±0.19	4.74±0.12	4.60±0.15

Soup's moisture content varied between 88.79±0.02 and 86.59±0.10 % which is in agreement with the values reported in the literature that vary between 61% and 96%, depending on the type of soup and its composition [127, 129, 131, 132]. Since soup is a product in which water is its major component, the values obtained were expected. This high moisture content is one of the main problems regarding the conservation of the soup because high moisture contents lead to a faster degradation.

Regarding to protein content, it ranged from 19.38±1.05 to 20.21±0.00 %, which is might be able to contribute significantly to the daily protein requirements of 46-56 g/d of protein, according to Dietary Reference Intakes (DRIs) [133]. Considering that each soup has 300 g, each one will have approximately 7 g of protein, which corresponds to about 14% of the recommended daily intake of protein. If in a meal we can have 14% of the daily dose of protein, considering that we must do at least 6 meals a day, 14% is a very positive value. It is also important to note that the amount of protein in both seaweed soups is superior to the respective controls (20.21±0.00 for SFFS > 19.98±0.73 for SFFS Control and 19.83±0.00 for UFS > 19.38±1.05 for UFS Control), so the incorporation of the seaweeds in the soups did not influenced the protein content of them. In some green seaweeds such as the species belonging to the genus *Ulva rigida*, the protein content can represent between 10 and 26% (dry weight) of the plant. This means that the incorporation of seaweeds allows to increase the protein level of the food product [25].

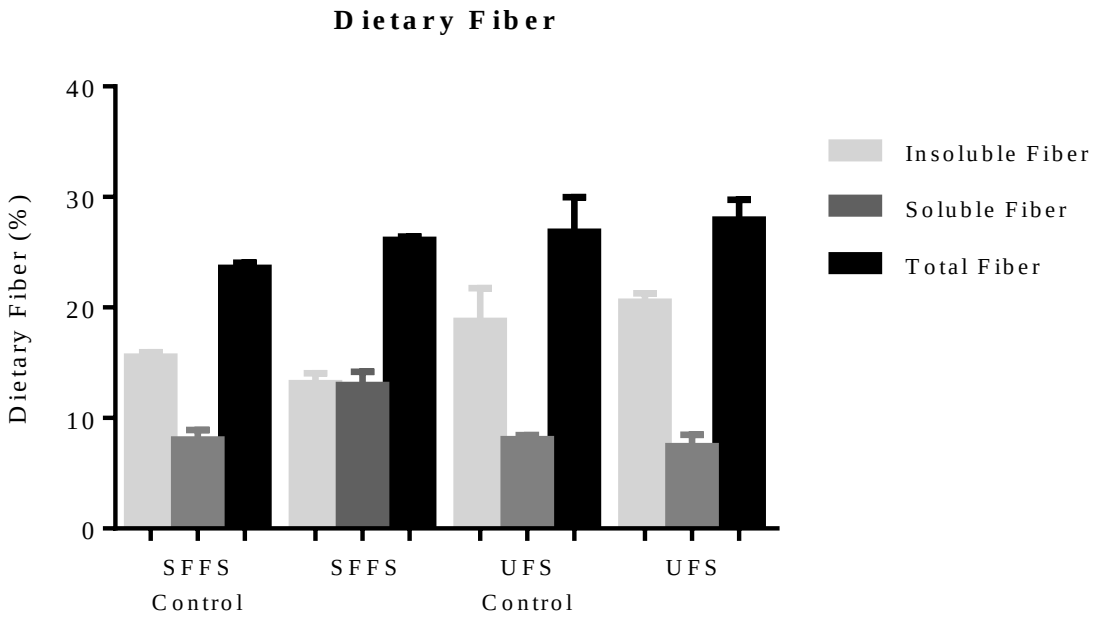


Figure 8 - Dietary Fiber content of SFFS Control, SFFS, UFS Control and UFS (%).

