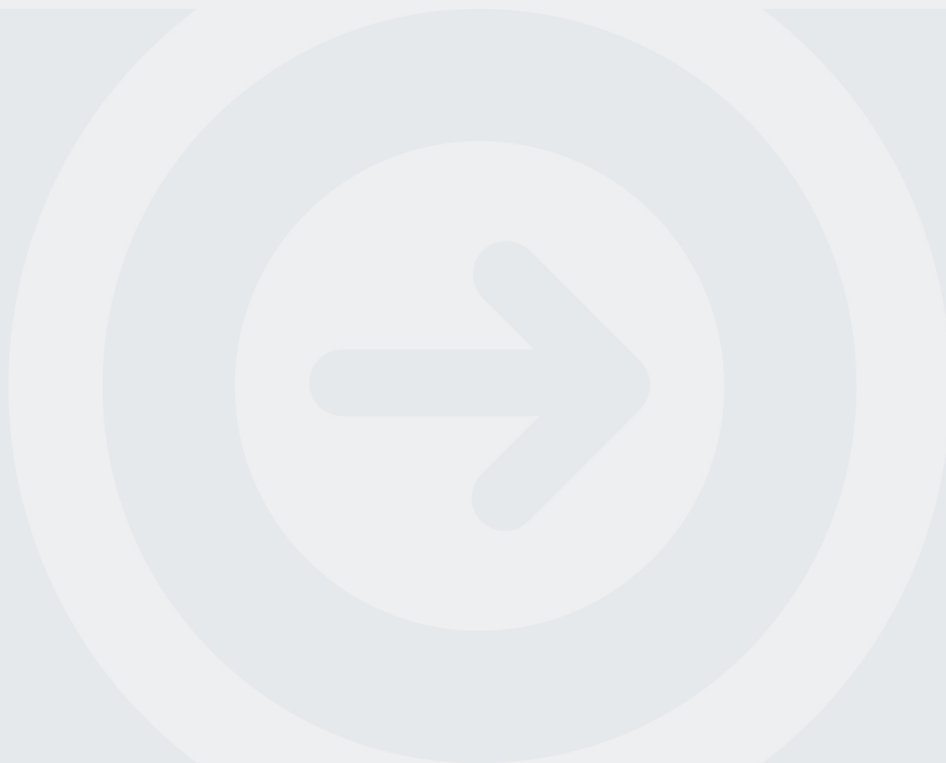




## **Desenvolvimento de novos produtos alimentares enriquecidos com algas**

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Outubro de 2019



# Development of new food products enriched with algae

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Dissertation submitted as a partial prerequisite to obtain a master's degree in Chemical Engineering

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October, 2019

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Dissertação submetida como requisito parcial para a obtenção do grau de Mestre em Engenharia Química

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# PREFACE

This Master's thesis concludes two rewarding years studying Chemical Engineering at ISEP. It was written and developed at the "Grupo de Reações e Análises Químicas (GRAQ) between September 2018 and October 2019.

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To all my friends, my sincere apologized for the long absence, I am back now!

I hope you enjoy your reading!

Helena Correia

Porto, October 2019

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## ABSTRACT

Climate change, current agriculture production, and consumption patterns have become the most significant problems of the food industry. Several studies proved the need to develop functional food as an alternative. Seaweeds are still not commonly used in Europe, but their several health benefits could improve food habits and essential mineral intakes. Lack of iodine has been documented as a global problem since the last century, and this mineral comprises a vital part of a healthy diet.

The main objectives of this work were the development of a new and accessible food product rich in iodine and other essential minerals in order to fill the gap that the current European diet has but, also with appealing sensory characteristics. To achieve these main objectives, a canned mackerel enriched with seaweeds was developed.

The selected edible macroalgae were: *S. latissima*, *L. digitata*, *U. pinnatifida*, and *C. crispus*. To quantify the minerals of interest (I, Se, Na, and K) and arsenic, the samples were digested by microwave-assisted acid digestion. The quantification of As, I and Se were performed by inductively coupled plasma - mass spectrometry; Na and K were determined by high-resolution continuum source flame atomic absorption spectrometer.

All the characterized species exhibited high iodine levels: *U. pinnatifida* ( $64.02 \pm 8.74 \mu\text{g I/g ww}$ ), *L. digitata* ( $643.5 \pm 13.0 \mu\text{g I/g ww}$ ), *S. latissima* ( $211.6 \pm 12.8 \mu\text{g I/g ww}$ ) and *C. crispus* ( $239.2 \pm 17.7 \mu\text{g I/g ww}$ ). The impact of processing steps, such as rehydration and boiling, was assessed. Several canned fish samples were prepared, with and without the addition of algae, and comparisons between them were made. All samples revealed a satisfactory contribution to the recommended daily allowances of nutritionally essential minerals (K: 0.53-3.64%, I: 310.32-1780.8 %, Se: 71.1-99.9%).

A sensory analysis was done after the canned samples production. The samples with macroalgae revealed the best textural aspect and aroma. Therefore, it was concluded that the sample with *S. latissima* has the best aroma, flavour, texture, and appearance.

In conclusion, all the main objectives of the work were achieved, and a healthy product was developed. Some future work is needed to optimize the canned product and to explore further the total macroalgae and canned beneficial properties.

Keywords: Seaweeds, Functional Food, Canned Fish, Iodine, Selenium

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# RESUMO

Fatores como as alterações climáticas, agricultura intensiva e crescimento do consumo tornaram-se alguns dos problemas da indústria alimentar. Diversos estudos provam que existe uma necessidade crescente de desenvolvimento de alimentos funcionais. Apesar dos seus vastos benefícios que poderiam melhorar os hábitos alimentares europeus as macroalgas não são muito utilizadas nesta zona do globo. A deficiência do iodo na população tem sido estudada e atualmente é considerada como um problema mundial.

Os principais objetivos deste trabalho são o desenvolvimento de um produto alimentar, mais barato e acessível rico em iodo e outros minerais essenciais, de modo a colmatar o défice deste mineral na dieta Europeia, mas também que apresente características sensoriais apelativas. De modo a cumprir estes objetivos projetou-se o desenvolvimento de uma conserva de cavala com macroalgas.

As macroalgas edíveis escolhidas foram: *S. latissima*, *L. digitata*, *U. pinnatifida* e *C. crispus*. De forma a quantificar os minerais de interesse (I, Se, Na e K) e arsénio as amostras foram previamente digeridas por micro-ondas. A quantificação de As, I e Se foi realizada por ICP-MS. Na e K por HR-CS-FAAS.

Todas as macroalgas selecionadas demonstraram altos teores em iodo: *U. pinnatifida* ( $64.02 \pm 8.74 \mu\text{g I/g ww}$ ), *L. digitata* ( $643.50 \pm 12.99 \mu\text{g I/g ww}$ ), *S. latissima* ( $211.60 \pm 12.79 \mu\text{g I/g ww}$ ) e *C. crispus* ( $239.21 \pm 17.69 \mu\text{g I/g ww}$ ). As amostras de conservas criadas revelaram altas percentagens de contribuição para o valor diário recomendado de alguns elementos (K: 0.53-3.64%, I: 310.32-1780.8 %, Se: 71.1-99.9%).

Após a produção de conservas foi efetuada a análise sensorial. Em geral todas as amostras com a presença da macroalga revelaram uma melhor textura e aroma marinho. Em concordância concluiu-se que a melhor conserva (sabor, aroma, aspeto e textura) seria a alga com a inserção da macroalga *S. latissima*.

Em conclusão, os principais objetivos do trabalho foram alcançados. Contudo, algum trabalho futuro é recomendado de forma a otimizar os produtos criados, testar mais amostras para validação do método e para explorar o benefício total do uso das macroalgas na conserva de cavala.

Palavras-chave: Macroalgas, Alimentos Funcionais, Conservas de Peixe, Iodo, Selénio

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## ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
<i>Chondrus crispus</i>	<i>C. crispus</i>
CONTAM Panel	Panel on Contaminants in the Food Chain
dw	dry weight
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization
HIV	Human immunodeficiency virus
HR-CS-FAAS	High-resolution continuum source flame atomic absorption spectrometer
ICP- MS	Inductively coupled plasma - mass spectrometry
<i>Laminaria digitata</i>	<i>L. digitata</i>
MAD	Microwave-assisted digestion
PTFE	Polytetrafluoroethylene
PUFAs	Polyunsaturated fatty acids
RDA	Recommended daily allowance
RDI	Recommended daily intake
<i>Saccharina latissima</i>	<i>S. latissima</i>
<i>Undaria pinnatifida</i>	<i>U. pinnatifida</i>
UL	Upper intake Level
wt	weight
ww	wet weight
yr	year

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# CHAPTER 1

## 1. INTRODUCTION

The combined impacts of climate change, current agriculture production, and consumption patterns are undermining our planetary habitat. It has been estimated that the equivalent of more than two planet Earth will be needed by 2050 to support the growing global population (Tiwari and Troy 2015). The study and development of seas and oceans resources could provide an alternative to overcome this global problem.

Oceans cover proximally 70% of the world's surface and have an extensive diversity of marine organisms (algae, fungi, cnidarian, among others), which are a rich source of food and bioactive natural products, that may have a significant role in health promotion, mainly in diseases prevention and treatment (Santos et al. 2015). Several dietary reports confirmed that seafood is an essential part of human health and life (Bajpai 2017).

Algae are quantitatively the primary biomass in the ocean, with 25 000 – 30 000 species already known (Qin 2018). The term algae refer to a large and diverse collection of organisms that contains chlorophyll and carry out photosynthesis (Qin 2018). They usually exist in complex habitats, under extreme environmental conditions, which lead to the development of resistance strategies such as the production of bioactive metabolites.

World production of algae comes from two sources: harvesting from wild stocks and aquaculture (including land-based culture and farming) (West, Calumpong, and Martin 2016). With the growth of the sustainable attitude, algae have become one of the primary raw material of interest, since ocean farms are seemingly more sustainable compared to land-based agriculture because the cultivation of seaweeds requires no freshwater, chemical fertilizer, or land. Considering energy conversion rates from one trophic level to the next, algae farming is much more efficient energetically and nutritionally than animal farming (Tiwari and Troy 2015). Harvests from wild populations are affected by overexploitation and climatic changes, with some cases, already reported (Moy and Christie 2012; T. R. Pereira et al. 2011) being aquaculture a much more appealing alternative.

Data from the Food and Agriculture Organization (FAO 2016) revealed that the world production of algae reached 31.2 million tonnes in 2016. Out of those values, 30.1 million tonnes (96.5%) were harvested from aquaculture. China is the largest seaweed cultivator in the world, with about 50% of the global output, followed by Europe that represents the second major producer (FAO 2018). The algae processing industry in Europe embraces several sectors including biopolymers, cosmetics, agri-foods, and functional food additives with various health properties (Tiwari and Troy 2015).

### 1.1. Marine algae

Marine algae can be divided into macroalgae, commonly known as seaweeds, and microalgae (phytoplankton) according to their physical sizes (Figure 1.1(a)) (Qin 2018). Microalgae are mostly found in both benthic and littoral habitats and throughout the ocean waters (Gupta and Abu-ghannam 2011). Seaweeds grow in the intertidal or the sub-tidal area up to a certain depth where very little photosynthetic light is available (Gupta and Abu-ghannam 2011).

Seaweeds are divided into four categories based on their characteristic pigmentation: brown, green, red, and blue-green (Figure 1-1(b)) (Qin 2018; Pereira 2016).

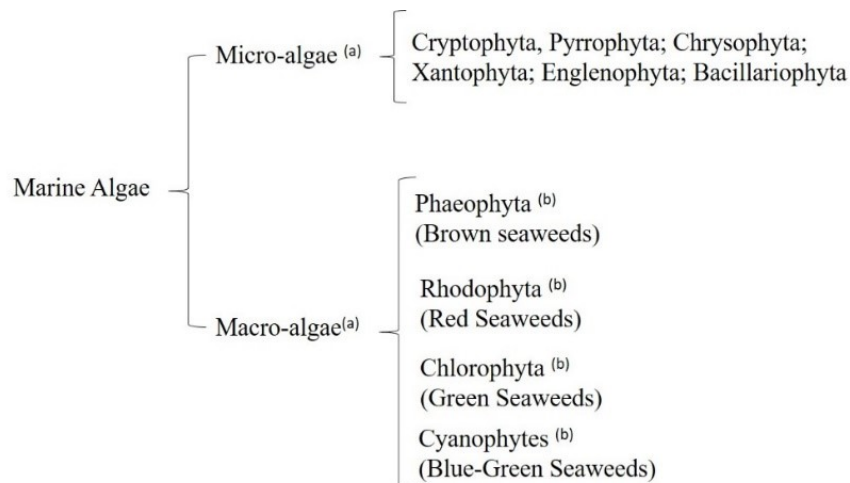


Figure 1-1-(a) Marine macroalgae characterization by size. (b) Macroalgae categories based on their pigmentation. (Adapted from Qin, 2018).

The division *Phaeophyta* comprises an extensive collection of plants that are classified in about 265 genera with more than 1500 species (Gupta and Abu-ghannam 2011).

Their characteristic brown colour derives from the vast amounts of the carotenoid fucoxanthin contained in their chloroplasts. Brown algae flourish at the temperate climate of subpolar

regions where they exhibit the most extensive diversity in species and morphological expression (Qin 2018).

The *Rhodophyta* division has photosynthetic pigments such as chlorophyll, carotenoids, and some phycobilin. Their morphology varies from unicellular, filamentous, to multicellular thalloid forms. These species live primarily in marine environments from the intertidal zone to deep waters, but they can be found in freshwater and terrestrial lineages as well (Yoon et al. 2017).

*Chlorophyta* group includes marine, freshwater, and terrestrial green algae with a wide morphological diversity, ranging from planktonic unicellular organisms, to colonial, multicellular, and siphonous algae (Santos et al. 2015).

Blue-green algae or *Cyanophytes* are photosynthetic and mostly aquatic prokaryotic organisms, related to bacteria. They are found almost everywhere but are most common in marine and freshwater habitats in humid terrestrial habitats (Guiry 2011).

## 1.2. Edible seaweeds

Most of the algae species are edible; however, some are poisonous for mammals (in particular some blue-green) (Mouritsen, Mouritsen, and Johansen 2013). Data from FAO, 2018 demonstrate that the vast majority (~83%) of the seaweeds harvested and cultured in the world are consumed by humans, either as a direct food source or as a food additive (White and Wilson 2015).

Data exhibited in Table 1-1 represent the total of seaweeds imports and exports in Europe during the years of 2015 and 2016. The analysis of Table 1-1 shows that contrary to what happens in East Asia, the West is more interested in thickeners and gelling properties of hydrocolloids extracted from seaweeds: carrageenan, agar, and alginate (E407, E406, and E400, respectively) (Pereira 2016). However, in recent years, the many bioactivities of seaweed-derived substances have found applications in functional foods, nutraceuticals, cosmetic products, biomedical materials, pharmaceutical products, fertilizers, and many other industries where health benefits can be achieved (Qin 2018). This fact predicts an exponential increase in food markets and applications.

Table 1-1- European Union: imports and exports of seaweed 2015-2016 (Adapted from FAO, 2018).

	Weight (tonnes)			
	Imports		Exports	
	2015	2016	2015	2016
<b>Seaweed for human consumption</b>	17 175	15 184	5 219	4 607
<b>Seaweed not for human consumption</b>	89 103	88 485	55 974	53 723
<b>Agar agar</b>	3 734	4 171	2 766	2 462
<b>Carrageenan</b>	57 158	70 627	37 587	40 802
<b>Total</b>	167 170	17 846	101 546	101 594

Edible seaweeds or sea vegetables are an essential item in Japanese, Korean and Chinese diets since pre-historic times. About 21 species are used in everyday cookery in Japan, six of them since the 8th century (Pereira 2011). In Europe, their use is more established among the Celtic cultures (Pereira 2016).

Some examples of the most common edible species are represented in Table 1-2.

Table 1-2- Common edible seaweeds (Pereira 2016; Mouritsen, Mouritsen, and Johansen 2013).

Class	Specie
<b><i>Phaeophyta</i></b>	<i>S. latissima sp.</i>
	<i>L. digitata sp.</i>
	<i>U. pinnatifida</i>
	<i>Fucus vesiculosus</i>
	<i>Sargassum fusiforme</i>
	<i>Himanthalia elongata</i>
<b><i>Rhodophyta</i></b>	<i>C. crispus</i>
	<i>Porphyra sp.</i>
	<i>Palmaria palmata</i>
	<i>Porphyra sp.</i>
<b><i>Chlorophyta</i></b>	<i>Ulva sp.</i>
	<i>Enteromorpha sp.</i>

France was one of the first European countries to establish regulation concerning the use of marine algae as food substance (with 21 macroalgae and 3 microalgae authorized as vegetables and condiments) (CEVA 2014). However, so far, there are only recommendations from the food safety authority and not legally binding (Circuncis et al. 2018).

Like all food products, sea vegetables need regulation to ensure the quality and safety of their use. Available data have been showing that seaweeds may contain significant concentrations of

arsenic, cadmium, iodine, lead, and mercury (The European Commission 2018). Thus, the European Commission recommends through (EU) 2018/464 of 19 March, the monitoring of those metals and metalloids, as well as iodine in seaweeds, halophytes, and products based on seaweeds. Table 1-3 presents the established maximum intake levels, recommended daily allowances for iodine and the current legislation.

Table 1-3- Maximum intake levels (MLs) for As, Cd, Pb, Hg and I (mg/kg dry weight) and recommended daily allowance (RDA) for iodine (mg/day).

	MLs		RDAs		Regulation
	Adults	Children	Adults	Children	
<b>Arsenic</b>	3 mg/kg dw		(*)		(EU) 2018/464 of 19 March
<b>Cadmium</b> <sup>(1)(2)</sup> (mg/kg dw)	<3		(*)		(EC) No 629/2008
<b>Lead</b> (mg/kg dw)	<3		(*)		FAO, 2018
<b>Mercury</b> (mg/kg dw)	0.01		(*)		(EC) No 396/2005
<b>Iodine</b> (mg/day)	0.6	0.2	0.15	0.09	(EU) 2018/464   (EC) No 100/2008

(\*) No data found.

(1) Seaweed accumulates cadmium naturally. Food supplements consisting exclusively or mainly of dried seaweed or products derived from seaweed can, therefore, contain higher levels of cadmium than other food supplements. Considering this, a higher maximum level for cadmium is needed for food supplements consisting exclusively or mainly of seaweed.

(2) Food supplements consisting exclusively or mainly of dried seaweed or products derived from seaweed.

### 1.2.1. Chemical composition

Seaweeds are a rich source of micronutrients such as vitamins (vitamin A, B1, B2, B3, B6, B12, C, D, E, pantothenic acid and folic acid) (Madhusudan et al. 2011; Ferraces-Casais, Lage-Yusty, and J.López-Hernández 2012; Gómez-ordóñez, Jiménez-escrig, and Rupérez 2010; Roohinejad et al. 2017), sterols (Hernández-Ledesma and Herrero 2014) and minerals (calcium, magnesium, potassium, iodine, sodium, phosphorus, nickel, chromium, selenium, iron, zinc, manganese, copper, lead, cadmium, mercury and arsenic) (Gupta and Abu-Ghannam 2011; Roohinejad et al. 2017). No land plant approaches seaweeds as sources of metabolically required minerals. Iodine is an essential mineral that is known to be very abundant in seaweeds (Romarís-Hortas et al. 2011; Roleda et al. 2018; Yeh, Hung, and Lin 2014). Although iodine levels differ from species to species, it has been reported to vary between 4.3 and 2660 mg/kg (Roohinejad et al. 2017). Minerals are in chelated and colloidal forms, which may enhance their bioavailability in the body (Pereira 2016).