As it was said before, seaweeds contain large amounts of dietary fiber, reaching higher values than in fruits or vegetables [134]. They appear to be good sources of fibers presenting great chemical, physicochemical and rheological diversities that may be beneficial in nutrition. Adequate intake of dietary fiber can lower the level of serum cholesterol and reduce the risk of developing hypertension, constipation, diabetes, colon, cancer and coronary heart disease [135]. As can be seen in Figure 8, different values are found for the percentage of insoluble, soluble and total dietary fiber for each sample. According to Dietary Reference Intakes (DRIs), the recommended daily fiber value is about 21-30 g/d [133]. The obtained values for total dietary fiber, ranged between 29.22±4.55 to 23.27±1.31 %. Considering that each soup has 300 g, each package will have approximately 10g of fiber, which corresponds to about 21% of the recommended daily intake of fiber. It is important to note that there is a greater contribution of the insoluble fiber, than the soluble one, to the total dietary fiber content in all the soups.

Concerning to total and reducing sugars, it was concluded that the method used was probably not sufficiently sensitive to detect them. We can only conclude that the amount of sugar in the soups is so small that it was not possible to quantify them properly (for both

reducing sugars and total sugars). It was expected because a soup is naturally low in sugars.

4.3.2 Ashes and Elemental composition (Minerals)

Table 12 – Ashes and Minerals content of SFFS Control, SFFS, UFS Control and UFS (%).

		SFFS Control	SFFS	UFS Control	UFS
Ashes Content (%)		8.5±0.07	8.3±0.03	9.9±0.55	8.0±0.15
Minerals					
Macroelements (mg/100g)	**Na**	171.2±20.3	123.0±4.7	302.4±6.3	177.0±8.0
	K	228.7±16.8	229.0±13.6	162.3±19.7	155.9±20.2
	Ca	12.8±0.2	18.3±2.7	16.1±0.2	16.3±2.3
	Mg	19.7±1.5	23.5±2.2	16.2±1.2	24.8±1.6
Microelements (mg/100g)	**Cu**	0.1±0.0	0.2±0.0	0.1±0.0	0.1±0.0
	Zn	0.5±0.1	0.6±0.2	0.2±0.0	0.2±0.1
	Fe	0.7±0.0	1.0±0.1	0.6±0.0	2.0±0.2
	Mn	0.3±0.0	0.5±0.0	0.1±0.0	0.2±0.0

As regards to the ash content, it was found that seaweed soup samples have a tendency to lower values, contrary to expectations. However, these values are not significantly different as compared to the control.

In this topic of discussion, the interpretation of minerals will be made as two main topics. First of all, the values obtained for each element (present in 300g of soup, since the package marketed by the company will have 300g of soup) are compared with the values of the recommended daily dose; then the values obtained in the soups with seaweeds will be compared with the respective controls and after all with literature reviews.

Since a pack of soup contains 300 g and the values in Table 12 are in mg/100 g it will be necessary to multiply them all by three in order to agree with the package that will be marketed. In addition, the Table 13 values are in different units, provided by DRIs, which will all be adjusted to mg/d in order to be a coherent discussion because I want to

show with which nutritional values each soup can contribute to the recommended daily dose.

In relation to SFFS soup, the percentage of Mn and Cu are the most significant, corresponding to approximately 73% and 67% of the recommended daily dose (RDD), respectively. In addition, Fe, Na and K also occur with high representativity. The present Fe corresponds to about 38% of the RDD and Na and K correspond to about 30% of the RDD, each. On the other hand, with respect to UFS, the value of Fe is greater than 50%, approximately 75% of the RDD. As regards the other elements, the high percentage of Na and Cu (42% and 33%, respectively) should be noted. Comparing both soups, the one that has the greatest contribution in the supply of minerals to RDD is SFFS.

Table 13 – Dietary reference intakes of element's recommended values for Male and Female elderly.