Seaweeds may also accumulate toxic metals (e.g., arsenic (As), cadmium (Cd), copper (Cu), mercury (Hg), and lead (Pb)) This fact is one crucial aspect to have in mind when considering seaweed consumption. Values of toxic metal in the majority of edible macroalgae have been reported, although, below the maximum concentrations allowed for human consumption (Circuncis et al. 2018). The adverse effects of the toxic metals depend on their physical state, for instance, As is much more toxic in inorganic form, even though, the predominant form in seaweeds is the organic As (Circuncis et al. 2018; Díaz et al. 2012; Rose et al. 2007). In general organic As exert low or no toxicity, while inorganic As is the most hazardous, with accumulation already associated to several disorders (nephrotoxicity, diabetes, hepatotoxicity, cardiovascular dysfunction, and cancer, mainly at the skin, lungs, and bladder level) (Circuncis et al. 2018).

Besides mineral content, seaweeds had been studied as a natural source of antioxidants (Wang, Jónsdóttir, and Ólafsdóttir 2009; Chew et al. 2008), with antimicrobial activity (Gurav et al. 2008; Besednova et al. 2015) and hydrocolloids compounds (carrageenan, alginate and agar agar) (Evans and Critchley, 2014).

### **1.3. Edible seaweeds under study**

The seaweeds chosen for this study were three brown species (*L. digitata*, *S. latissima*, and *U. pinnatifida*) and one red species (*C. crispus*) since these four macroalgae have high iodine levels; human deficiency of this element covers a vast geographical area. Between the four macroalgae classes, several studies proved that brown is the richer in iodine (Gupta and Abughannam 2011; Pereira 2015; Wells et al. 2017; Funahashi et al. 2001). Although *C. crispus* is a red algae, the iodine levels are also high and have the advantage of a high percentage of the hydrocolloid carrageenan (Pina and Costa 2014).

### 1.3.1. *Laminaria digitata*



Figure 1-2- Representation of *L. digitata* (Adapted from Pereira, 2016).

**Common names:** Tangle, Sea Girdles, Tangle Tail, Wheelbangs, Sea Wand, Sea Ware, Sea Tangle, Horsetail Kelp, Kelp, Strap Wrack, Oarweed, Oar Weed, Horsetail Tangle, Sea Girdle, Kombu Breton.

**Geographic distribution:** North Atlantic, Atlantic Islands (Canary Is, Greenland, Iceland), Baltic Sea.

**Curiosity:** Is used for accelerating the cooking time of vegetables such as beans and lentils.

*L. digitata* is a dark brown macroalgae with a smooth, flexible stipe and can reach 3 to 4 m in length; it can grow to be 4 to 5 years old. This kelp grows on rocky bottoms in the upper subtidal zone in sheltered to moderately exposed areas from 1 m to 25 m in depth. Extracts of this species have antibacterial and antioxidant activity (Pereira 2016; Mouritsen, Mouritsen, and Johansen 2013).

Contain minerals, vitamins, and trace elements; these include iodine, calcium, potassium, iron, carotene, alginic acid, laminarin, mannitol, protein, carotene, niacin, phosphorus, the B complex vitamins, vitamin C and many other trace elements. This species stores flavour-enhancing glutamic acid, or sodium glutamate, which imparts a mellow, silky taste to dishes (Pereira 2014).

### 1.3.2. *Saccharina latissima*



Figure 1-3- Representation of *S. latissima* (Adapted from Pereira, 2016).

**Common names:** Sea Belt, Poor Man's Weather Glass, Sweet Wrack, Sugar wrack, Sugar Tang, Oarweed, Tangle, Kelp, Sugar Sea Belt, Sweet Tangle, Sugarwrack, Zuckertang, Royal or Sweet Kombu, Laminaire Sucrée, See-Palme, Karafuto Kombu, Karafuto Tororo Kombu, Kan-Hoa, Rabeiro, Kombu-Real.

**Geographic distribution:** N and NE Atlantic (Greenland to Portugal, N Sea, Baltic), NW Atlantic (Canadian Arctic to Massachusetts), NE Pacific (Alaska to California).

**Curiosity:** Can be served as a snack, fried with dip sauce.

*S. latissima* has blades, stipes, and holdfasts, which is attached to substrates; it has a vibrant medium-brown colour. The stipe of *S. latissima* can be 50 cm long. Extracts of this species have anticoagulant, antifouling, antimicrobial, and antioxidant activity (Pereira 2015, 2016, 2014).

### 1.3.3. *Undaria pinnatifida*



Figure 1-4- Representation of *U. pinnatifida* (Adapted from Pereira, 2016).

**Common names:** Qun Dai Cai, Asia Kelp, Apron-Ribbon Vegetable, Sea Mustard, Precious Sea Grass, Wakame, Miyok, Miyeouk

**Geographic distribution:** Indigenous to the NW Pacific Ocean and the cold temperate coastal regions of Japan, China, Korea, and Southeast Russia. Has been spread around the world by international shipping and mariculture and has extended its range to include four continents since the 1980s.

**Curiosity:** It is traditionally served as a luxury food among Japanese and Korean people.

In Oriental medicine, *U. pinnatifida* has been used for blood purification, abdominal strength, skin, hair, reproductive organs, and menstrual regularity.

*U. pinnatifida* is large brown kelp with a branched holdfast giving rise to a stipe. It can reach an overall length of 1 m to 3 m. *U. pinnatifida* is an annual species with two separate life stages.

It has a high nutritional value, containing high levels of calcium, iodine, thiamine, niacin, iron, vitamin B12, and protein. It is also a rich source of eicosapentaenoic acid, an omega-3 fatty acid, and also of polysaccharides (Pereira 2014).

Extracts of this species have antihypertensive, immunomodulating, antidiabetic, antiviral, cytotoxic, antioxidant, antitumor, antiedema, antiplasmodial, anti-osteoporotic, anti-inflammatory, anti-obesity, antihypertensive, and antithrombotic activity (Pereira 2015, 2016, 2014).

#### 1.3.4. *Chondrus crispus*



Figure 1-5- Representation of *C. crispus* (Adapted from Guiry 2011).

**Common names:** Irish moss, Carrageen moss, Pearl moss. Carrageen, Jelly moss

**Geographic distribution:** Has a wide distribution, can be found all around the globe (Spain, Portugal, Russia, Canada, New Jersey, English Channel, the Faroes, and West Africa).

**Curiosity:** “Irish Moss” has been used in Ireland since the beginning of the 19th century as a remedy for respiratory disorders; It is the source of carrageenan’s (Guiry 2011; Pereira 2016).

*C. crispus* is harvested as a common source of the polysaccharide carrageenan, which is extracted from cell walls and forms a gel that is used commercially as a thickener. The gelling and thickening properties of carrageenan are used widely in the cosmetics, food, and pharmaceutical industries (Guiry 2011).

A non-extensive review of the mineral and nonessential or toxic metals levels in the seaweeds used in this study are reported in Table 1-4. These values result from bibliographic research and refer to fresh marine macroalgae. Several studies refer that some environmental factors like temperature, season and geographic distribution, have a significant impact on mineral composition (Mohamed, Hashim, and Rahman 2012; Nitschke and Stengel 2016; Pereira 2018a) of wild macroalgae, while a similar influence is not observed on the macroalgae from aquaculture.

Table 1-4- *L. digitata*, *S. latissima*, *U. pinnatifida* and *C. crispus* principal nutrient composition and nonessential or toxic metals composition.

	Macronutrient composition (mg/g dw)			Micronutrient composition (µg/g dw)		Nonessential or toxic metals (µg/g dw)			Reference
	Na	K	Ca	I	Se	As	Hg	Cd	
<i>L. digitata</i>	27-38.18	52-113	10-15	10×10 <sup>6</sup>	0.07-0.3	27-49	0.54	0.1-3.4	(Biancarosa et al. 2018; Kolb et al. 2004; Pereira 2016; Circuncis et al. 2018)
<i>S. latissima</i>	24	25-120	9.59-17	957.6-10×10 <sup>6</sup>	0.06	28-120	0.05-0.117	0.1-5	(Biancarosa et al. 2018; Cabrita et al. 2016; Circuncis et al. 2018)
<i>U. pinnatifida</i>	48.80-64.94	56.91-68.10	6.8-13.80	220-300	0.03-0.05	13-26	0.01-0.22	0.1-5	(Kolb et al. 2004; Pereira 2016; Rupérez 2002; Circuncis et al. 2018)
<i>C. crispus</i>	12-42.70	13.50-31.84	4.20-11.20	245-20×10 <sup>4</sup>	0.14-0.6	0.4-2.6	0.006	0.3-1	(Biancarosa et al. 2018; Pereira 2016; Rupérez 2002)

dw- dry weight

### 1.3. Seaweeds as additives to functional food

A functional food can be described as a product that has beneficial effects on one or more physiological functions, increases the welfare, or/and decreases the risk of disease (Madhusudan et al. 2011). In addition to their fundamental nutritive value, the idea of a functional product is to add balance to the ingredients, to make them more effective, including in the prevention and treatment of disease and illness (Goldberg 1994). As it can be observed in Figure 1-6, the applications and benefits of seaweeds used as functional food are diverse, and they can be applied in various food types to expand their beneficial properties (Hanjabam et al. 2016; Rioux, Beaulieu, and Turgeon 2017; Roohinejad et al. 2017; Rico et al. 2018).

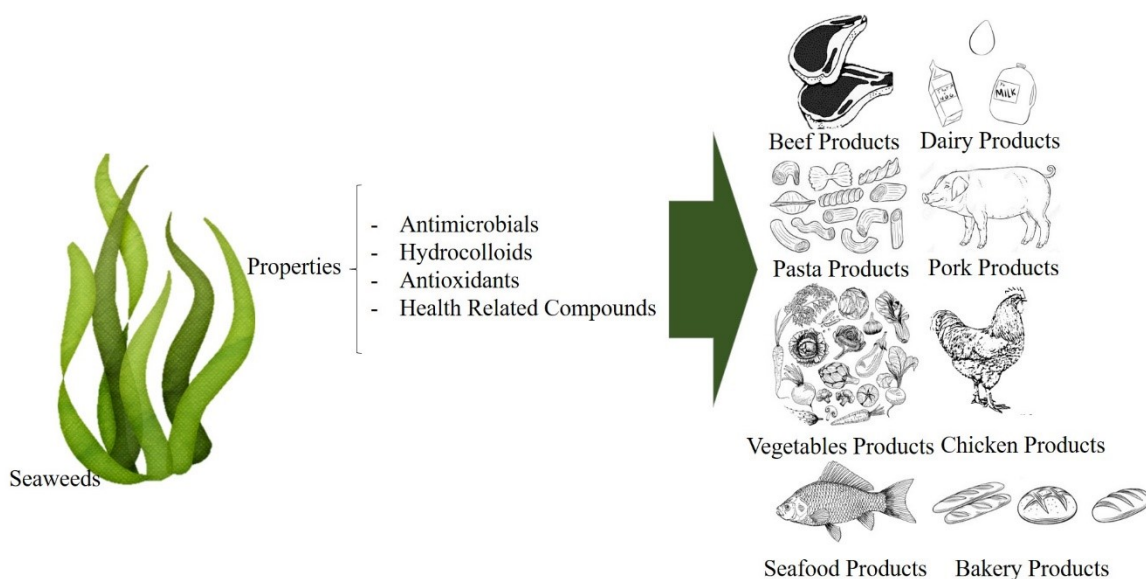


Figure 1-6- Application of seaweeds and seaweed extracts to develop new food products (Adapted from Roohinejad *et al.*, 2017).

Studies have been demonstrating the promising properties of macroalgae extracts as anticoagulant, anti-proliferative, antiviral, antimicrobial, among others (Wells *et al.* 2017; Funahashi *et al.* 2001; Mohamed, Hashim, and Rahman 2012; Gupta and Abu-ghannam 2011; Santos *et al.* 2015; Qin 2018), mainly due to their low content in lipids, high concentration in polysaccharides, natural richness in minerals, polyunsaturated fatty acids and vitamins as well as their content in bioactive molecules (Gupta and Abu-ghannam 2011).

A summary of some studies about the nutraceutical activity of compounds of brown seaweeds is presented in Table 1-5.

Table 1-5- Biologically active compounds (nutraceuticals) present in seaweed used as therapeutic agents (Adapted from Pereira, 2016).

Category	Compounds	Seaweed	Potential health benefit	Reference
<b>Lipids and fatty acids</b>	Omega 3	<i>U. pinnatifida</i>	Prevention of cardiovascular diseases, osteoarthritis and diabetes	(Mišurcová, Ambrožová, and Samek 2011)
	Omega 6 acids	<i>S. fusiforme</i>		
<b>Carotenoids</b>	Fucoxanthin	Brown seaweeds	Antiangiogenic; Protective effects against retinol deficiency	(Miyashita and Hosokawa 2007; Sangeetha, Bhaskar, and Baskaran 2009)
	$\beta$ -carotene, lutein	<i>C. crispus</i>	Antimutagenic; Protective against breast cancer	(Lohrmann, Logan, and Johnson 2004)
<b>Minerals</b>	Iodine	<i>L. digitata spp.</i>	Treating thyroid goiter	(Holdt and Kraan 2011)
	Calcium	<i>U. pinnatifida</i>	Useful in the case of expectant mothers, adolescents and elderly that are exposed to a risk of calcium deficiency	
<b>Polyphenols</b>	Phlorotannins	Brown algae	Algicidal and bactericidal effect	(Nagayama et al. 2003; Ngo et al. 2011)
	Fucoidan	Brown algae	Anticoagulant and antithrombotic activity; Antitumor and immunomodulatory activity	(Béress et al. 1993; Witvrouw and De Clercq 1997; Feldman et al. 1999)
<b>Polysaccharides and dietary fibers</b>	Carrageenan, fucoidan	Red Algae, Brown algae	Antitumor	(Smit 2004)
	Carrageenan	Red algae (carrageenophyte)	Antiviral and anti-HIV; Antitumor	

<b>Vitamins</b>	Vitamin E	<i>Fucus spp.</i>	Vitamin E inhibits the oxidation of the low-density lipoproteins	(MacArtain et al. 2007)
	Vitamin C	<i>H. elongata</i> <i>P. palmata</i>	Strengthens the immune defence system, activates the intestinal absorption of iron, controls the formation of conjunctive tissue and the protidic matrix of bony tissue, and acts in trapping free radicals and regenerates	
<b>Proteins and amino acids</b>	Proteins, amino acids	<i>U. pinnatifida</i>	The high balance between the essential amino acids, which gives a high biological value to their proteins. Proteins, also, with high bioavailability (85-90%)	(Holdt and Kraan 2011)

### 1.4. Iodine health aspects

Iodine deficiency has been documented as a global problem since the last century (Lazarus 2014). The primary natural sources of iodine include seafood (e.g., seaweed, fish and shellfish), dairy products, eggs, fruits and vegetables grown on iodine-rich soil, and products made from grains (e.g., bread and cereals) (Roleda et al. 2018).

The Global Scorecard of Iodine Nutrition (evaluated in 2017) in the general population and pregnant women, (in Iodine Global Network - IGN ) showed that, in Portugal, although the general population registers an adequate iodine intake, the pregnant women had an insufficient iodine consumption. An inadequate iodine supply in pregnancy is prevalent in approximately 30% of the European countries (Lazarus 2014).

Iodine is a necessary part of a healthy diet, but the food supply contains very little of this chemical element (Pacquette et al. 2013). It is an essential element required in trace amounts for the biosynthesis of the thyroid hormones triiodothyronine (T3) and thyroxine (T4), that are involved in growth, bone formation, metabolism and brain development (Taylor et al. 2014).

Taylor et al. 2014, in “*Impact of iodine supplementation in mild-to-moderate iodine deficiency: systematic review and meta-analysis*” revealed that iodine deficiency in the first trimester of pregnancy was associated with increased odds of the intelligence quotient of offspring being affected, with the highest negative impact associated with the verbal intelligence quotient.

The recommended daily intake (RDI) of iodine for different age groups (yr), pregnant women and lactating is included in Table 1-6.

Table 1-6-Recommended daily intake of iodine for different age groups as defined by the World Health Organisation (WHO), International Council for the Control of the Iodine Deficiency Disorders (ICCIDD) and United Nations Children Fund (UNICEF).

<b>Population</b>	<b>WHO, UNICEF and ICCIDD <sup>(1)</sup> (µg/d)</b>
<b>Adults and children ≥ 12 yr</b>	150
<b>Pregnant woman</b>	250
<b>Lactating</b>	250
<b>Children 0-5 yr</b>	90
<b>Children 6-12 yr</b>	120

<sup>(1)</sup>Adapted from WHO 2001 and WHO et al. 2007.

On the other hand, the ingestion of iodine at levels above the RDI can also negatively affect human health. A thyroid dysfunction upon high iodine exposure can occur in vulnerable people with specific risk factors, including those with pre-existing thyroid conditions, the elderly, fetuses, and neonates (Roleda et al. 2018). Due to that fact, a tolerable upper intake level (UL) of iodine was established. UL refers to the highest average daily nutrient intake level, unlikely to pose a risk of adverse health effects to almost all (97.5%) healthy individuals (Andersson et al. 2007). Table 1-7 represented the UL values in Europe.

Table 1-7-Tolerable upper limit intake of iodine (µg/d).

<b>Population</b>	<b>Europe <sup>(1)</sup> (µg/d)</b>
<b>1-3 yr</b>	200
<b>4-6 yr</b>	250
<b>7-10 yr</b>	300
<b>11-14 yr</b>	450
<b>15-17 yr</b>	500
<b>Adults</b>	600
<b>Pregnant Woman</b>	600

<sup>(1)</sup>(European Food Safety Authority 2006)

#### 1.4.1. Iodine in macroalgae

The oceans are the most abundant source of bioavailable iodine due to leaching from the upper Earth’s crust (Roleda et al. 2018). So, all the seafood and seaweeds are a rich source of iodine.

The genus *Saccharina* alongside *Laminaria* has species that can accumulate iodine in the range of 3-10 g/kg dw (Lüning and Mortensen 2015), which result in the most active accumulators of iodine among living organisms (Küpper et al. 2008).

Besides family and species, several factors are responsible for iodine accumulation in macroalgae, such as the location of the cultivation site that will affect the content through varying latitude, salinity, tidal amplitude, and temperature (Schiener et al. 2015).

Preservation and storage conditions, such as desiccation and deep-freezing that are used to increase macroalgae shelf life, can affect the iodine content (Teas et al. 2004). The packaging material and whether the product is vacuumed or not, as well as the storage time, can allow to release volatile iodine species.