Elements		Recommended Values for elderly M/F
Macroelements	**Na (g/d)**	1.2 – 1.3 *
	K (g/d)	4.7 *
	Ca (mg/d)	*1000 – 1200*
	Mg (mg/d)	*320 – 420*
Microelements	**Cu (μg/d)**	*900*
	Zn (mg/d)	*8 – 11*
	Fe (mg/d)	*8*
	Mn (mg/d)	1.8 – 2.3 *

NOTE: This table (taken from the DRI reports, presents Recommended Dietary Allowances (RDAs) in italic type and Adequate Intakes (AIs) in ordinary type followed by an asterisk (*). An RDA is the average daily dietary intake level; sufficient to meet the nutrient requirements of nearly all (97-98 percent) healthy individuals in a group. It is calculated from an Estimated Average Requirement (EAR). If sufficient scientific evidence is not available to establish an EAR, and thus calculate an RDA, an AI is usually developed. For healthy breastfed infants, an AI is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all healthy individuals in the groups, but lack of data or uncertainty in the data prevent being able to specify with confidence the percentage of individuals covered by this intake.

Comparing the values obtained for each element in each seaweed soup, with the values obtained in the controls, we can conclude that, in general, the introduction of both seaweeds led to an increase of the mineral contribution in the two soups. In relation to SFFS, the only element in which there was a decrease compared to the SFFS Control, was Na. There was a decrease of the reason Na/K (Figure 9), which is good because nowadays most of the diets are rich in Na. In the remainder, there was an increase with the introduction of *Fucus vesiculosus* (0.75%), more significant in Ca (which increased from 12.8 to 18.3 mg/100g) and Mg (which increased from 19.7 to 23.5 mg/100g). Regarding to the UFS, although the introduction of this seaweed also led to a greater contribution of minerals, the difference was not as significant as that of *Fucus vesiculosus* (0.75%). In UFS, Na and K were found in smaller amounts than in control (Na decreased from 302.4 to 177 mg/100g and K decreased from 162.3 to 155.9), so there was also a decrease of the reason Na/K such as like what happened on SFFS. On the other hand, in UFS, Mg and Fe were the elements that showed a greater increase when compared to control: Mg increased from 16.2 to 24.8 mg/100g and Fe from 0.6 to 2 mg/100g.

Regarding once again to Na, considering [Na] in mg/100g of fresh soup, it is possible to calculate the amount of salt of the soup (g). Thus, the values obtained are: SFFS Control 0.43±0.10 g; SFFS 0.31±0.00 g; UFS Control 0.76±0.00 g; UFS 0.44±0.00 g. From these values, we can conclude that the amount of salt in the soups with seaweeds is significantly lower than the amount in the controls. This is due to the salty taste associated with seaweeds. These results are quite positive, not only for the reason given above regarding excess Na in the diets nowadays but also because a traditional soup has on average about 0.60-0.70 g of salt. The values obtained for SFFS and UFS are well below these mean values, reaching about half of them.

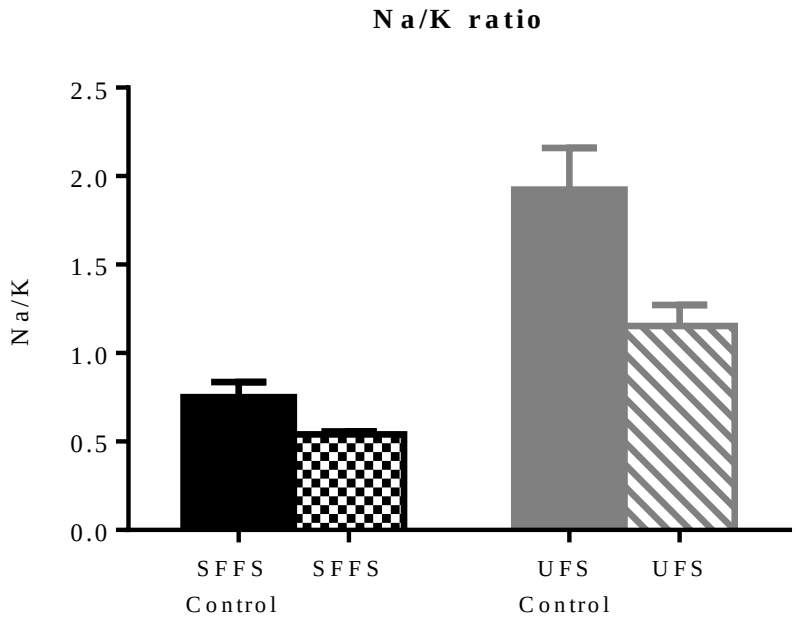


Figure 9 – Ratio Na/K of SFFS Control, SFFS, UFS Control and UFS.

According to the literature, it is not easy to see if the amounts of minerals obtained are the expected ones for a soup since most articles refer to instant soups and not to traditional vegetables or seaweeds soups. However, according to A. Krejčová et al., the values the values found in a vegetable soup base cube are similar to those obtained for seaweed soups, especially the value of Mg, Fe and Mn. For example, the Mg value found in the article soup is around 16.9 mg/100g, whereas in the soups obtained it varies between 16.2 and 24.8 mg/100g [136].

4.4 Phytochemical analysis

4.3.1 Phenolic compounds and Antioxidant activity

Among the three seaweeds phyla, several studies consider brown seaweed, including *Fucus vesiculosus*, as the seaweed richest in total phenolic compounds (TPC) [17, 89, 90]. This information is according with the obtained results: the TPC found in the soup with the brown seaweed *Fucus* (0.029±0.000 mGAE/mL extract), is higher than the TPC found in UFS (0.024±0.001 mGAE/mL extract), which has the green seaweed *Ulva*. On the other side, in the study by Matanjun et al., of the eight species of seaweeds studied,

the highest AA was recorded for green seaweeds, followed by brown and red [137]. According to the obtained results, the highest AA was also verified in the presence of the green seaweed *Ulva* (0.008±0.000 mTE/mL extract), followed by the presence of the brown seaweed *Fucus* (0.006±0.001 mTE/mL extract) [90, 138]. Therefore, TPC and AA content was determined for SFFS (brown seaweed) and for UFS (green seaweed) and for their controls, since the seaweeds present in these soups have a high TPC and AA because they are seaweeds with large potential and applicability in the market area. It is important to note that despite the differences observed with the presence of one or the other seaweed, comparing each soup with its control, it was verified that the introduction of the seaweed did not practically change the values of TPC and AA. The changes noted are so minimal that they are not considered relevant (Figure 10 and 11).