Seaweed products are commonly consumed dried, rehydrated, or boiled. However, these procedures can affect the nutritional value, chemical composition, and associated bioactivity of the macroalgal material and their influence on macroalgal iodine is still not well characterized. Some previous studies have indicated that an iodine loss occurs during processing (Nitschke and Stengel 2016; Teas et al. 2004; Lüning and Mortensen 2015).

## **1.5. Canned Fish**

It is not surprising that nowadays diets have become less balanced, and several studies have shown the relationships between dietary patterns and lifestyle habits with chronic illnesses (Galli and Risé 2009; Richards et al. 2017).

Fish has many health benefits and its high consumption may decrease the risk of chronic diseases, namely coronary heart disease, stroke, type two diabetes and certain cancers (Zhao et al. 2016). Consumption of fish and seafood should be promoted on a global scale (Galli and Risé 2009).

Seafood is well known to be a great source of iodine. In fish, iodine content is available both in organic and inorganic form. The organic form of iodine in fish varies with the species, age, size, growth, and gender of the animals (Leloup and Fontaine 1960; Huang and Hickman 1968).

In Portugal, mackerel is the species that reached the largest national fishing quota since 2013, followed by horse mackerel and sardine (PORDATA 2018). Due to the decrease of the sardine fishery, the mackerel has been the most promising substitute product in the canning industry.

Mackerel is an excellent source of omega-3 polyunsaturated fatty acids (PUFAs), which makes it a beneficial choice regarding coronary artery diseases, as well as affecting psychological health such as depression (Kristinsd, Kristinsson, and Karlsd 2016). Currently, it is possible to find several mackerel canned products, in the Portuguese market, with lemon, spices, tomato, among others.

Although canned tuna is the most consumed product, this fish is a top chain species. Consequently, it yields large heavy metal bioaccumulation. It is well known to accumulate a substantial amount of mercury compared to some other fish species, for example, sardine or mackerel (Sobhanardakani, Hosseini, and Tayebi 2018; Ikem and Egiebor 2005).

Many studies have shown that the nutritional value of canned food is as good as that of its fresh or frozen counterparts (Featherstone 2015). Canned foods have several advantages for the fish consumers around the world: long preservation time, availability all year, favorable cost and nutrition compared with fresh and frozen forms, and short cooking time. Contrary to popular belief, canned fish can be a part of a healthy balanced diet (Featherstone 2015).

Recently, the intake of canned foods has increased and has become one of the fastest-growing practices around the world (Park et al. 2018; Richards et al. 2017). This trend is because it is a good substitute for fresh fish while accessible to the population who cannot afford to buy fresh or frozen fish. Europe is the largest canned market due to the high density of the community and consumers with a health-conscious lifestyle (García-del-hoyo, Jiménez-toribio, and Guillotreau 2017).

## **1.6. Thesis objectives**

The purpose of this thesis was the study of the impact of macroalgae processing (boiling and rehydration) to assess the effect on the levels of iodine and other essential minerals (Se, Na, and K) and arsenic.

The main objectives of this work are the development of a new and accessible food product rich in iodine and other essential minerals in order to fill the gap that the current European diet has, but also to develop a product with appealing sensory qualities. To achieve these main objectives, a canned mackerel enriched with seaweeds was developed.

# CHAPTER 2

## 2. MATERIALS AND METHODS

### 2.1. Apparatus

Ultrapure water, resistivity 18.2 M $\Omega$  cm, obtained from a Milli-Q Simplicity 185 system, (Millipore, Molsheim, France), was used throughout this work.

Sample drying was performed in an Excalibur 4900 food dryer (Sacramento, USA).

An ultra-centrifugal mill ZM 200 grinder (Rech, Germany), with a 2.0 mm fixed ring sieve, was used to grind and homogenize the dehydrated samples.

The moisture content was determined using in an oven J.P. Selecta, S.A. (Barcelona, Spain).

Microwave-assisted acid digestion was performed in a MARS-X 1500 W (Microwave Accelerated Reaction System for Extraction and Digestion, CEM, Mathews, NC, USA), using 12 PTFE digestion vessels and temperature (Probe RTP - 300 Plus, CEM, Mathews, NC, USA;  $\pm 3^{\circ}\text{C}$ ) and pressure (Digital Pressure Gauge ESP 1500 Plus, CEM, Mathews, NC, USA;  $\pm 10$  psi) control sensors.

The analysis of iodine, selenium, and total arsenic was performed in an inductively coupled plasma - mass spectrometry iCAP<sup>TM</sup> Q ICP-MS instrument (Thermo Fischer Scientific, Bremen, Germany).

A ContrAA 700 high-resolution continuum source flame atomic absorption spectrometer (HR-CS-FAAS, Analytik Jena, Germany) was used for the analysis of sodium and potassium.

The autoclave used was Uniclave 88, ajc, Cacém, Portugal, to do steam sterilization under pressure of the canned fish samples.

## 2.2. Reagents

The following reagents were used: Suprapure 65% (v/v) nitric acid (HNO<sub>3</sub>) from Merck, (Darmstadt, Germany), nitric acid 69% (v/v) *p.a.* (HNO<sub>3</sub>) from Panreac (Barcelona, Spain), Ammonium hydroxide 25% (v/v) (NH<sub>4</sub>OH) from Sigma-Aldrich, (Steinheim, Germany), stock standard solutions of Na and K (1000 mg/L) from Carlo Erba Reagents (Barcelona, Spain), cesium chloride (CsCl) from Panreac, (Barcelona, Spain), ICP-MS 200.8-CAL1-1 (Isostandards Material, Madrid, Spain), ICP-MS 200.8-CAL2-1 (AccuTrace Reference Standard from AccuStandard, USA), and Plasma CAL Q.C.N.3 (SCP Science, Canada).

All the container materials were washed before use with regular and deionized water, were soaked in a 10% (v/v) nitric acid bath, then rinsed several times with deionized water before use to avoid contamination.

## 2.3. Seaweeds collection

The species of seaweed used in this study were: *C. crispus*, *S. latissima*, *L. digitata* ('Kombu') and *U. pinnatifida* ('Wakame'). The last two species were sold by ALGAMAR and Trevijano S.L, respectively, in the dehydrated form, *S. latissima* was obtained in multitrophic salmon aquaculture in Norway. *C. crispus* was sold dehydrated by ALGA+.

The seaweeds were stored in sealed bags at room temperature in the dark during the entire study.

## 2.4. Seaweeds processing

As illustrated in Figure 2-1, seaweed samples (*ca.* 5 g) were subjected to several sequential treatments. After 5 min of hydration in 50 mL Milli-Q water, samples were drained for 5 minutes. Then, samples were boiled in 50 mL Milli-Q water during 1 to 20 min being subsequently dehydrated for 11 hours at 42 °C in a food dryer. Finally, samples were homogenized by grinding at 8000-12000 rpm using a 2.0 mm sieve and stored until analysis.

The water used in this study was also analyzed for the same elements as all the samples.

Due to the interest of the study, the hydration and boiling waters were kept frozen (- 4°C) for analysis. All the procedures were made in triplicate.

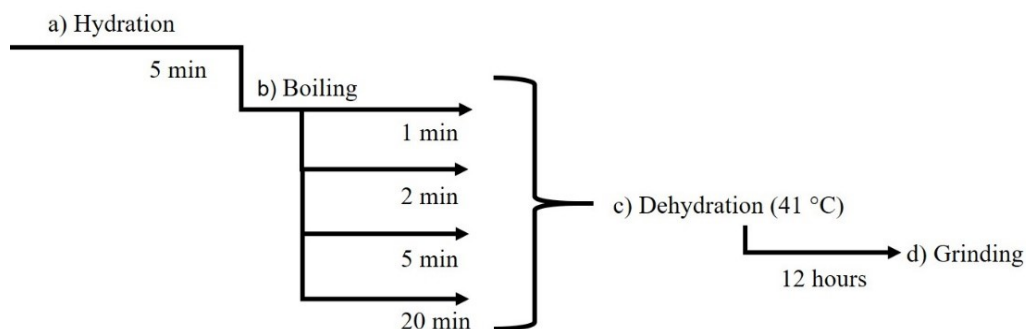


Figure 2-1-Schematic flow chart summarising the processing of the samples: a) Hydration b) Boiling c) Dehydration and d) Grinding.

## 2.5. Fish collection

The mackerel was chosen take into account its weight and freshness. All samples were from the Atlantic Ocean and weighed around 400 g each. The samples were kept frozen in sealed bags before the processing step.

## 2.6. Fish processing

After the removal of viscera, mackerel was immersed in a 15% brine (182.0 g of salt per litre of water) for 15 minutes.

The fish was cooked with the skin and head at 100°C in a steam pan for 30 min. Finally, it cooled until room temperature. After this period, the fishes were divided into uniform fillets.

## 2.7. Canning production

Canning production is presented in Figure 2-2. Samples were made in triplicate targeting 120 g as the final mass of the canned product.

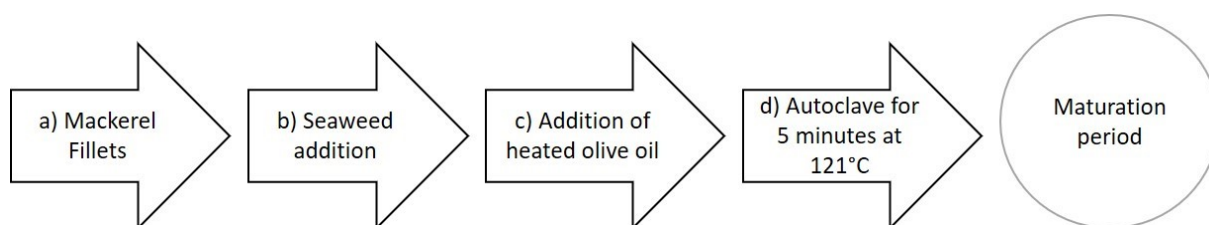


Figure 2-2- Schematic flow chart summarising the canning production: a) Mackerel fillets b) Seaweed addition c) Heated olive oil addition until all the fish and seaweeds are covered, d) Autoclave sterilization and maturation period.

The prepared samples are represented in Table 2-1. The quantity and macroalgae type, as well as the processing applied, were based on the preliminary tests previously performed (please see chapter 3, section 3.1) in the present study.

Table 2.1- Canned products prepared with seaweeds and mackerel with an average mass of 120 g.

<b>Samples</b>			
<b>Control mackerel with salt treatment</b>	84 g of mackerel (min 70% <sup>(1)</sup> )	130 mL of refined olive oil	
<b>Control Canned mackerel without salt treatment</b>	84 g of mackerel (min 70% <sup>(1)</sup> )	130 mL of refined olive oil	
<b>Canned mackerel with dehydrated <i>Saccharina latissima</i></b>	82 g of mackerel	250 mL of refined olive oil	2 g commercial dried <i>S. latissima</i>
<b>Canned mackerel with hydrated <i>Undaria pinnatifida</i></b>	80 g of mackerel	115 mL of refined olive oil	5 g ww rehydrated <i>U. pinnatifida</i>
<b>Canned mackerel without salt treatment and hydrated <i>Undaria pinnatifida</i></b>	72 g of mackerel	200 mL of refined olive oil	12 g of <i>Undaria</i> (wet weight)

<sup>(1)</sup> Legislation applied to canned products (Jornal Oficial das Comunidades Europeias 1992).

## 2.8. Sensory analysis

After the maturation period, a sensory analysis was performed in the laboratory. A sensory form/questionnaire (presented in Appendix A) was elaborated and distributed to all the volunteers that were going to taste the product, in order to register each opinion.

The analysis consisted of opening the canned product, identifying the differences between the samples, and finally, the flavour of the product. The tasting process involved the evaluation of:

- 1) visual aspect of the product in general (fish coloration, algae colour, and overall impact);
- 2) aroma of the macroalgae and fish (individually);
- 3) flavour and texture of macroalgae and fish (tasted mixed and independently).

The general opinions were recorded using a scale of 1 to 6 (consider 1-Did not like at all and 6- Liked very much).

## 2.9. Moisture

Moisture was evaluated using *ca.* 2 g of the homogenized sample according to the official AOAC method (AOAC 2007).

All the pans were prepared, washed in an acid bath, and dried in the oven at 100 °C before used. The sample was heated under the specified conditions of the AOAC method (AOAC 2007), and the loss of weight was used to calculate the moisture content of the sample.

### 2.10. Microwave-assisted acid digestion

All the samples were homogenized, as previously described, before carrying out the digestion procedures.

For acid digestion, *ca.* 0.2 g of homogenized sample was weighed in a microwave PTFE vessel, and 10,0 mL of suprapure nitric acid were added. The applied microwave program was as follows: 15 min at 50 °C and 150 psi (10.2 atm) (stage 1), 10 min at 100 °C and 200 psi (13.6 atm) (stage 2) and 15 min at 150 °C and 200 psi (13.6 atm) (stage 3). For olive oil, to avoid vessel overheating, the final temperature was changed to 140 °C.

After cooling down until below 40 °C, the vessels were opened, and 2.5 mL of the sample acidic extract were added to 20 mL of NH<sub>4</sub>OH (1:1; v/v) solution to performed iodine analysis. The remaining volume was stored in polyethylene flasks until metal analysis.

In the canned fish samples, seaweed, fish, and olive oil were digested separately.

### 2.11. Selenium, iodine and arsenic determination

For selenium and arsenic quantification, the following isotopes were monitored by Inductively Coupled Plasma - mass spectrometry (ICP-MS): <sup>75</sup>As, <sup>82</sup>Se, including the yttrium isotope (<sup>89</sup>Y), which was used as an internal standard. An external standard curve was constructed with seven standards with concentrations between 0.5 e 100 µg/L (parameters of the standard curve presented in the Appendix). The calibration standards were prepared from 100 mg/L multielement standard solutions, according to Cabrita *et al.* (2016).

For the quantification of iodine, the AOAC Official First Action Method was considered. After digestion of the sample with nitric acid in a closed vessel microwave oven, alkalisation of the samples was immediately performed. This method was applied because iodine is volatile in acidic medium (I<sub>2</sub> or HI), so the alkalisation through ammonium hydroxide promotes the conversion of volatile iodine to a more stable form (I<sup>-</sup> or IO<sub>3</sub><sup>-</sup> species). Iodine was measured by ICP- MS, according to Leite *et al.* (2017); the elemental isotope <sup>127</sup>I was used for quantification, and the tellurium (<sup>125</sup>Te) isotope was monitored as the internal standard.

### **2.12. Sodium and potassium analysis**

Calibration standards of sodium and potassium for HR-CS-FAAS analysis were prepared from single element standard solutions. External calibration curves were constructed daily, with eight standards solutions, between 0.1 mg/L and 4.5 mg/L or 6 mg/L to potassium and sodium, respectively (Appendix C).

Analytical blanks (CsCl 0.1% and HNO<sub>3</sub> 1%) and standard solutions were analysed along with the samples to check instrument performance. Appropriate dilutions of samples were prepared with a solution of 1% HNO<sub>3</sub> and 0.1% CsCl. All measurements were performed in triplicate.

# CHAPTER 3

## 3. RESULTS AND DISCUSSION

### 3.1. Characterization of Seaweeds and Extracts

The macroalgae available in the market undergo processing by the consumer, i.e., rehydration or boiling to be more edible. During the rehydration process, the particles will swell as they bind with water. At the same time, some hydro-soluble components can be transferred to the water, representing a loss of total solids. The seaweed samples were submitted to preliminary processes that intended to mimic the homemade cooking techniques performed by consumers. The samples were rehydrated and boiled during different periods, usually according to the label instructions of the products. Minerals were quantified in the extracts and seaweeds in order to assess the amount of minerals still present in the algae as consumed after processing.

Seaweeds are abundant in essential minerals, but in many species, there is also the presence of toxic metals that can hinder human health benefits. The contents of total arsenic, iodine, selenium, potassium, and sodium were quantified in the macroalgae and treatment water (extract) to assess the safety of their consumption. The iodine content was the main focus of the study, and even though the ingestion of this component is essential, the excess can be dangerous to human health as well. One of the purposes of this quantification was to assure that the RDI of iodine was not exceeded. As described in chapter 1 one-third of the population is at risk of iodine deficiency, particularly those who lives in areas that only have a small amount of iodine in the soil (including European countries). The quantification of arsenic and sodium had the same purpose. These minerals can be found at concentrations in macroalgae that can be linked to several health problems. Besides iodine, selenium and potassium were also analysed in macroalgae due to their known benefits to well-being (Pereira 2018b).

#### 3.1.1. Moisture content

The water content in seafood is critical since it affects their sensorial quality, microbiological stability, physical characteristics and shelf life (Mota et al. 2008). The water level in the

processed samples was determined before MAD, and HR-CS-FAAS and ICP-MS quantifications. The moisture results obtained for the four characterized species of algae, after and before processing, are presented in Table 3-1.

Table 3-1- Moisture (%) (n=3) for the species of macroalgae in the study (*C. crispus*, *U. pinnatifida*, *L. digitata* and *S. latissima*).

Process	Macroalgae species			
	<i>U. pinnatifida</i>	<i>L. digitata</i>	<i>S. latissima</i>	<i>C. crispus</i>
<b>% Moisture (w/w), (n=3)</b>				
<b>Dehydrated sample (commercial)</b>	11.2	13.4	8,5	12,98
<b>Rehydrated 5 min</b>	75.7	79.0	93.0	69.2
<b>Boiled 1 min</b>	89.0	84.5	91.4	83.4
<b>Boiled 2 min</b>	89.8	87.5	92.7	81.1
<b>Boiled 5 min</b>	92.5	87.5	90.6	84.6
<b>Boiled 20 min</b>	89.3	86.2	94.3	85.5

The values obtained for samples rehydrated for 5 min were very similar to the results found in the literature for the *Laminaraceae* (kelp) family (73-90%), which includes the *L. digitata*, *S. latissima* and *U. pinnatifida* (Guiry 2011). Previous studies have proved that some brown algae have moisture-absorbent polysaccharides that help retaining water and excrete a thick layer of a viscous liquid on their surfaces to keep them moist (Mouritsen, Mouritsen, and Johansen 2013). This detail was more evident in the brown macroalgae *L. digitata*, where this polysaccharide layer was clearly observed.

There are no significant variations of the water levels when comparing the different boiling periods, even among different seaweed species. This may occur since hot water promotes the diffusion of water to the cell walls increasing its humidity levels in all species. *S. latissima* was the more challenging species to dehydrate after boiling because it presented the formation of a crystallized layer during the drying process. This observation could be justified by its characteristic composition (commonly named ‘sugar kelp’ because of a whitish, sweet-tasting powder on the dried frond) (White and Marshall 2007).

Morphology, as well as composition, also influences water percentage. Some Rhodophyta species may have a relatively low water content when compared to other macroalgae types (Mota et al. 2008). The red algae *C. crispus* presented a value of 69% lower than the reported value of 80% (USDA National Nutrient Database for Standard Reference, 2015).

The (commercial) dehydrated samples presented humidity values close to the label information and were around 10% of total weight.

### 3.1.2. Arsenic

Seaweeds are known to contain high concentrations of arsenic in comparison to terrestrial plants owing to the ability of marine plants to concentrate the arsenic derive from seawater (Rose et al. 2007). Arsenic was quantified in the rehydration and boiled waters for each species of macroalgae. The results are presented in Table 3-2, and Figure 3-1 as mean  $\pm$  SD mg As/L (n=9).