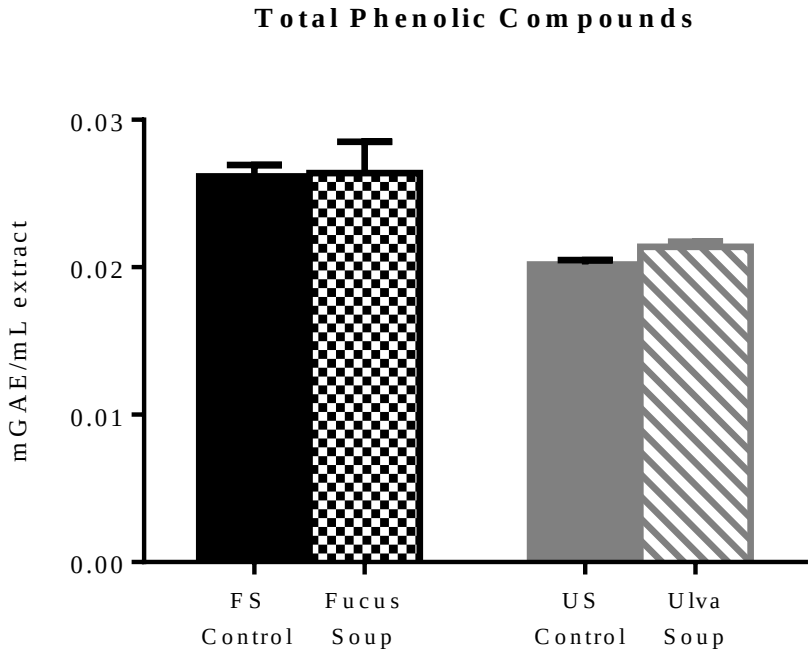


Figure 10 - Total phenolic compounds (TPC) of SFFS Control, SFFS, UFS Control and UFS expressed in gallic acid milliequivalents per mL of extract (mGAE/mL extract).

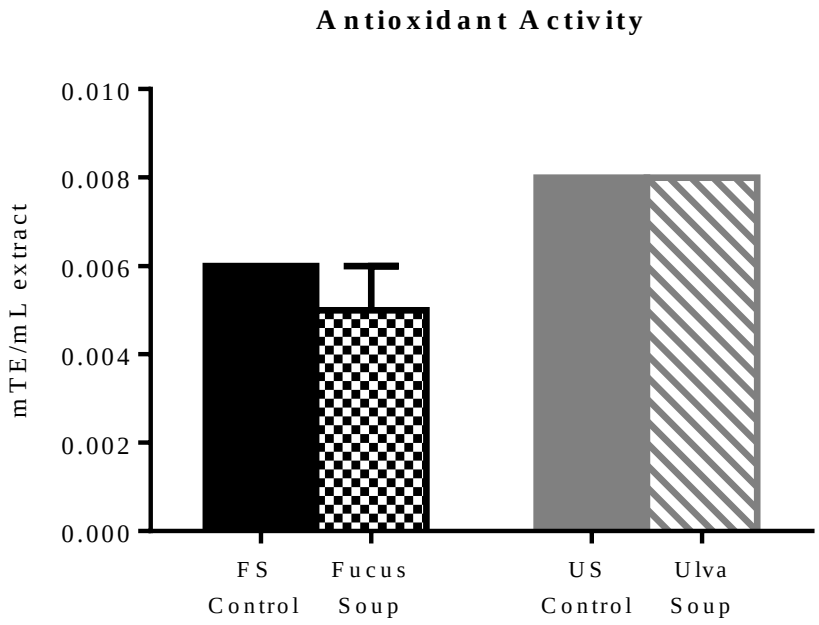


Figure 11 - Antioxidant activity (AA) of SFFS Control, SFFS, UFS Control and UFS expressed in mili Trolox equivalents per mL of extract (mTE/mL extract).

4.3.2 Pigments and carotenoids content

Seaweeds are photosynthetic organisms capable of synthesizing 3 types of pigments: chlorophylls, carotenoids and phycobiliproteins, being classified in *Chlorophyceae* (green seaweeds), *Phaeophyceae* (brown seaweeds) and *Rhodophyceae* (red seaweeds, according to the type of pigments they have. The green color of *Ulva rigida* is due to the presence of chlorophyll a and b, however this seaweed also contains carotenoids [139].

Figure 12 represents the representative chromatographic profiles of SFFS Control, SFFS, UFS Control and UFS, at 655 nm. Observing the chromatograms we can conclude that even after boiling, the soups still have carotenoids and chlorophylls. It is important to note which pigments are visible in each of the soups, as well as the differences between the controls and respective samples. In SFFS Control, we verified that only carotenoids and no chlorophylls are visible. Therefore, we can already conclude that it is a soup rich in carotenoids, such as β-carotene that appears at 18.7 min and is quite abundant. With the

addition of algae, we have already been able to verify the presence of chlorophylls and other compounds in SFFS. At 3.5 min we checked for the presence of fucoxanthin, as well as for 2.9 min since the peak leaves a bit dragged. Finally, with the presence of the seaweed *Fucus vesiculosus* (0.75%), at 5.8 min a chlorophyll derivative appears and at 20.3 min a pheophytin A appears. Regarding the UFS it is important to note that the presence of *Ulva rigida* (0.32%), being a green seaweed, introduces several pigments, namely chlorophylls. Therefore, in UFS there is an intensification of chlorophylls, comparing with UFS Control. In the chromatogram referring to UFS, we verified that the main differences to be noted in the contribution of *Ulva rigida* are as follows: at 17.3 min there is the presence of a chlorophyll derivative and at 20.2 min and 20.8 min there is pheophytin A.

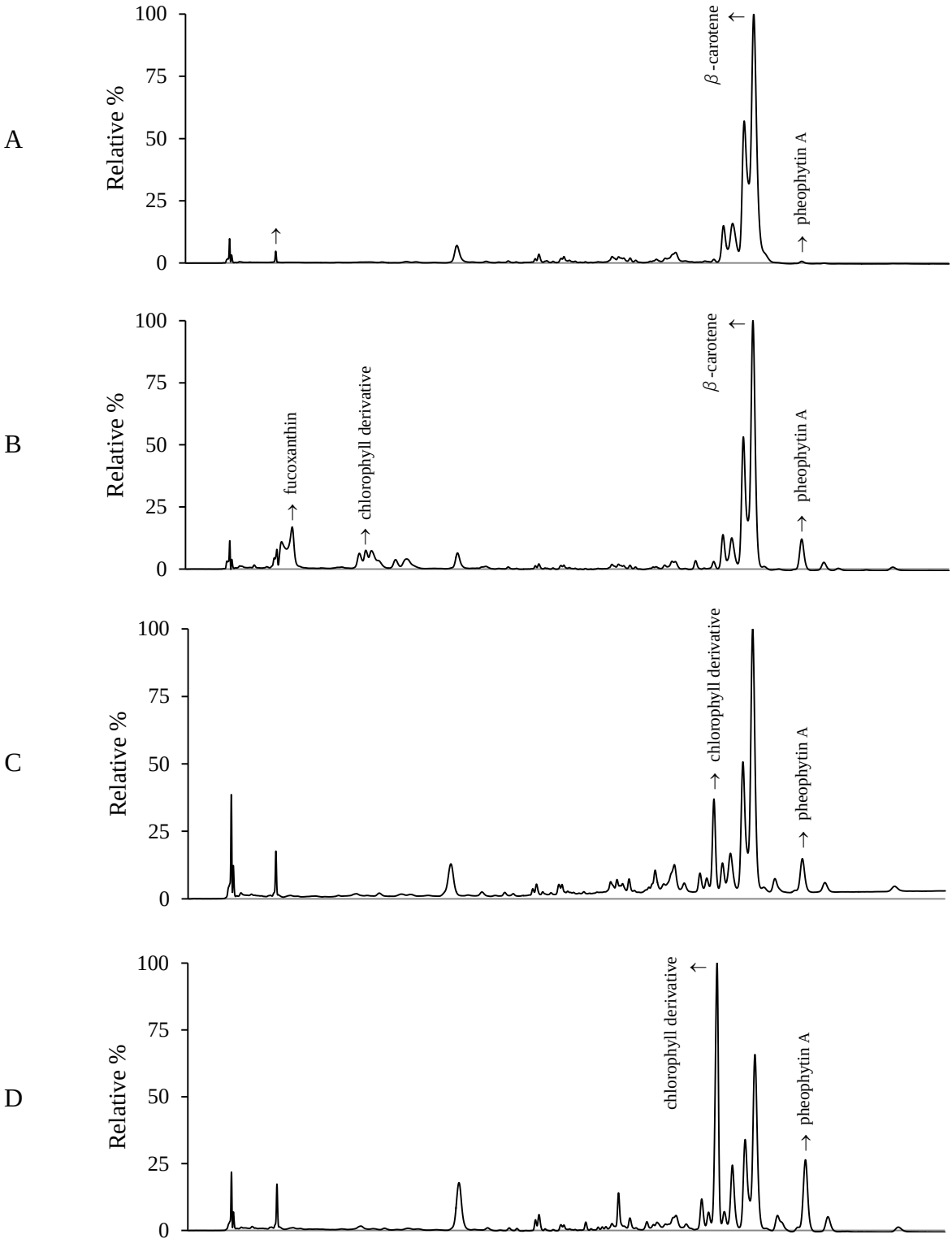

A
B
C
D
Relative %
100
75
50
25
0
β-carotene
pheophytin A
fucoxanthin
chlorophyll derivative

Figure 12 - Representative chromatographic profiles of SFFS Control (A), SFFS (B), UFS Control (C) and UFS (D), at 655 nm.

4. CONCLUSION AND FUTURE PERSPECTIVES

The main aim of the present work was to incorporate the. brown seaweed *Fucus vesiculosus* and the green seaweed *Ulva rigida*, in soups with added value. These soups should have enough nutritional information to replace a meal, particularly with the elderly, and should also be organoleptically similar to a Portuguese traditional soup.

In the first place, two soups were developed, one containing *Fucus vesiculosus* seaweed and soybean water-soluble extract and another one containing *Ulva rigida* seaweed without the soybean water-soluble extract. Next, the soups and respective controls were submitted to physical-chemical analysis in order to perceive their nutritional composition and other parameters.