Table 3-2- Total arsenic (mg As/L) content in water extracts of macroalgae species *C. crispus*, *U. pinnatifida*, *L. digitata* and *S. latissima* (n=9).

Process	Mean $\pm$ SD mg As/L			
	<i>U. pinnatifida</i>	<i>L. digitata</i>	<i>S. latissima</i>	<i>C. crispus</i>
H 5 min	0.74 $\pm$ 0.07	1.95 $\pm$ 0.18	1.42 $\pm$ 0.14	0.63 $\pm$ 0.18
B 1 min	0.44 $\pm$ 0.09	0.62 $\pm$ 0.02	1.41 $\pm$ 0.12	0.30 $\pm$ 0.07
B 2 min	0.47 $\pm$ 0.05	0.94 $\pm$ 0.05	1.12 $\pm$ 0.10	0.47 $\pm$ 0.05
B 5 min	0.64 $\pm$ 0.11	1.13 $\pm$ 0.13	2.71 $\pm$ 0.11	0.36 $\pm$ 0.02
B 20 min	0.54 $\pm$ 0.09	1.10 $\pm$ 0.02	1.75 $\pm$ 0.26	0.57 $\pm$ 0.12

Process: H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

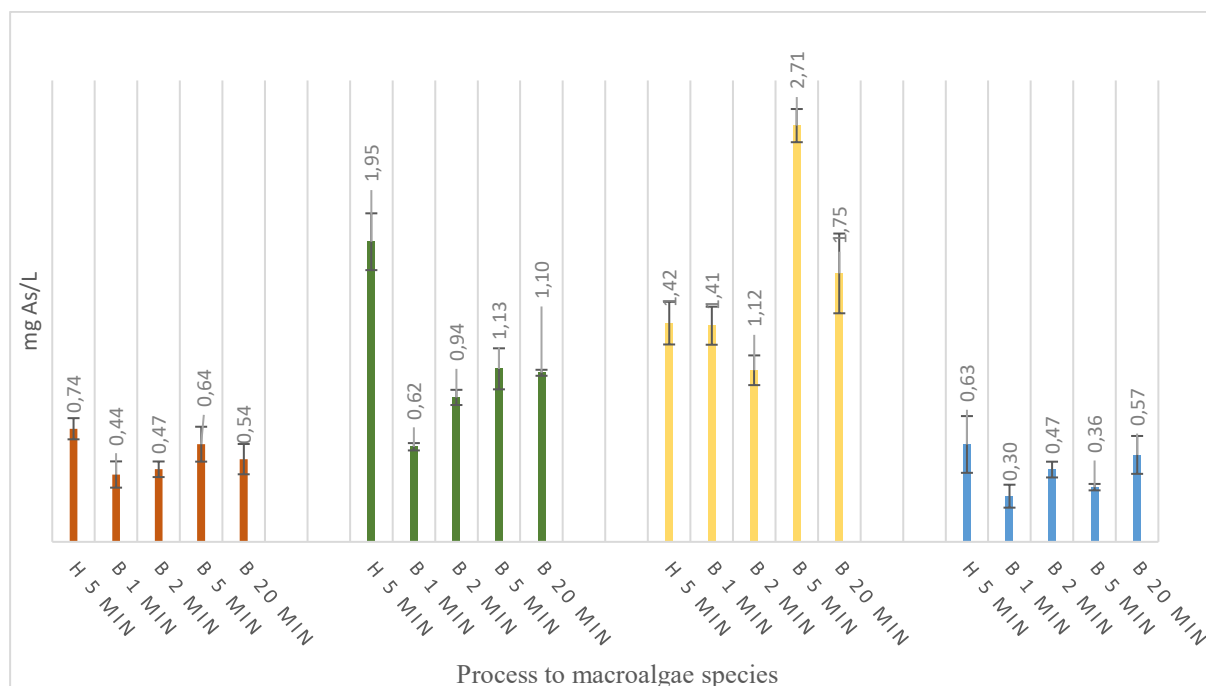


Figure 3-1 - Arsenic concentration (mg As/L) in water extracts of species *U. pinnatifida* (orange); *L. digitata* (green); *S. latissima* (yellow); *C. crispus* (blue).

The results obtained for the water extracts of the species *U. pinnatifida* show that no significant variations occur after the different treatments. Rehydration water displays an amount of 0.74 mg As/L and 0.54 mg As/L after 20 min boiling. These data suggest that loss of As to the water occurs mainly in the rehydration step, and the values obtained were consistent throughout all the process.

The results for the brown species *S. latissima* and *L. digitata* present a higher variability. This was expected since small variations in the mass, soaking time, and exposed surfaces can result in higher variations. However, a major As increase can be observed in the rehydration water of the *L. digitata* and on the boiling water for the *S. latissima* species.

The red algae species, *C. crispus*, presented a behaviour similar to *U. pinnatifida*, which could be justified by the soft form of this species compared with the other studied macroalgae.

As a conclusion, As levels can be minimized with a simple immersion or cleaning step, since there is a transfer between macroalgae and water. Arsenic is water-soluble, as described, so it was expected that in arsenic-free water, the transfer occurs to the water.

The total arsenic content in the sample species *U. pinnatifida*, *L. digitata*, *S. latissima*, and *C. crispus* are presented in Table 3-3, Figure 3-2, and Figure 3-3. The dry commercial form (dw commercial) represents the dry weight normalized with the humidity value found in the commercialized seaweed.

Table 3-3-Total arsenic content ( $\mu\text{g As/g}$ ) present as mean  $\pm$  SD (n=9) in seaweeds in wet weight (ww), dry weight (dw) and commercial dry weight (dw commercial).

	$\mu\text{g As/g ww}$	$\mu\text{g As/g dw}$	$\mu\text{g As/g dw commercial}$
<i>U. pinnatifida</i>			
<b>U</b>	8.83 $\pm$ 0.59	40.91 $\pm$ 2.73	36.37 $\pm$ 2.43
<b>H 5 min</b>	7.62 $\pm$ 0.62	31.41 $\pm$ 2.54	27.93 $\pm$ 2.26
<b>B 1 min</b>	3.82 $\pm$ 0.27	34.71 $\pm$ 2.44	30.86 $\pm$ 2.17
<b>B 2 min</b>	2.61 $\pm$ 0.18	25.69 $\pm$ 1.76	22.84 $\pm$ 1.56
<b>B 5 min</b>	2.06 $\pm$ 0.16	27.56 $\pm$ 2.18	24.50 $\pm$ 1.94
<b>B 20 min</b>	2.72 $\pm$ 0.14	25.40 $\pm$ 1.34	22.58 $\pm$ 1.19
<i>L. digitata</i>			
<b>L</b>	11.37 $\pm$ 1.24	73.95 $\pm$ 8.09	64.01 $\pm$ 7.00
<b>H 5 min</b>	7.98 $\pm$ 1.17	51.91 $\pm$ 5.59	44.93 $\pm$ 4.83
<b>B 1 min</b>	5.39 $\pm$ 0.30	35.04 $\pm$ 1.94	30.33 $\pm$ 1.68
<b>B 2 min</b>	5.82 $\pm$ 0.51	37.84 $\pm$ 4.13	32.75 $\pm$ 3.57
<b>B 5 min</b>	5.66 $\pm$ 0.29	36.85 $\pm$ 2.31	31.90 $\pm$ 2.00
<b>B 20 min</b>	1.41 $\pm$ 0.11	21.97 $\pm$ 1.65	24.71 $\pm$ 1.86
<i>S. latissima</i>			
<b>S</b>	5.18 $\pm$ 0.55	64.08 $\pm$ 6.79	58.61 $\pm$ 6.21
<b>H 5 min</b>	3.23 $\pm$ 0.13	46.04 $\pm$ 1.84	42.10 $\pm$ 1.68
<b>B 1 min</b>	3.63 $\pm$ 0.12	42.40 $\pm$ 1.38	38.78 $\pm$ 1.27
<b>B 2 min</b>	2.92 $\pm$ 0.12	39.90 $\pm$ 1.60	36.49 $\pm$ 1.46
<b>B 5 min</b>	4.49 $\pm$ 0.29	47.64 $\pm$ 3.12	43.57 $\pm$ 2.85
<b>B 20 min</b>	3.95 $\pm$ 0.09	25.23 $\pm$ 0.58	28.72 $\pm$ 0.66
<i>C. crispus</i>			
<b>C</b>	4.50 $\pm$ 0.63	14.59 $\pm$ 2.04	12.97 $\pm$ 1.82
<b>H 5 min</b>	3.53 $\pm$ 0.07	11.44 $\pm$ 0.23	10.17 $\pm$ 0.20
<b>B 1 min</b>	1.51 $\pm$ 0.08	9.13 $\pm$ 0.47	8.11 $\pm$ 0.42
<b>B 2 min</b>	0.97 $\pm$ 0.22	8.32 $\pm$ 0.38	7.39 $\pm$ 0.34
<b>B 5 min</b>	0.98 $\pm$ 0.07	9.16 $\pm$ 0.67	8.14 $\pm$ 0.60
<b>B 20 min</b>	1.57 $\pm$ 0.07	10.83 $\pm$ 0.46	9.63 $\pm$ 0.41

C: *C. crispus* (commercialized dehydrated form); U: *U. pinnatifida* (commercialized dehydrated form); L: *L. digitata* (commercialized dehydrated form); S: *S. latissima* (commercialized dehydrated form); C: *C. crispus* (commercialized dehydrated form);

Process: H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

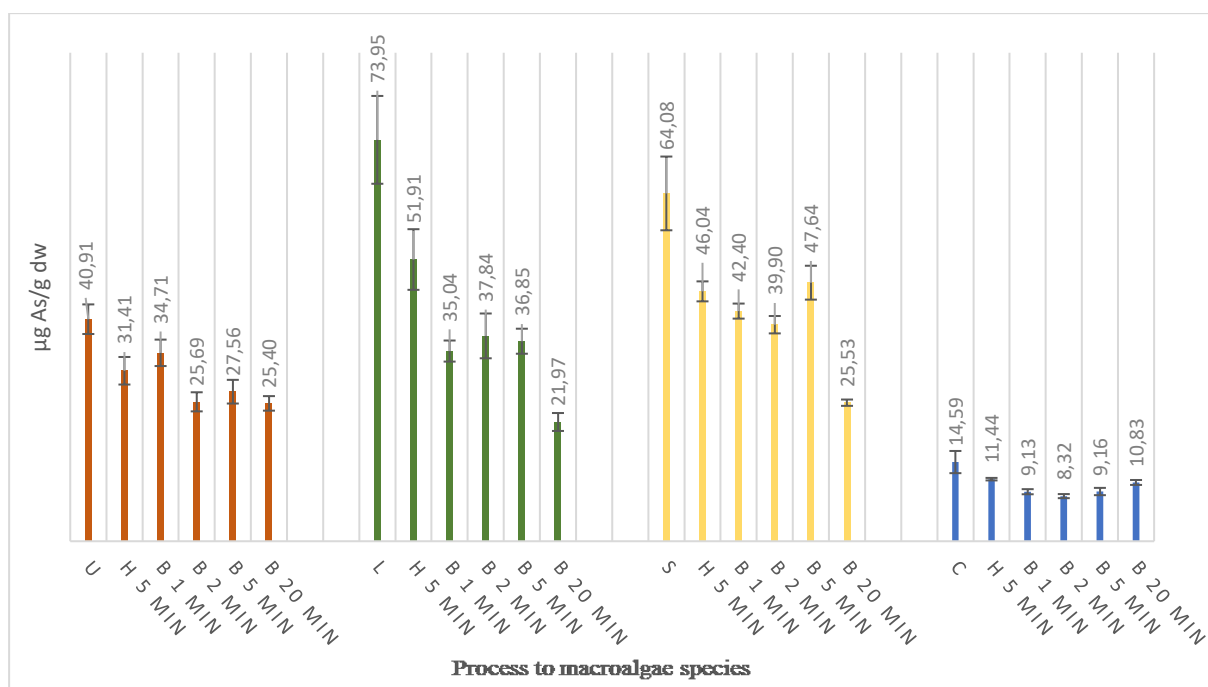


Figure 3-2- Arsenic concentration ( $\mu\text{g As/g dw}$ ) in the macroalgae *U. pinnatifida* (orange), *L. digitata* (green), *S. latissima* (yellow), and *C. crispus* (blue) after hydration and boiled for 1, 2, 5, and 20 minutes.

Processing applied to the macroalgae species: U: *U. pinnatifida* (commercialized dehydrated form); L: *L. digitata* (commercialized dehydrated form); S: *S. latissima* (commercialized dehydrated form); C: *C. crispus* (commercialized dehydrated form); H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

The reported values for As in brown algae vary between 21-120  $\mu\text{g/g dw}$  (Biancarosa et al. 2018). Different levels of arsenic in algae could be attributed to the arsenic retention, absorption and excretion capacities associated with each species (Fariás et al. 2007). The predominance of this element in brown macroalgae when compared with Chlorophyta and Rhodophyta was also observed. Overall the highest levels of As were found in brown seaweeds, while red and green macroalgae showed the lowest values (Circuncis et al. 2018).

A literature review of total As (tAs) concentration previously reported for each species (Table I-4) shows for *L. digitata*, 27-49  $\mu\text{g/g dw}$ , *S. latissima*, 28-120  $\mu\text{g/g dw}$ , *U. pinnatifida*, 32-70  $\mu\text{g/g dw}$  and *C. crispus*, 4-26  $\mu\text{g/g dw}$  of As. In the present study, the values obtained were: 40.91  $\mu\text{g/g dw}$  for *U. pinnatifida*, 73.95  $\mu\text{g/g dw}$  for *L. digitata*, 64.08  $\mu\text{g/g dw}$  for *S. latissima* and 14.59  $\mu\text{g/g dw}$  for *C. crispus*. The results are in general agreement with the literature (Biancarosa et al. 2018; Cabrita et al. 2016; Rupérez 2002; Pereira 2016; Kolb et al. 2004; Circuncis et al. 2018).

The first step that the consumer usually take before macroalgae consumption is washing and rehydrating by following the seller instructions. Total As after rehydration was superior in the

*L. digitata* (61.91  $\mu\text{g tAs/ g dw}$ ) and inferior in *C. crispus* (11.44  $\mu\text{g tAs/ g dw}$ ) as already reported in some studies (Desideri et al. 2016; Kolb et al. 2004; Rose et al. 2007). The values obtained after five minutes of rehydration shows that this step is very efficient in As removal for all the algae species. *C. crispus* showed the highest loss in rehydration, keeping values uniform during the entire process. In contrast, the three brown species revealed the highest loss when boiled for 20 minutes.

In Table 3-4, the total leachable fraction (considering 20 minutes of boiling) is presented using the ratio between the concentration of As lost throughout the process and As present in the dehydrated commercial algae.

Table 3-4- Total leachable fraction (%) for macroalgae species in this study after 20 min boiling.

	Macroalgae species			
	<i>U. pinnatifida</i>	<i>L. digitata</i>	<i>S. latissima</i>	<i>C. crispus</i>
<b>Total leachable fraction (%)</b>	37.9	70.3	60.2	25.7

The quantity of arsenic that leach out from *C. crispus* was the lowest. Sample *S. latissima* and *L. digitata* were the species in which the process had more impact on the arsenic amount. *U. pinnatifida* had an intermediate leachable fraction, as was expected due to the values found in the extracts and described previously.

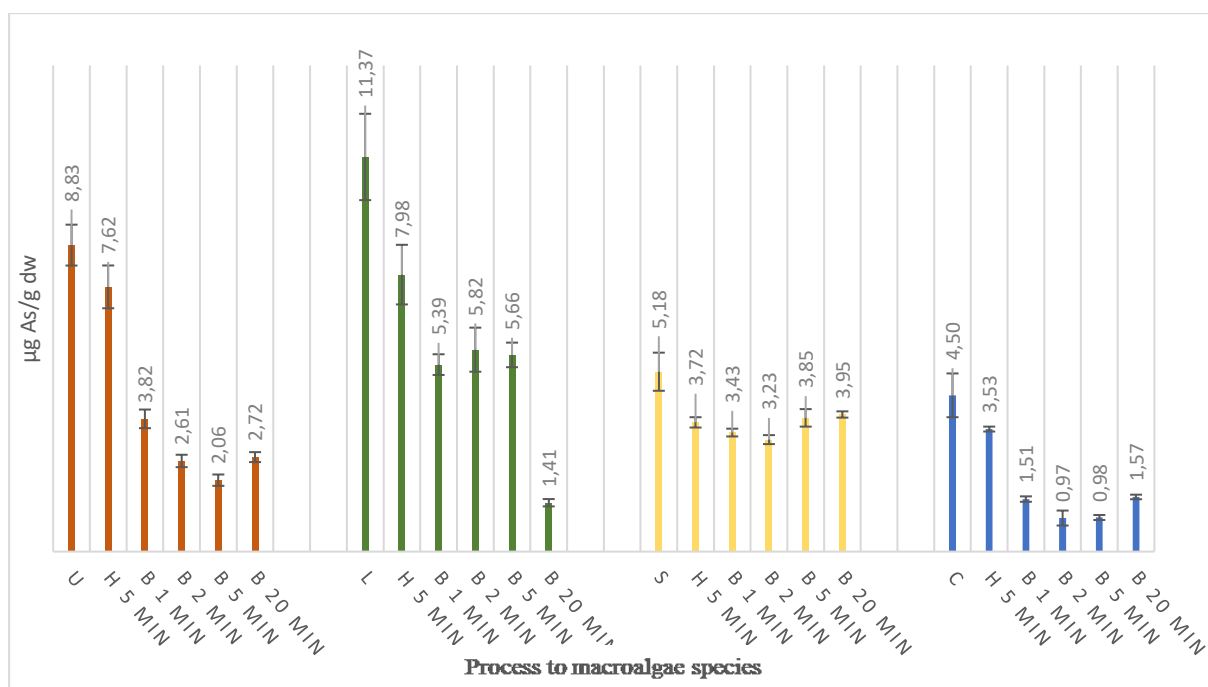


Figure 3-3- Arsenic concentration ( $\mu\text{g As/g ww}$ ) in the macroalgae *U. pinnatifida* (orange), *L. digitata* (green), *S. latissima* (yellow), and *C. crispus* (blue) after hydration and boiled for 1, 2, 5, and 20 minutes.

Processing applied to the macroalgae species: U: *U. pinnatifida* (commercialized dehydrated form); L: *L. digitata* (commercialized dehydrated form); S: *S. latissima* (commercialized dehydrated form); C: *C. crispus* (commercialized dehydrated form); H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

The results presented in Figure 3-3, are for macroalgae in the wet weight to demonstrate the tAs ingestion in a ready to consume product. In their fresh form, all the species followed the same pattern as in dry weight, as expected. *L. digitata* was the species where arsenic levels in commercial form were the highest ( $11.37 \mu\text{g As/g ww}$ ). However, after a boiling period of 20 minutes, this species displayed the lowest content ( $1.41 \mu\text{g As/g ww}$ ).