As expected, the introduction of the seaweeds mainly altered the values of minerals, since seaweeds are rich in several essential elements. It is important to emphasize the increase of the Fe, Mg and Ca values, in this order, with the introduction of the seaweeds in the soups. On the other hand, the introduction of the seaweeds lead to a decrease of Na which is positive because nowadays most of the diets are rich in Na and diets rich in Na are harmful to health. Regarding the nutritional values, besides the minerals, a reasonable increase of the percentage of fiber in the SFFS and UFS was verified.

As regards the nutritional values of the soups, we can conclude that the nutritional values present in both soup packages (300 g each package) are enough to nutritionally provide a meal for an elderly person. Regarding protein values, each soup contains approximately 7 g of protein. Considering that this corresponds to about 14% of RDD, we can consider a good percentage. However, improvements in soups recipe may and should be made to further increase protein per soup so that a higher percentage of RDD can be covered. As regards to fiber, bearing in mind the fiber values of the soups, these cover about 21% of the RDD. These values slightly exceed the fiber values of a standard soup, which can be regarded as a positive result. This is because, in the third age, people tend

more and more to opt for foods rich in fiber, for health reasons like, for example, intestinal malfunction.

Considering these results as a first approach, it would be interesting to carry out further nutritional labeling analysis, such as carbohydrates and lipids. In addition, it is also relevant in the future to carry out microbiological analyzes and to understand how long it takes to conserve the seaweed soups through the various conservation methods available on the market.

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6. APPENDIX

6.1 Appendix A - Inverted sugar mass

- Extracted from NP-1420 of 1987

Table 14 - Required table for determination of total sugars and reducers.

Solução de Tiossulfato de sódio 0,1N	Glucose, frutose ou açúcar invertido $C_6H_{12}O_6$	
$\Delta V\ cm^3$	mg	Δ
1	2,4	2,4
2	4,8	2,4
3	7,2	2,5
4	9,7	2,5
5	12,2	2,5
6	14,7	2,5
7	17,2	2,6
8	19,8	2,6
9	22,4	2,6
10	25,0	2,6
11	27,6	2,7
12	30,3	2,7
13	33,0	2,7
14	35,7	2,8
15	38,5	2,8
16	41,3	2,9
17	44,2	2,9
18	47,1	2,9
19	50,0	3,0
20	53,0	3,0
21	56,0	3,1
22	59,1	3,1
23	62,2	-