Arsenic in biological matrices exists in organic or inorganic (iAs) form, and while organoarsenic forms are considered to be non-toxic or of low toxicity, iAs is regarded as the most toxic form of As for living organisms (Biancarosa et al. 2018). Chronic exposure to inorganic arsenic leads to a higher incidence of several cancers, including skin, lung, and urinary tract (Wells et al. 2017). To ensure the safe consumption of the studied species, the As levels were presented as tAs and iAs (Table 3-5). The maximum intake of the fresh macroalgae per day (g ww/day) considering an adult with 69 kg is also presented.

Table 3-5- Total arsenic concentration and inorganic arsenic concentration ( $\mu\text{g As/g}$ ) in the *C. crispus*, *U. pinnatifida*, *L. digitata* and *S. latissima*; Maximum intake of a fresh algae portion for an adult (g ww/day) in accordance with the reference minimum level (0.3 to 8  $\mu\text{g iAs/kg}$  body weight per day; EU 2015/1006).

	<b>As total</b>	<b>iAs<sup>(1)</sup></b>	<b>Maximum portion of algae per day that can be consumed to reach 0.3 <math>\mu\text{g iAs/kg}</math> bodyweight per day<sup>(2)</sup></b>
	Mean $\pm$ SD $\mu\text{g tAs/g ww}$	Mean $\pm$ SD $\mu\text{g iAs/g ww}$	Mean $\pm$ SD g algae ww/day
<b><i>U. pinnatifida</i></b>			
<b>U</b>	8.83 $\pm$ 0.59	1.82 $\pm$ 0.121	47.11 $\pm$ 3.14
<b>H 5 min</b>	7.62 $\pm$ 0.62	0.38 $\pm$ 0.031	54.66 $\pm$ 4.42
<b>B 1 min</b>	3.82 $\pm$ 0.27	0.19 $\pm$ 0.013	108.83 $\pm$ 7.64
<b>B 2 min</b>	2.61 $\pm$ 0.18	0.13 $\pm$ 0.009	159.32 $\pm$ 10.91
<b>B 5 min</b>	2.06 $\pm$ 0.16	0.10 $\pm$ 0.008	202.51 $\pm$ 16.05
<b>B 20 min</b>	2.72 $\pm$ 0.14	0.14 $\pm$ 0.007	152.56 $\pm$ 8.04
<b><i>L. digitata</i></b>			
<b>L</b>	11.37 $\pm$ 1.24	0.57 $\pm$ 0.06	36.87 $\pm$ 5.21
<b>H 5 min</b>	10.91 $\pm$ 1.17	0.40 $\pm$ 0.04	52.50 $\pm$ 5.45
<b>B 1 min</b>	5.45 $\pm$ 0.30	0.27 $\pm$ 0.02	77.11 $\pm$ 5.15
<b>B 2 min</b>	4.71 $\pm$ 0.51	0.29 $\pm$ 0.03	72.05 $\pm$ 11.45
<b>B 5 min</b>	4.59 $\pm$ 0.29	0.28 $\pm$ 0.02	73.38 $\pm$ 6.99
<b>B 20 min</b>	1.41 $\pm$ 0.11	0.07 $\pm$ 0.005	295.84 $\pm$ 22.23
<b><i>S. latissima</i></b>			
<b>S</b>	5.18 $\pm$ 0.55	0.26 $\pm$ 0.03	80.82 $\pm$ 8.57
<b>H 5 min</b>	3.72 $\pm$ 0.15	0.12 $\pm$ 0.01	111.41 $\pm$ 4.45
<b>B 1 min</b>	3.43 $\pm$ 0.11	0.17 $\pm$ 0.01	120.90 $\pm$ 3.95
<b>B 2 min</b>	3.23 $\pm$ 0.13	0.16 $\pm$ 0.01	128.56 $\pm$ 5.16
<b>B 5 min</b>	3.85 $\pm$ 0.25	0.19 $\pm$ 0.01	107.96 $\pm$ 7.07
<b>B 20 min</b>	3.95 $\pm$ 0.09	0.20 $\pm$ 0.005	104.89 $\pm$ 2.40
<b><i>C. crispus</i></b>			
<b>C</b>	4.50 $\pm$ 0.22	0.23 $\pm$ 0.03	93.83 $\pm$ 13.15
<b>H 5 min</b>	3.53 $\pm$ 0.02	0.18 $\pm$ 0.004	117.36 $\pm$ 2.35
<b>B 1 min</b>	1.51 $\pm$ 0.05	0.08 $\pm$ 0.004	274.42 $\pm$ 14.13
<b>B 2 min</b>	0.97 $\pm$ 0.04	0.05 $\pm$ 0.01	450.94 $\pm$ 100.67
<b>B 5 min</b>	0.98 $\pm$ 0.07	0.05 $\pm$ 0.004	424.02 $\pm$ 31.00
<b>B 20 min</b>	1.57 $\pm$ 0.05	0.08 $\pm$ 0.003	263.52 $\pm$ 11.10

<sup>(1)</sup> Maximum % levels for inorganic As, i.e. 5% (Regulation (EU) 2015/1006; EFSA 2010; AFSSA 2009). <sup>(2)</sup> Considering a reference intake of 0.3  $\mu\text{g iAs/kg}$  bodyweight for day and 69 kg as the mean weight for an adult.

The scientific literature reports that 0.4–5.3% of As present in sea products is in the form of inorganic arsenic (Leblanc et al. 2007), and therefore, a percentage of 5% was considered in

this study to ensure that, in the worst scenario, the consumption was still safe. The Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) of the European Food Safety Authority (EFSA) adopted in 2015 the benchmark dose lower confidence limit (BMDL01) of 0.3 to 8  $\mu\text{g}$  iAs/kg body weight per day. As demonstrated in Table 3-4, by increasing the boiling time (20 min), the amount of macroalgae that can be safely consumed per day also increases because the iAs concentration levels decrease.

The seaweed with lower iAs after 20 min boiling was *L. digitata* with 0.07  $\mu\text{g}$  iAs/g ww. *L. digitata* was the species which revealed the more effective As elimination with the treatment: 0.57  $\mu\text{g}$  iAs/g ww present in the dehydrated commercial sample and 0.07  $\mu\text{g}$  iAs/g ww in the sample boiled for 20 minutes. The samples of *S. latissima* revealed the highest iAs even after 20 minutes boiling (0.20  $\mu\text{g}$  iAs/g ww).

Since it was estimated that levels of iAs could vary between 0.3 and 8  $\mu\text{g}$  iAs/ kg body weight per day, none of the species in the study represents a risk to human health. As it can be seen in Table 3-5 and considering that iAs is the most toxic element of seaweeds, it is possible to consume a significant quantity of algae per day without risk. For example, without any boiling, the consumption of *S. latissima* can reach a minimum of 111.4 g/ per day for an adult. The same can be verified in the red seaweed, *C. crispus*, which represents the species with the lower levels of iAs and with the highest possible consumption (117.36 g/ per day). Still, it is crucial to keep in mind that there are other potentially harmful components in seaweeds, so this analysis is only based on iAs.

Only a few studies have been evaluating the impact of processing in the macroalgae mineral content. In “*Arsenic in seaweed — Forms, concentration, and dietary exposure,*” tAs and iAs were quantified after preparation and cooking following the product package labels. The authors reported that for every treated macroalgae species, iAs and tAs decreased during preparation and cooking (Rose et al. 2007). Consider that cooking implies a boiling period, the obtained results were in accordance with this study.

### 3.1.3. Iodine

There is a wide diversity in iodine content according to macroalgae species (Hou et al. 1997). Exposure to seaweed-derived iodine through the consumption of macroalgae, as described in chapter 1, can positively or negatively influence human health, depending on whether adequate or excessive quantities are ingested. The iodine contents (mg I/L) in the water extracts of the characterized seaweeds are presented in Table 3-6 and Figure 3-4.

Table 3-6- Iodine (mg I/L) content in waters extracts for the macroalgae species *U. pinnatifida*, *L. digitata*, *S. latissima*, and *C. crispus*.

Process	Mean $\pm$ SD mg I/L			
	<i>U. pinnatifida</i>	<i>L. digitata</i>	<i>S. latissima</i>	<i>C. crispus</i>
H 5 min	37.38 $\pm$ 11.7	192.3 $\pm$ 25.9	51.90 $\pm$ 4.71	0.53 $\pm$ 0.34
B 1 min	49.74 $\pm$ 2.30	58.62 $\pm$ 6.21	97.25 $\pm$ 7.53	0.60 $\pm$ 0.07
B 2 min	56.46 $\pm$ 23.4	46.49 $\pm$ 3.51	56.58 $\pm$ 5.49	0.47 $\pm$ 0.05
B 5 min	89.98 $\pm$ 18.4	64.34 $\pm$ 7.22	121.9 $\pm$ 11.5	0.57 $\pm$ 0.02
B 20 min	122.7 $\pm$ 20.5	214.1 $\pm$ 33.7	211.9 $\pm$ 9.36	1.15 $\pm$ 0.12

Process: H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

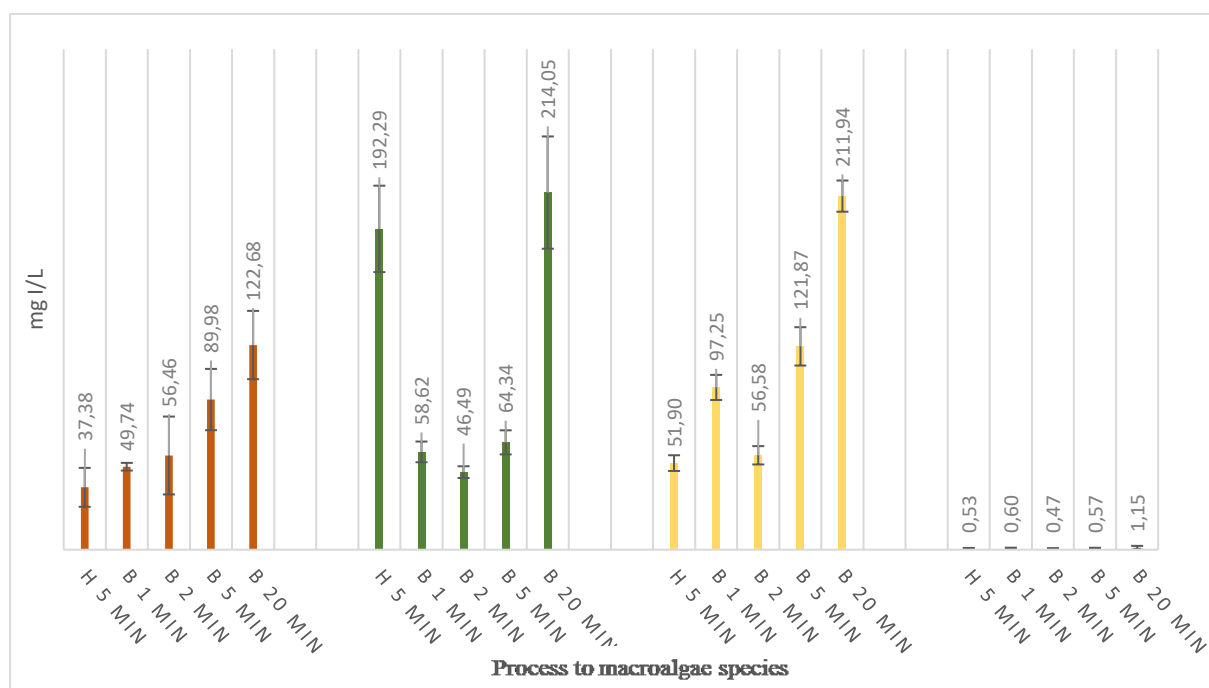


Figure 3-4- Iodine concentration (mg I/L) in water extracts for the species *U. pinnatifida* (orange); *L. digitata* (green); *S. latissima* (yellow); *C. crispus* (blue).

Processing of the macroalgae species: U: *U. pinnatifida* (commercialized dehydrated form); L: *L. digitata* (commercialized dehydrated form); S: *S. latissima* (commercialized dehydrated form); C: *C. crispus* (commercialized dehydrated form); H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

Edible macroalgae are considered rich in iodine, but total iodine content depends on species and taxonomy. Brown seaweeds usually present the highest levels of iodine. During the described processing, iodine values, when compared to arsenic, exhibited a gradual decline during the boiling period, being the minimum loss obtained in the samples boiled for 20 min. Both inorganic and organic iodine has been reported to be present in marine macroalgae (Nitschke and Stengel 2016; Meguro et al. 1967; Gall, Kupper, and Kloareg 2004). Depending on algal species, 9 to 99% of iodine may be water-soluble (mostly as inorganic iodine) (Hou et al. 1997). *Laminariales* are reported to have the highest inorganic iodine levels, more than 94%

existing mainly as I<sup>-</sup> (Hou et al. 1997). The iodine in its inorganic form can be completely dissolved in water. On the other hand, iodine remaining in the residues can be considered organic iodine. The obtained results of iodine content for the studied species are presented in Table 3-7, Figure 3-5, and Figure 3-6.

Table 3-7- Iodine content in seaweeds ( $\mu\text{g I/g}$ ) presented as mean  $\pm$  SD (n=9) in wet weight (ww), dry weight (dw) and commercial dry weight (dw commercial)

	$\mu\text{g I/g ww}$	$\mu\text{g I/g dw}$	$\mu\text{g I/g dw commercial}$
<i>U. pinnatifida</i>			
<b>U</b>	64.02 $\pm$ 8.74	263.8 $\pm$ 36.0	234.5 $\pm$ 32.0
<b>H 5 min</b>	30.87 $\pm$ 7.25	127.2 $\pm$ 29.9	154.1 $\pm$ 15.4
<b>B 1 min</b>	17.67 $\pm$ 1.79	160.4 $\pm$ 16.2	142.6 $\pm$ 14.4
<b>B 2 min</b>	10.49 $\pm$ 2.59	118.2 $\pm$ 17.1	105.1 $\pm$ 15.2
<b>B 5 min</b>	12.74 $\pm$ 3.69	170.6 $\pm$ 21.3	151.7 $\pm$ 18.9
<b>B 20 min</b>	15.54 $\pm$ 2.46	145.0 $\pm$	123.9 $\pm$ 20.4
		22.9	
<i>L. digitata</i>			
<b>L</b>	643.5 $\pm$ 13.0	4187 $\pm$ 84.5	3624 $\pm$ 73.2
<b>H 5 min</b>	548.0 $\pm$ 65.2	2607 $\pm$ 310.1	2257 $\pm$ 268.4
<b>B 1 min</b>	258.7 $\pm$ 14.6	1665 $\pm$ 93.9	1441 $\pm$ 81.3
<b>B 2 min</b>	196.8 $\pm$ 8.8	1580 $\pm$ 70.9	1368 $\pm$ 61.4
<b>B 5 min</b>	206.8 $\pm$ 21.9	1660 $\pm$ 175.4	1437 $\pm$ 151.8
<b>B 20 min</b>	58.2 $\pm$ 6.20	907.8 $\pm$ 96.8	1021 $\pm$ 108.8
<i>S. latissima</i>			
<b>S</b>	211.6 $\pm$ 12.8	2617 $\pm$ 158	2394 $\pm$ 145
<b>H 5 min</b>	157.4 $\pm$ 8.0	2247 $\pm$ 114	2055 $\pm$ 105
<b>B 1 min</b>	161.1 $\pm$ 8.8	1880 $\pm$ 103	1719 $\pm$ 94.2
<b>B 2 min</b>	129.2 $\pm$ 14.1	1763 $\pm$ 193	1612 $\pm$ 176
<b>B 5 min</b>	208.7 $\pm$ 17.9	2212 $\pm$ 189	2023.15 $\pm$ 172
<b>B 20 min</b>	17.87 $\pm$ 1.13	1037 $\pm$ 65.5	1169.49 $\pm$ 73.7
<i>C. crispus</i>			
<b>C</b>	239.2 $\pm$ 17.7	775.4 $\pm$ 57.4	689.3 $\pm$ 51.0
<b>H 5 min</b>	232.5 $\pm$ 7.14	753.6 $\pm$ 85.6	670.0 $\pm$ 20.6
<b>B 1 min</b>	123.9 $\pm$ 11.3	747.3 $\pm$ 62.8	664.3 $\pm$ 55.8
<b>B 2 min</b>	104.2 $\pm$ 3.69	551.9 $\pm$ 19.6	490.7 $\pm$ 17.4
<b>B 5 min</b>	104.9 $\pm$ 9.80	680.3 $\pm$ 63.6	604.8 $\pm$ 56.5
<b>B 20 min</b>	99.89 $\pm$ 5.47	687.3 $\pm$ 133	611.1 $\pm$ 118

U: *U. pinnatifida* (commercialized dehydrated form); L: *L. digitata* (commercialized dehydrated form); S: *S. latissima* (commercialized dehydrated form); C: *C. crispus* (commercialized dehydrated form)

Process: H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

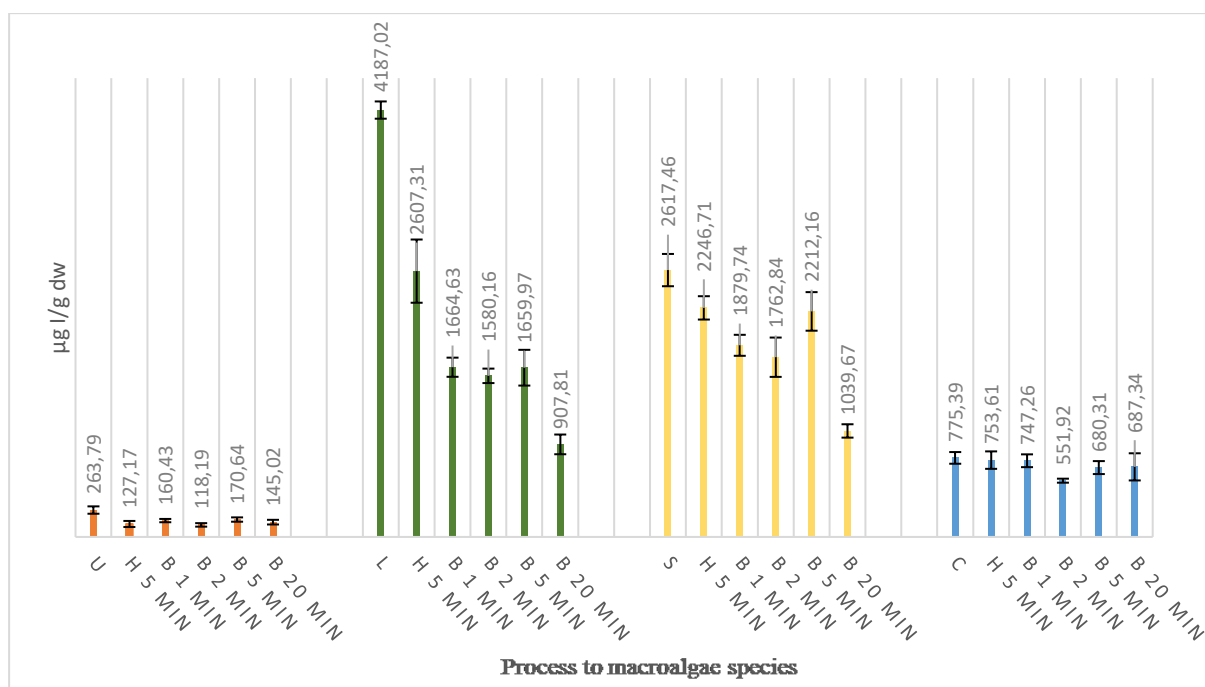


Figure 3-5- Iodine concentration ( $\mu\text{g I/g dw}$ ) in the macroalgae *U. pinnatifida* (orange), *L. digitata* (green), *S. latissima* (yellow), and *C. crispus* (blue) after hydration and boiled for 1, 2, 5, and 20 minutes.

Processing applied to the macroalgae species: U: *U. pinnatifida* (commercialized dehydrated form); L: *L. digitata* (commercialized dehydrated form); S: *S. latissima* (commercialized dehydrated form); C: *C. crispus* (commercialized dehydrated form); H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

The commercial sample of *L. digitata* presented a lower quantity ( $4187 \mu\text{g I/g dw}$ ) when compared with other values reported in the literature that can reach  $10 \times 10^6 \mu\text{g I/g dw}$ . This could be justified by the variations on the iodine level in the marine environment or losses during the processing of the commercial seaweed. As described in chapter 1, iodine concentration in the water is primarily affected by the surroundings (location, temperature, salinity, among others). The species analysed in this study was cultivated, so the expected iodine level was lower (Lüning and Mortensen 2015).

*S. latissima* presented iodine values of  $2617 \mu\text{g I/g dw}$ . In the literature the reported quantities varied between  $957.6$  and  $46 \times 10^5 \mu\text{g I/g dw}$ . This variation could be justified by the environmental factors, as previously described (Pereira 2018b).

The iodine values found in the literature for the species *U. pinnatifida* are between  $220$ - $300 \mu\text{g I/g dw}$ . The results obtained in this study,  $263.8 \mu\text{g I/g dw}$ , are in accordance with the reported values.

Considering the *Rhodophyta* class, *C. crispus* is one of the algae with the highest iodine level (Biancarosa et al. 2018). The obtained value of 775.4  $\mu\text{g I/g dw}$  was in accordance with those found in literature,  $245\text{-}20 \times 10^4 \mu\text{g I/g dw}$ .

The applied process was able to reduce a considerable amount of iodine on the macroalgae, due to its water-soluble form. To ensure that the amount ingested does not represent a health risk, the boiling or even the rehydration procedures can be applied by consumers, mainly in the brown species.

The total leachable fractions (considering 20 minutes of boiling) were determined based on the ratio between the iodine concentration remaining in the extract and the iodine present in the dehydrated commercial algae (Table 3-8). Data show that after 20 minutes of boiling, a higher amount of soluble iodine can be leached out.

Table 3-8- Total leachable fraction (%) for macroalgae species in this study after 20 min boiling.

	Macroalgae species			
	<i>U. pinnatifida</i>	<i>L. digitata</i>	<i>S. latissima</i>	<i>C. crispus</i>
<b>Total leachable fraction (%)</b>	45.0	80.7	60.3	11.4

The attained leachable fractions revealed that brown algae *L. digitata* and *S. latissima* contain a significant amount of water-soluble inorganic iodine (leachable values of 80.7% and 60.3% respectively). On the contrary, *U. pinnatifida* revealed a smaller fraction (45.0%). The species *L. digitata* presented the greater decrease during rehydration, as it was expected. Some studies confirm that this species, besides having the highest amount of iodine, is also the species in which iodine occurs mainly in the inorganic form (*ca.* 93%) (Hou et al. 1997). The *Rhodophyta* species, *C. crispus*, showed a very different behaviour. Besides the lowest loss of iodine to water, the variation throughout the processing was 11.4%. These differences, were likely caused by differences in speciation, complexation, and subcellular localisation of iodine in the species investigated (Hou et al. 1997; Nitschke and Stengel 2016).

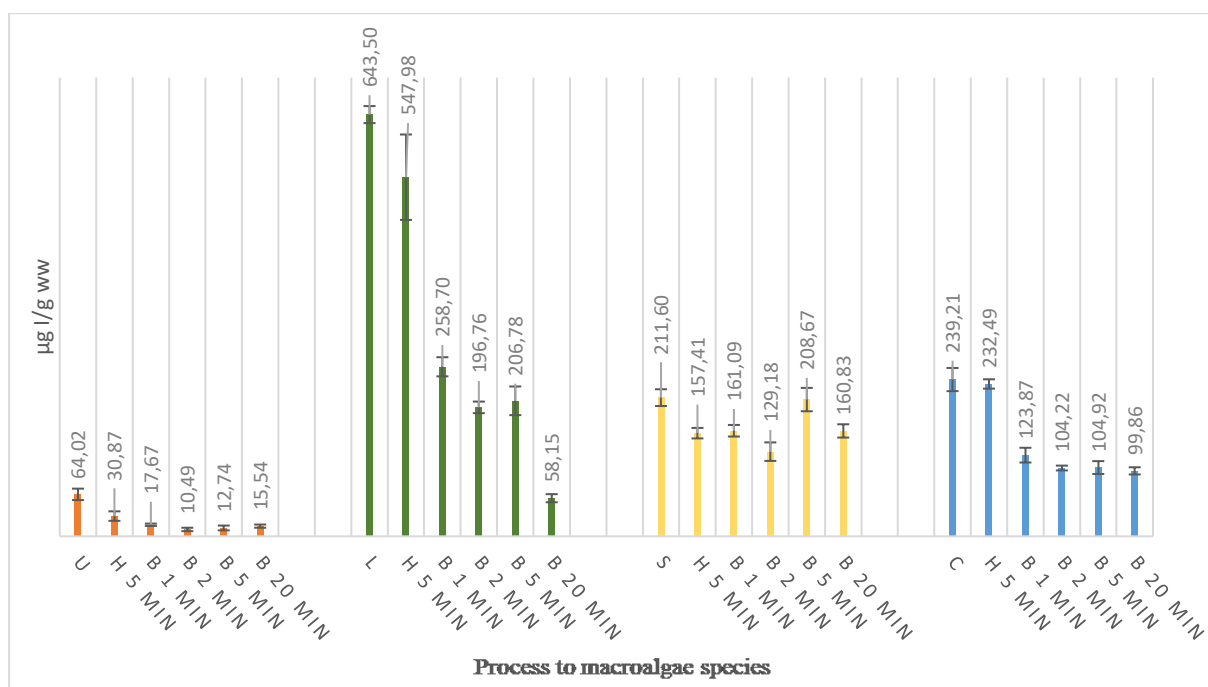


Figure 3-6- Iodine concentration ( $\mu\text{g I/g ww}$ ) in the macroalgae *U. pinnatifida* (orange), *L. digitata* (green), *S. latissima* (yellow), and *C. crispus* (blue) after hydration and boiled for 1, 2, 5, and 20 minutes.

Processing applied to the macroalgae species: U: *U. pinnatifida* (commercialized dehydrated form); L: *L. digitata* (commercialized dehydrated form); S: *S. latissima* (commercialized dehydrated form); C: *C. crispus* (commercialized dehydrated form); H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

The purpose of this study was the development of a product that could ensure the recommended daily intake (RDI) of iodine. In chapter 1, the values of RDIs for different population groups, as well as the upper limit (UL), were presented. Adults and adolescents RDI are  $150 \mu\text{g/d}$ , pregnant and lactating woman  $250 \mu\text{g/d}$  and UL for all age groups is  $600 \mu\text{g/d}$ . Values of algae (g ww) necessary to ensure the RDI levels without posing a health risk are depicted in Table 3-9. Considering that the consumption of macroalgae always follows a process of rehydration or boiling, the real values of consumption must be evaluated in the macroalgae fresh form.

Table 3-9-Estimated daily consumption of fresh seaweed to comply with recommended daily intake (RDI 150 µg/d) and the tolerable upper limit (UL 600 µg/d).

Process	RDI and UL (g ww/day)							
	<i>U. pinnatifida</i>		<i>L. digitata</i>		<i>S. latissima</i>		<i>C. crispus</i>	
	RDI	UL	RDI	UL	RDI	UL	RDI	UL
<b>Dehydrated sample</b>	2.34	9.37	0.23	0.93	0.71	2.84	0.63	2.51
<b>Rehydrated 5 min</b>	4.86	19.4	0.27	1.09	0.95	3.81	0.65	2.58
<b>Boiled 1 min</b>	8.49	34.0	0.58	2.32	0.93	3.72	1.21	4.84
<b>Boiled 2 min</b>	14.3	57.2	0.76	3.05	1.16	4.64	1.44	5.76
<b>Boiled 5 min</b>	11.8	47.1	0.73	2.90	0.72	2.88	1.43	5.72
<b>Boiled 20 min</b>	9.65	38.6	2.58	10.3	0.93	3.73	1.50	6.01

These results represent a range of algae (g ww) that an adult can consume per day. The values increase linearly with the process of boiling, which was expected since the iodine level decrease through the same process. Some macroalgae species represent a higher risk of consumption when only rehydrated. To consume *C. crispus* and *S. latissima* without boiling, the calculated value varies between 0.63-2.51 g/day and 0.95-3.81 g/day, respectively. *U. pinnatifida*, with the lowest iodine level, allows consumption of 4.86 to 19.44 g per day only with rehydration. *L. digitata* is the species that allows the lowest consumption with just a rehydration step (0.27 – 1.09 g/day). However, as described previously, *L. digitata* revealed the more significant loss of iodine when boiled for 20 minutes. The species in which the allowed consumption was higher after 20 min boiling was *C. crispus*. However, it must be taken in consideration that the quantities proposed in this work only consider the iodine levels and no other potentially hazardous components of seaweeds.

#### 3.1.4. Selenium

Selenium is a trace mineral essential for humans (Moreda-piñeiro et al. 2013). Its bioavailability ratios exhibit a positive correlation with the carbohydrate and dietary fiber contents. Macroalgae are known as excellent sources of these nutrients, and Moreda-Piñeiro et al. (2013) reported that among the analysed seafood, macroalgae presented the most satisfactory Se bioavailability results. Table 3-10 and Figure 3-7 exhibit the Se values obtained in the water extracts throughout the processing (µg Se/L).

Table 3-10- Selenium ( $\mu\text{g Se/L}$ ) content in water extracts for the macroalgae species *C. crispus*, *U. pinnatifida*, *L. digitata* and *S. latissima* presented as mean  $\pm$  SD (n=9).

Process	Mean $\pm$ SD $\mu\text{g Se/L}$			
	<i>U. pinnatifida</i>	<i>L. digitata</i>	<i>S. latissima</i>	<i>C. crispus</i>
<b>H 5 min</b>	55.9 $\pm$ 26.44	76.7 $\pm$ 8.02	75.5 $\pm$ 7.47	14.0 $\pm$ 7.30
<b>B 1 min</b>	20.0 $\pm$ 11.19	9.66 $\pm$ 0.89	95.8 $\pm$ 8.46	3.84 $\pm$ 4.51
<b>B 2 min</b>	10.6 $\pm$ 6.56	15.7 $\pm$ 2.02	56.5 $\pm$ 6.36	0.73 $\pm$ 4.45
<b>B 5 min</b>	17.3 $\pm$ 9.56	18.4 $\pm$ 1.54	140 $\pm$ 16.5	4.46 $\pm$ 6.18
<b>B 20 min</b>	4.47 $\pm$ 3.48	57.3 $\pm$ 8.60	81.4 $\pm$ 2.22	10.3 $\pm$ 9.17

Process: H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

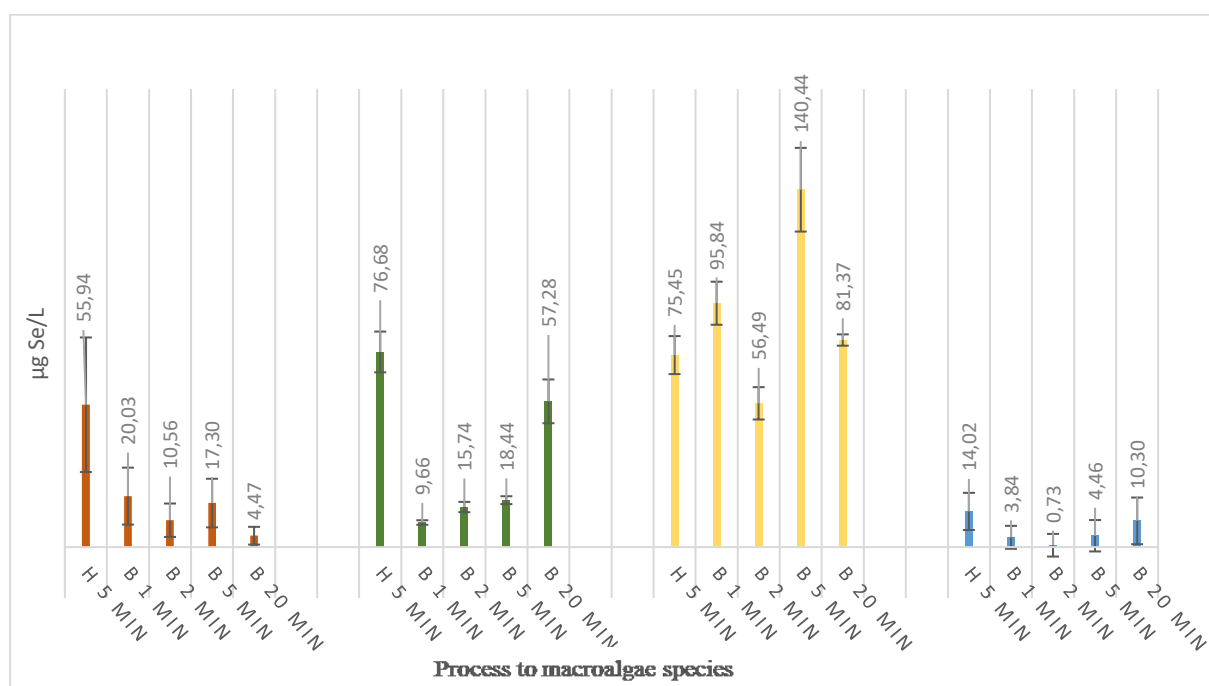


Figure 3-7- Selenium concentration ( $\mu\text{g Se/L}$ ) in water extracts for the species *U. pinnatifida* (orange); *L. digitata* (green); *S. latissima* (yellow); *C. crispus* (blue).

Processing applied to macroalgae species: H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

Macroalgae's selenium content was low compared to the other minerals and nutrients in the study. Water extracts presented varying results, which could be justified by the generally low levels of this element. In all the analysed, macroalgae the primary transfer occurred during the rehydration process. The obtained results of selenium content for the studied species are presented in Table 3-11, Figure 3-8, and Figure 3-9, in dry and fresh weight, respectively.

Table 3-11- Selenium content ( $\mu\text{g Se/g}$ ) presented as mean  $\pm$  SD (n=9) in wet weight (ww), dry weight (dw) and commercial dry weight (dw commercial).

	$\mu\text{g Se/g ww}$	$\mu\text{g Se/g dw}$	$\mu\text{g Se/g dw commercial}$
<i>U. pinnatifida</i>			
<b>U</b>	0.72 $\pm$ 0.12	2.96 $\pm$ 0.51	2.63 $\pm$ 0.46
<b>H 5 min</b>	0.61 $\pm$ 0.06	2.50 $\pm$ 0.25	2.22 $\pm$ 0.22
<b>B 1 min</b>	0.17 $\pm$ 0.08	1.55 $\pm$ 0.71	1.38 $\pm$ 0.63
<b>B 2 min</b>	0.16 $\pm$ 0.01	1.56 $\pm$ 0.11	1.39 $\pm$ 0.10
<b>B 5 min</b>	0.12 $\pm$ 0.02	1.63 $\pm$ 0.27	1.45 $\pm$ 0.24
<b>B 20 min</b>	0.08 $\pm$ 0.01	0.77 $\pm$ 0.13	0.68 $\pm$ 0.11
<i>L. digitata</i>			
<b>L</b>	1.45 $\pm$ 0.13	9.47 $\pm$ 0.82	8.19 $\pm$ 0.71
<b>H 5 min</b>	0.51 $\pm$ 0.03	3.31 $\pm$ 0.20	2.86 $\pm$ 0.17
<b>B 1 min</b>	0.39 $\pm$ 0.04	2.54 $\pm$ 0.29	2.20 $\pm$ 0.25
<b>B 2 min</b>	0.33 $\pm$ 0.03	2.17 $\pm$ 0.22	1.88 $\pm$ 0.19
<b>B 5 min</b>	0.46 $\pm$ 0.04	2.97 $\pm$ 0.28	2.57 $\pm$ 0.25
<b>B 20 min</b>	0.04 $\pm$ 0.01	0.67 $\pm$ 0.19	0.75 $\pm$ 0.21
<i>S. latissima</i>			
<b>S</b>	0.87 $\pm$ 0.10	10.72 $\pm$ 1.21	9.80 $\pm$ 1.10
<b>H 5 min</b>	0.25 $\pm$ 0.02	3.06 $\pm$ 0.27	2.80 $\pm$ 0.25
<b>B 1 min</b>	0.27 $\pm$ 0.02	3.33 $\pm$ 0.25	3.05 $\pm$ 0.22
<b>B 2 min</b>	0.21 $\pm$ 0.02	2.54 $\pm$ 0.30	2.32 $\pm$ 0.28
<b>B 5 min</b>	0.14 $\pm$ 0.01	1.67 $\pm$ 0.14	1.53 $\pm$ 0.13
<b>B 20 min</b>	0.16 $\pm$ 0.08	1.04 $\pm$ 0.52	1.17 $\pm$ 0.58
<i>C. crispus</i>			
<b>C</b>	1.75 $\pm$ 0.27	5.67 $\pm$ 0.86	5.04 $\pm$ 0.77
<b>H 5 min</b>	2.59 $\pm$ 0.27	8.40 $\pm$ 0.89	7.47 $\pm$ 0.79
<b>B 1 min</b>	1.55 $\pm$ 0.55	9.37 $\pm$ 3.34	8.33 $\pm$ 2.97
<b>B 2 min</b>	0.77 $\pm$ 0.13	4.10 $\pm$ 0.68	3.65 $\pm$ 0.60
<b>B 5 min</b>	1.37 $\pm$ 0.46	8.89 $\pm$ 2.99	7.90 $\pm$ 2.65
<b>B 20 min</b>	1.52 $\pm$ 0.13	10.48 $\pm$ 0.88	9.32 $\pm$ 0.78

U: *U. pinnatifida* (commercialized dehydrated form); L: *L. digitata* (commercialized dehydrated form); S: *S. latissima* (commercialized dehydrated form); C: *C. crispus* (commercialized dehydrated form)

Processing: H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

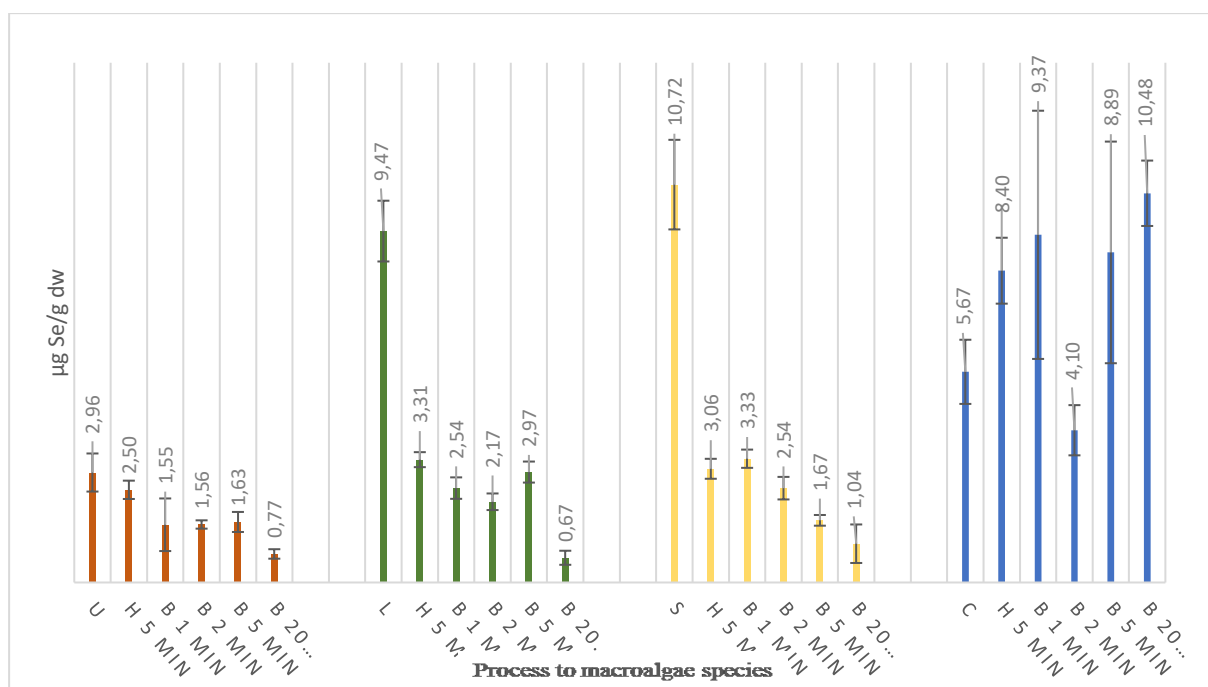


Figure 3-8- Selenium concentration ( $\mu\text{g Se/g dw}$ ) in the macroalgae *U. pinnatifida* (orange), *L. digitata* (green), *S. latissima* (yellow), and *C. crispus* (blue) after hydration and boiled for 1, 2, 5, and 20 minutes.

Processing applied to the macroalgae species: U: *U. pinnatifida* (commercialized dehydrated form); L: *L. digitata* (commercialized dehydrated form); S: *S. latissima* (commercialized dehydrated form); C: *C. crispus* (commercialized dehydrated form); H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

*S. latissima* was the species with the highest Se levels followed by *C. crispus*, as it was expected when compared with literature. However, in this work, a higher content was quantified for these species, ( $10.72 \mu\text{g Se/g dw}$  and  $9.37 \mu\text{g Se/g dw}$ , respectively) when compared to the reported values ( $0.06\text{-}1.3 \mu\text{g Se/g dw}$  and  $0.14\text{-}0.6 \mu\text{g Se/g dw}$ , respectively). As previously described, the composition of Se in seaweeds varies significantly with the environment conditions (water) and with origin (wild or farmed). *U. pinnatifida* and *L. digitata* revealed the lowest amounts of Se. Values found in the literature were  $0.03\text{-}0.05$  and  $0.07\text{-}0.3 \mu\text{g Se/g dw}$ , respectively, and are comparable to the values found in this work ( $2.96$  and  $5.67 \mu\text{g Se/g dw}$ ).

Table 3-12 presents the leachable ratios of selenium in the species under study.

Table 3-12- Total leachable fraction (%) for macroalgae species in this study.

	Macroalgae species			
	<i>U. pinnatifida</i>	<i>L. digitata</i>	<i>S. latissima</i>	<i>C. crispus</i>
<b>Total leachable fraction (%)</b>	88.6	97.1	81.4	13.0

The total leachable fraction of Se corroborates the values obtained in the extracts. The algae *C. crispus* features the lowest leachability (13.0%). The selenium present in this species does not display a significative transfer to water when boiled.

*L. digitata* was the species with the most substantial leachability value of 97.1%. This was the species that exhibited more changes throughout the process. There are not values in the literature to compare with the leachable obtained values.

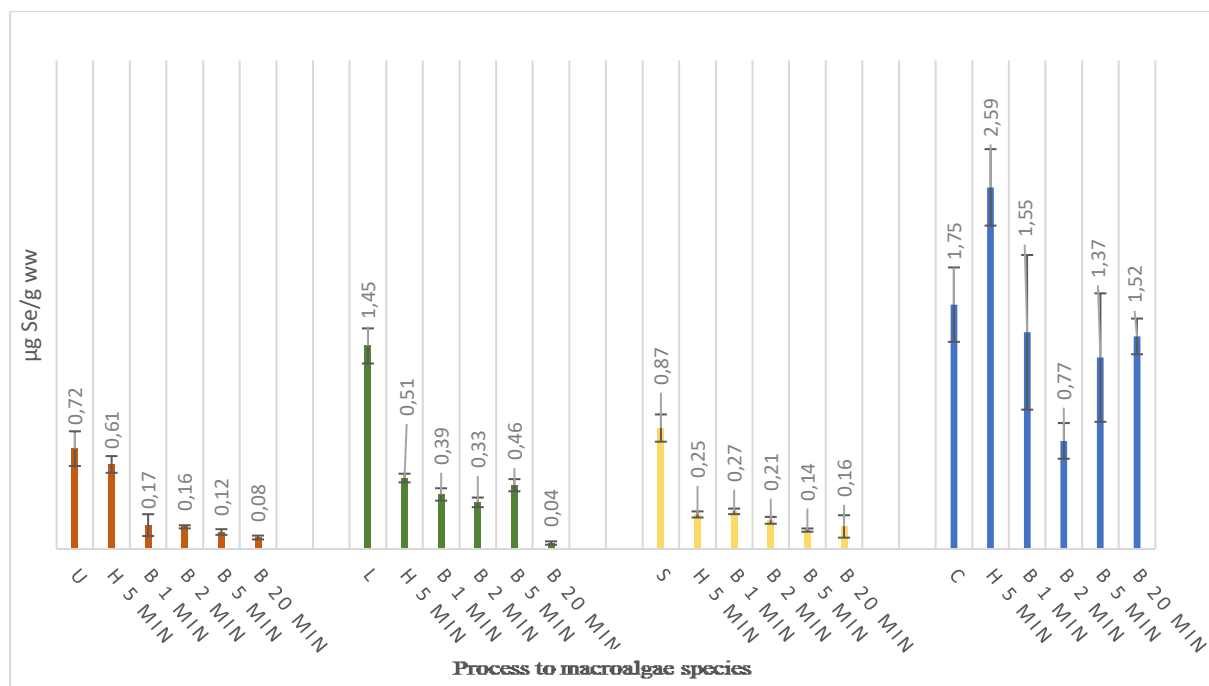


Figure 3-9- Selenium concentration ( $\mu\text{g Se/g ww}$ ) in the macroalgae *U. pinnatifida* (orange), *L. digitata* (green), *S. latissima* (yellow), and *C. crispus* (blue) after hydration and boiled for 1, 2, 5, and 20 minutes.

Processing applied to the macroalgae species: U: *U. pinnatifida* (commercialized dehydrated form); L: *L. digitata* (commercialized dehydrated form); S: *S. latissima* (commercialized dehydrated form); C: *C. crispus* (commercialized dehydrated form); H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

Selenium has an important role in development, reproduction, cardiovascular disease, and mood disorders (Ralston and Raymond 2010). However, the amount of dietary selenium required to reach human need is not very high.

The European Food Safety Authority has set a recommendation to dietary reference values (DRV) for Se (Table 3-13).

Table 3-13-Summary of the Dietary Reference Values for selenium ( $\mu\text{g}/\text{d}$ ) ( EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA) 2014).

Population	Adequate intake ( $\mu\text{g Se}/\text{d}$ )
1-3 yr	15
4-6 yr	20
7-10 yr	35
11-14 yr	55
$\geq 15$ yr	70
Lactation	85
Pregnant Woman	70

The two primary dietary forms of Se are selenocysteine derived from animal-sourced foods and selenomethionine obtained from animal-sourced foods and cereal products grown on Se-rich soil (Ralston and Raymond 2010). Animal-derived foods tend to be a better dietary source, which represents the difficulty in vegan and vegetarian population. Macroalgae could be an alternative to animal food sources. Table 3-14 demonstrates the values of macroalgae consumption needed to ensure the recommended dietary reference values for an adult ( $70 \mu\text{g Se}/\text{d}$ ).

Table 3-14- Quantity of macroalgae necessary to reach the recommended dietary reference daily intake for an adult ( $70 \mu\text{g Se}/\text{d}$ ).

Process	DRV (g ww/day)			
	<i>U. pinnatifida</i>	<i>L. digitata</i>	<i>S. latissima</i>	<i>C. crispus</i>
Dehydrated sample	97,37	48,12	80,79	40.0
Rehydrated 5 min	115.6	137.7	282.8	27.0
Boiled 1 min	408.9	179.2	259.7	45.1
Boiled 2 min	440.9	209.5	340.8	90.4
Boiled 5 min	575.3	153.2	517.3	51.1
Boiled 20 min	850.2	1642	433.5	46.0

The values obtained are high but merely indicative when considering macroalgae as the only source of Se in a day. Nonetheless, Se is an essential mineral to the human body, so a diversified diet with the inclusion of macroalgae could guarantee a significant level of Se ingested.

### 3.1.5. Sodium

Marine seaweeds contain higher amounts of macro minerals (i.e., Mg, Na, K, Ca) than those reported for edible land plants (Parjikolaei et al. 2016). Sodium is an essential regulator of blood pressure, with higher intakes correlated with raised blood pressure, and reduced intakes to excessively low blood pressure. Both low and excessive sodium intake was shown to be associated with higher mortality rates (Chau et al. 2017; Grimes et al. 2018). In developed

countries, excessive sodium intake is considered a bigger problem than low sodium intake. Therefore, The National Academies of Sciences and Engineering recommend a daily sodium intake of less than 1.5 g (National Academies of Sciences, Engineering 2019). Sodium content in macroalgae can reach very high values, so, to ensure the safe use of macroalgae in the developed product, this mineral was analyzed. This characteristic may, however, be advantageous if considering seaweeds as a salt replacer in processed foods, since their high mineral content would contribute to the maintenance of cooking salty taste (Circuncis et al. 2018). Table 3-15 and Figure 3-10 show the attained sodium contents in the water extracts.

Table 3-15-Sodium (g Na/L) content in water extracts of macroalgae species *C. crispus*, *U. pinnatifida*, *L. digitata* and *S. latissima* (n=9).

Process	Mean $\pm$ SD mg Na/L			
	<i>U. pinnatifida</i>	<i>L. digitata</i>	<i>S. latissima</i>	<i>C. crispus</i>
<b>H 5 min</b>	4.30 $\pm$ 0.18	2.87 $\pm$ 0.11	2.38 $\pm$ 0.20	4.79 $\pm$ 0.43
<b>B 1 min</b>	1.50 $\pm$ 0.06	0.76 $\pm$ 0.11	1.49 $\pm$ 0.28	1.14 $\pm$ 0.07
<b>B 2 min</b>	1.90 $\pm$ 0.09	0.73 $\pm$ 0.05	1.44 $\pm$ 0.20	1.13 $\pm$ 0.12
<b>B 5 min</b>	2.28 $\pm$ 0.07	0.92 $\pm$ 0.07	2.18 $\pm$ 0.18	1.01 $\pm$ 0.12
<b>B 20 min</b>	2.18 $\pm$ 0.11	1.40 $\pm$ 0.21	1.20 $\pm$ 0.07	1.51 $\pm$ 0.02

Processing: H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

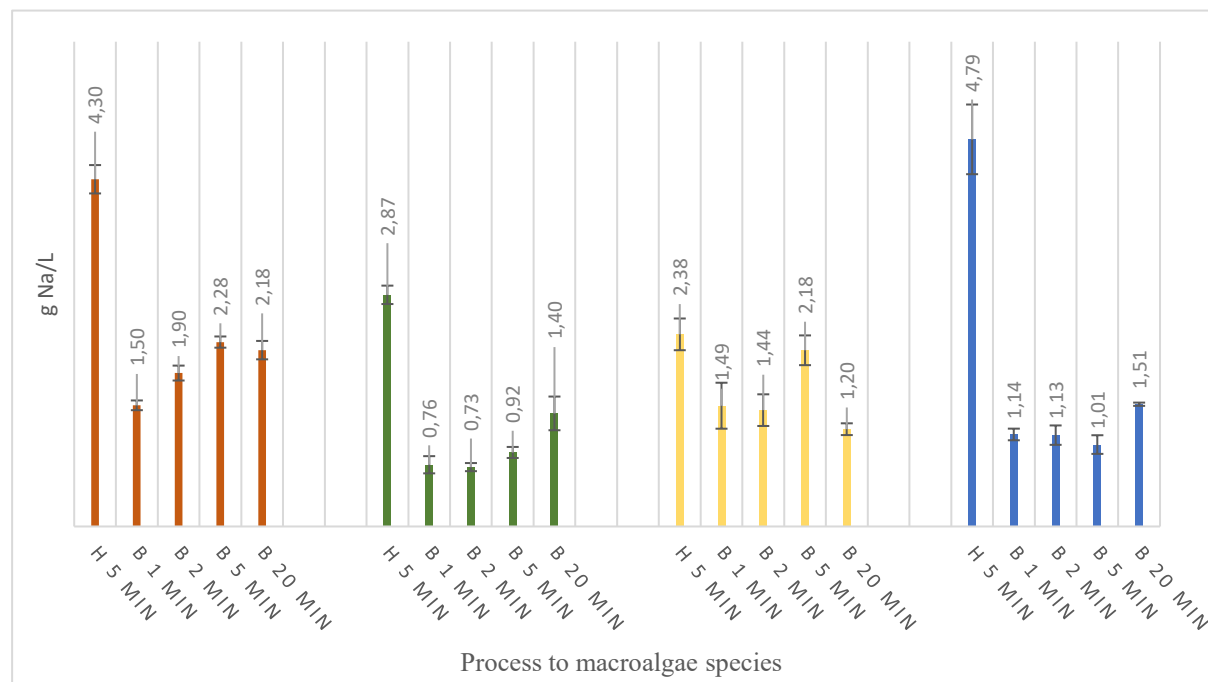


Figure 3-10- Sodium concentration (g Na/L) in water extracts for species *U. pinnatifida* (orange); *L. digitata* (green); *S. latissima* (yellow); *C. crispus* (blue).

Processing applied to macroalgae species: H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

In the extracts, it was possible to measure a considerable amount of sodium. Considering the values obtained, the species *U. pinnatifida* and *C. crispus* revealed the highest amount of Na in the rehydration water, 4.30 and 4.79 g Na/L, respectively, followed by *L. digitata* (2.87 g Na/L) and *S. latissima* (2.38 g Na/L). Although these elevated values were measured, throughout the process, there was a significant loss of sodium. The sodium content in the sample species *U. pinnatifida*, *L. digitata*, *S. latissima*, and *C. crispus* are presented in Table 3-16, Figure 3-11, and Figure 3-12.

Table 3-16- The sodium content in species *U. pinnatifida*, *L. digitata*, *S. latissima*, and *C. crispus* presented as mean  $\pm$  SD (n=9) in wet weight (ww), dry weight (dw), and dry commercial weight (dw commercial).

	mg Na/g ww	mg Na/g dw	mg Na/g dw commercial
<i>U. pinnatifida</i>			
<b>U</b>	23.60 $\pm$ 1.32	97.22 $\pm$ 5.43	86.33 $\pm$ 2.43
<b>H 5 min</b>	22.96 $\pm$ 0.53	97.45 $\pm$ 2.20	86.53 $\pm$ 1.95
<b>B 1 min</b>	5.81 $\pm$ 0.72	52.74 $\pm$ 6.52	46.83 $\pm$ 5.79
<b>B 2 min</b>	4.81 $\pm$ 0.31	47.29 $\pm$ 3.09	41.99 $\pm$ 2.74
<b>B 5 min</b>	3.45 $\pm$ 0.32	46.21 $\pm$ 4.35	41.03 $\pm$ 3.86
<b>B 20 min</b>	4.28 $\pm$ 0.21	39.92 $\pm$ 1.97	35.45 $\pm$ 1.75
<i>L. digitata</i>			
<b>L</b>	9.98 $\pm$ 0.52	47.47 $\pm$ 2.47	41.09 $\pm$ 2.13
<b>H 5 min</b>	6.53 $\pm$ 0.30	31.05 $\pm$ 1.43	26.88 $\pm$ 1.23
<b>B 1 min</b>	2.93 $\pm$ 0.20	18.84 $\pm$ 1.31	16.31 $\pm$ 1.13
<b>B 2 min</b>	2.72 $\pm$ 0.18	21.84 $\pm$ 1.44	18.90 $\pm$ 1.24
<b>B 5 min</b>	2.67 $\pm$ 0.12	21.39 $\pm$ 0.94	18.51 $\pm$ 0.82
<b>B 20 min</b>	1.17 $\pm$ 0.08	20.49 $\pm$ 1.35	18.22 $\pm$ 1.20
<i>S. latissima</i>			
<b>S</b>	3.62 $\pm$ 0.34	51.73 $\pm$ 4.81	47.31 $\pm$ 4.40
<b>H 5 min</b>	2.10 $\pm$ 0.51	29.97 $\pm$ 5.63	27.41 $\pm$ 5.15
<b>B 1 min</b>	2.73 $\pm$ 0.18	31.82 $\pm$ 2.16	29.10 $\pm$ 1.97
<b>B 2 min</b>	1.55 $\pm$ 0.08	21.12 $\pm$ 1.16	19.32 $\pm$ 1.06
<b>B 5 min</b>	3.99 $\pm$ 0.54	42.32 $\pm$ 5.77	35.52 $\pm$ 5.28
<b>B 20 min</b>	2.70 $\pm$ 0.72	26.08 $\pm$ 1.05	23.18 $\pm$ 0.94
<i>C. crispus</i>			
<b>C</b>	12.68 $\pm$ 0.91	41.09 $\pm$ 2.96	36.49 $\pm$ 2.62
<b>H 5 min</b>	5.97 $\pm$ 0.17	19.36 $\pm$ 0.55	17.19 $\pm$ 0.49
<b>B 1 min</b>	2.85 $\pm$ 0.18	17.19 $\pm$ 1.10	15.26 $\pm$ 0.97
<b>B 2 min</b>	3.47 $\pm$ 0.19	18.36 $\pm$ 1.00	16.30 $\pm$ 0.89
<b>B 5 min</b>	3.02 $\pm$ 0.18	19.56 $\pm$ 1.17	17.37 $\pm$ 1.04
<b>B 20 min</b>	3.01 $\pm$ 0.13	20.70 $\pm$ 0.92	18.38 $\pm$ 0.81

U: *U. pinnatifida* (commercialized dehydrated form); L: *L. digitata* (commercialized dehydrated form); S: *S. latissima* (commercialized dehydrated form); C: *C. crispus* (commercialized dehydrated form)

Processing: H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

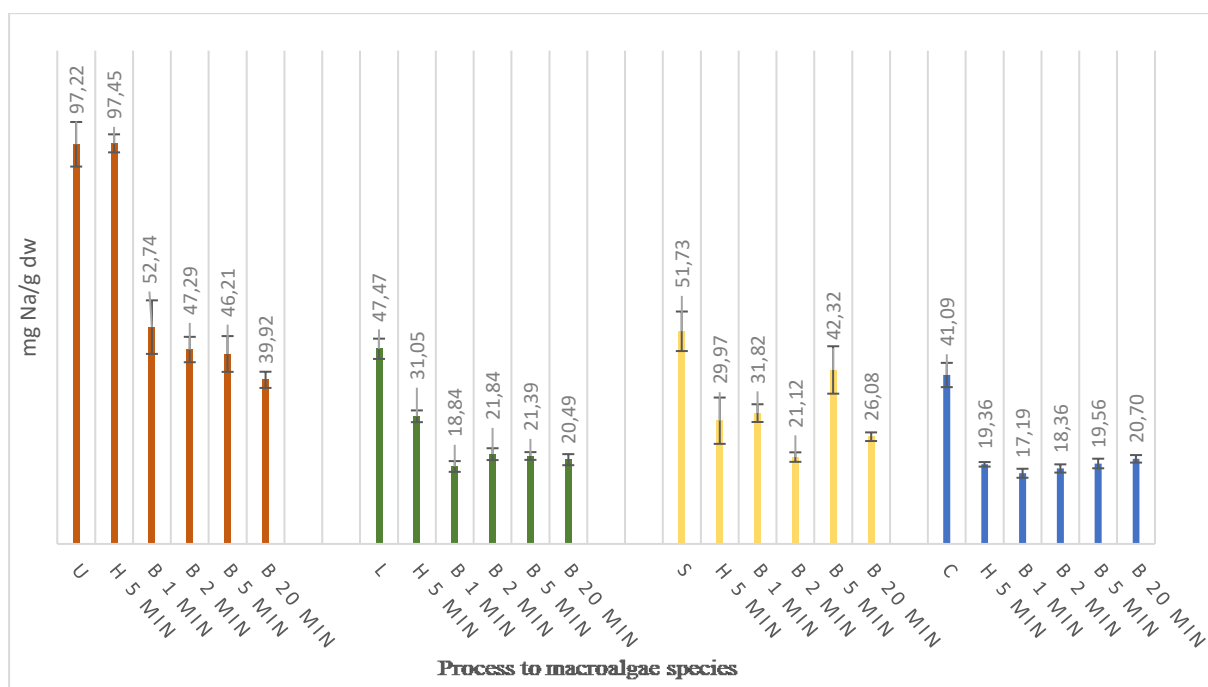


Figure 3-11- Sodium concentration (mg Na/g dw) in the macroalgae *U. pinnatifida* (orange), *L. digitata* (green), *S. latissima* (yellow), and *C. crispus* (blue) after hydration and boiled for 1, 2, 5, and 20 minutes.

Processing applied to the macroalgae species: U: *U. pinnatifida* (commercialized dehydrated form); L: *L. digitata* (commercialized dehydrated form); S: *S. latissima* (commercialized dehydrated form); C: *C. crispus* (commercialized dehydrated form); H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

In chapter 1, Na values found in the literature for: *U. pinnatifida* 48.80-64.94 mg Na/g dw; *L. digitata* 27-38.18 mg Na/g dw; *S. latissima* 24 mg Na/g dw; *C. crispus* 12-42.70 mg Na/g dw were already reported (Biancarosa et al. 2018; Kolb et al. 2004; Circuncis et al. 2018; Cabrita et al. 2016; Pereira 2016; Rupérez 2002). As presented in Table III-16 and Figure III-11, the values obtained for the samples in this study were, in general, higher compared with the ones found in the literature. *U. pinnatifida* 97.22 mg Na/g dw; *L. digitata* 47.47 mg Na/g dw; *S. latissima* 51.73 mg Na/g dw; *C. crispus* 41.09 mg Na/g dw. As described previously for other minerals, the macroalgae composition varies with environmental factors. The packaging process and dehydration method could justify some variations in the specific case of Na in commercial dehydrated samples (Nitschke and Stengel 2016) since a significant amount of Na is found in the algae surface. The macroalgae discussed in most reviews are mostly wild species, so they do not suffer changes with the manipulation process.

The leachable ratio of sodium is presented in Table 3-17.

Table 3-17- Total leachable fraction (%) for the macroalgae species in this study.

Total leachable fraction (%)	Macroalgae species			
	<i>U. pinnatifida</i>	<i>L. digitata</i>	<i>S. latissima</i>	<i>C. crispus</i>
	58.9	56.8	49.6	49.6

The leachable fraction for the species *S. latissima* was the lowest. The Na location in the macroalgae could justify this. For example, *U. pinnatifida* and *L. digitata* (58.9% and 56.8%) revealed the highest leachable fraction, and a significant loss of this mineral during the rehydration, since the Na in these species appears to be mainly in the superficial area of the algae as suggested by other studies (Raven 1976; Gutknecht 1965). *C. crispus* and *S. latissima* exhibited the same value of the leachable ratio of 49.6%, but still, a considerable amount of Na was removed throughout the process. There are no published values in the literature to allow comparison with the obtained results.

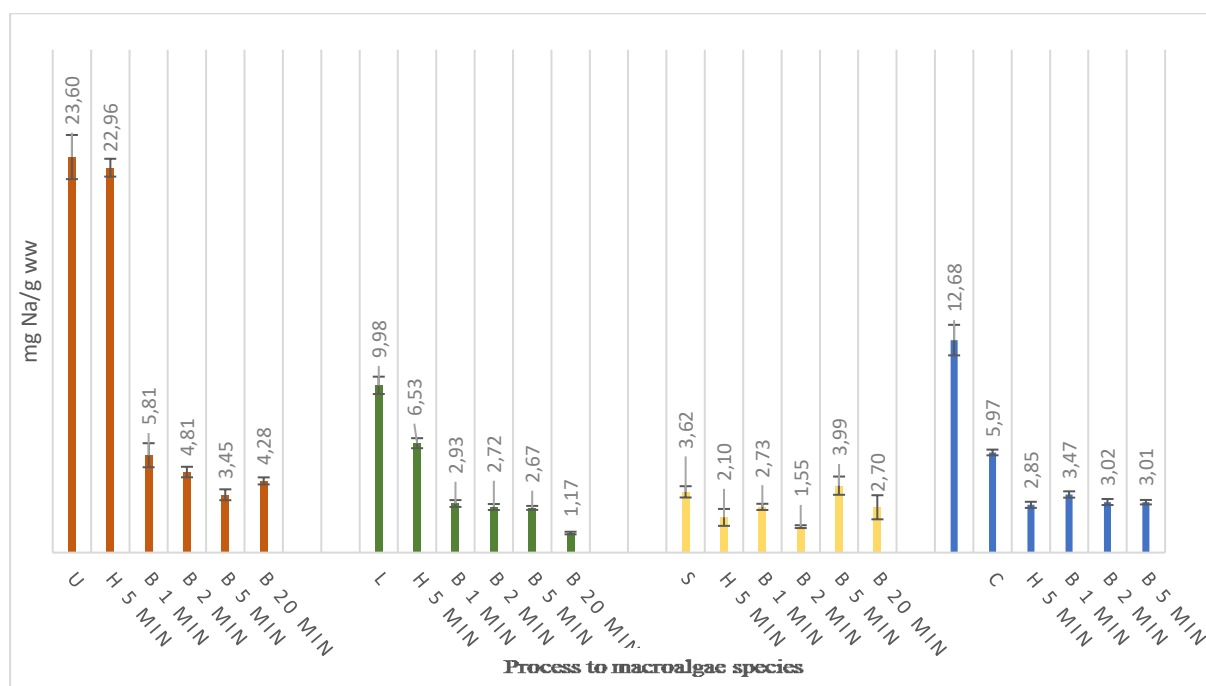


Figure 3-12-Sodium concentration (mg Na/g ww) in the macroalgae *U. pinnatifida* (orange), *L. digitata* (green), *S. latissima* (yellow), and *C. crispus* (blue) after hydration and boiled for 1, 2, 5, and 20 minutes.

Processing applied to the macroalgae species: U: *U. pinnatifida* (commercialized dehydrated form); L: *L. digitata* (commercialized dehydrated form); S: *S. latissima* (commercialized dehydrated form); C: *C. crispus* (commercialized dehydrated form); H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

A distinct decrease of Na levels throughout the hydration process, was more noticeable for *C. crispus*, *U. pinnatifida*, and *L. digitata*, in this order. The sample *U. pinnatifida*, was the species

with a lower loss during rehydration, losing the higher amount of Na during the boiling period. *S. latissima* demonstrated non-uniform behaviour throughout the process.

Considering the intake of 1.5 g of Na a day as the upper limit (UL), following the recommendation described (National Academies of Sciences, Engineering 2019), it is possible a safe consumption of every species. The suggested values of consumption throughout the process and for each species are presented in Table 3-18.

Table 3-18- Upper limit of safe use to macroalgae species in the study, consider a sodium consumption of 0.75 g a day.

	UL (g macroalgae/day)			
	<i>U. pinnatifida</i>	<i>L. digitata</i>	<i>S. latissima</i>	<i>C. crispus</i>
<b>Dehydrated sample</b>	31,78	75,17	206,96	59,17
<b>Rehydrated 5 min</b>	32,66	114,93	357,18	125,55
<b>Boiled 1 min</b>	129,14	256,16	275,05	263,22
<b>Boiled 2 min</b>	156,05	275,81	484,46	216,33
<b>Boiled 5 min</b>	217,40	281,23	187,87	248,59
<b>Boiled 20 min</b>	175,36	642,61	277,38	249,36

The obtained values indicate that considering half of the recommendation value (0.75 g/day), the portion of macroalgae that can be ingested per day is very high. These results confirmed that it is safe to use the characterized species in the proposed product.

### 3.1.6. Potassium

Besides being a physiologically essential nutrient to humans, K is a healthy alternative to the consumption of Na. Their coexistence is essential to prevent adverse health effects, the high Na to low K ratio became the most critical factor in the modern era of hypertension risk. Clinical trials confirm the capacity of dietary Na reduction and the use of K supplements to reduce blood pressure without any harmful side effects (Whelton 2014; Pereira 2018b). The use of edible seaweeds is a delicious high-K, salt-replacement in most foods (Pereira 2018b). Most of the macroalgae display higher Na and K concentrations than those reported for other vegetables, but usually low Na to K ratios, which is an essential aspect for proper maintenance of cardiovascular health (Circuncis et al. 2018).

The values of K obtained in the water extracts during macroalgae processing are presented in Table 3-19 and Figure 3-13.

Table 3-19- Potassium (g K /L) content in water extracts of macroalgae species *C. crispus*, *U. pinnatifida*, *L. digitata* and *S. latissima* (n=9).

Process	Mean $\pm$ SD mg K/L			
	<i>U. pinnatifida</i>	<i>L. digitata</i>	<i>S. latissima</i>	<i>C. crispus</i>
H 5 min	4.35 $\pm$ 0.56	5.27 $\pm$ 1.01	11.34 $\pm$ 1.83	1.88 $\pm$ 0.27
B 1 min	1.68 $\pm$ 0.14	5.15 $\pm$ 1.04	3.64 $\pm$ 0.25	0.47 $\pm$ 0.06
B 2 min	2.35 $\pm$ 0.37	3.65 $\pm$ 0.94	3.41 $\pm$ 0.10	0.55 $\pm$ 0.06
B 5 min	2.47 $\pm$ 0.17	7.48 $\pm$ 0.09	3.93 $\pm$ 0.34	0.48 $\pm$ 0.04
B 20 min	2.26 $\pm$ 0.25	4.35 $\pm$ 0.62	2.92 $\pm$ 0.08	0.75 $\pm$ 0.03

Processing: H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

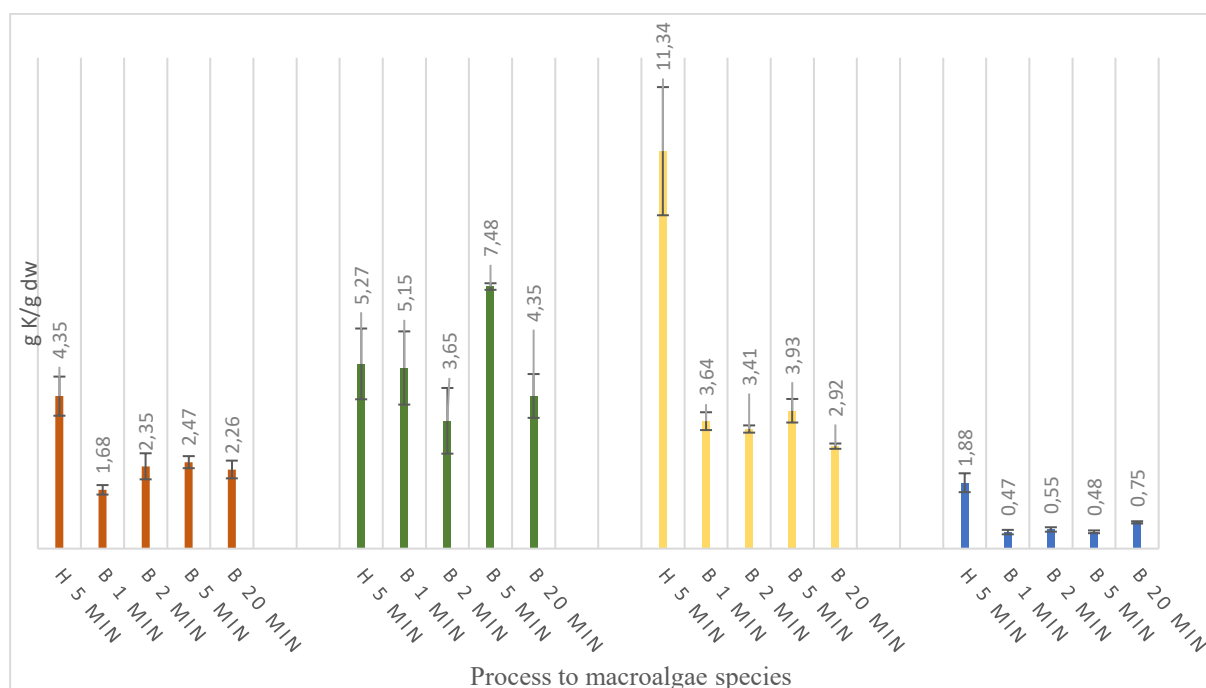


Figure 3-13- Potassium concentration (g K/L) in water extracts for species *U. pinnatifida* (orange); *L. digitata* (green); *S. latissima* (yellow); *C. crispus* (blue).

Processing applied to macroalgae species: H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

There are no studies on seaweed K behaviour during cooking. However, an example of its behaviour in some vegetables (carrot, potato, and broccoli) and the cooking process lead in all the cases to a its decrease (WoRMS Editorial Board 2019; Louis and Dolan 1970).

Considerable amounts of K were found in the rehydration extracts of *S. latissima*, followed by *U. pinnatifida*. In Table 3-20, K content is presented in wet weight (ww), dry weight (dw) and commercial dry weight (dw commercial), for the species in the study.

Table 3-20- Potassium content (mg K/g) presented as mean  $\pm$  SD (n=9) wet weight (ww), dry weight (dw) and commercial dry weight (dw commercial) in the studied seaweeds.

	mg K/g ww	mg K/g dw	mg K/g dw commercial
<i>U. pinnatifida</i>			
<b>U</b>	12.48 $\pm$ 0.71	45.65 $\pm$ 2.61	86.33 $\pm$ 2.43
<b>H 5 min</b>	8.72 $\pm$ 1.64	31.93 $\pm$ 5.99	86.53 $\pm$ 1.95
<b>B 1 min</b>	3.79 $\pm$ 0.24	30.60 $\pm$ 1.92	46.83 $\pm$ 5.79
<b>B 2 min</b>	3.76 $\pm$ 2.24	31.54 $\pm$ 1.92	41.99 $\pm$ 2.74
<b>B 5 min</b>	2.18 $\pm$ 0.04	19.03 $\pm$ 0.34	41.03 $\pm$ 3.86
<b>B 20 min</b>	2.50 $\pm$ 0.0	20.74 $\pm$ 1.64	35.45 $\pm$ 1.75
<i>L. digitata</i>			
<b>L</b>	24.36 $\pm$ 3.10	115.91 $\pm$ 14.73	100.33 $\pm$ 12.75
<b>H 5 min</b>	19.21 $\pm$ 2.17	91.40 $\pm$ 10.32	79.11 $\pm$ 8.93
<b>B 1 min</b>	11.29 $\pm$ 0.75	72.63 $\pm$ 4.82	62.87 $\pm$ 4.17
<b>B 2 min</b>	9.03 $\pm$ 0.20	72.50 $\pm$ 1.60	62.75 $\pm$ 1.39
<b>B 5 min</b>	9.03 $\pm$ 0.51	72.42 $\pm$ 4.05	62.69 $\pm$ 3.51
<b>B 20 min</b>	3.60 $\pm$ 0.31	63.18 $\pm$ 5.51	56.17 $\pm$ 4.90
<i>S. latissima</i>			
<b>S</b>	6.25 $\pm$ 0.55	89.16 $\pm$ 7.92	81.54 $\pm$ 7.24
<b>H 5 min</b>	5.85 $\pm$ 0.37	83.47 $\pm$ 10.27	76.34 $\pm$ 9.39
<b>B 1 min</b>	7.25 $\pm$ 0.69	83.57 $\pm$ 8.00	77.35 $\pm$ 7.31
<b>B 2 min</b>	5.86 $\pm$ 0.50	79.99 $\pm$ 24.80	73.15 $\pm$ 6.30
<b>B 5 min</b>	7.22 $\pm$ 0.60	76.56 $\pm$ 6.37	70.02 $\pm$ 5.82
<b>B 20 min</b>	6.18 $\pm$ 1.20	60.53 $\pm$ 2.34	53.81 $\pm$ 2.08
<i>C. crispus</i>			
<b>C</b>	15.83 $\pm$ 0.98	51.31 $\pm$ 3.18	45.56 $\pm$ 2.83
<b>H 5 min</b>	14.48 $\pm$ 1.75	46.92 $\pm$ 5.66	41.67 $\pm$ 5.02
<b>B 1 min</b>	5.49 $\pm$ 0.64	33.13 $\pm$ 3.85	27.10 $\pm$ 3.42
<b>B 2 min</b>	6.72 $\pm$ 0.14	35.58 $\pm$ 0.35	31.72 $\pm$ 0.31
<b>B 5 min</b>	6.10 $\pm$ 0.69	39.57 $\pm$ 4.50	35.14 $\pm$ 3.99
<b>B 20 min</b>	5.72 $\pm$ 0.14	39.36 $\pm$ 0.99	34.95 $\pm$ 0.88

U: *U. pinnatifida* (commercialized dehydrated form); L: *L. digitata* (commercialized dehydrated form); S: *S. latissima* (commercialized dehydrated form); C: *C. crispus* (commercialized dehydrated form)

Processing: H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

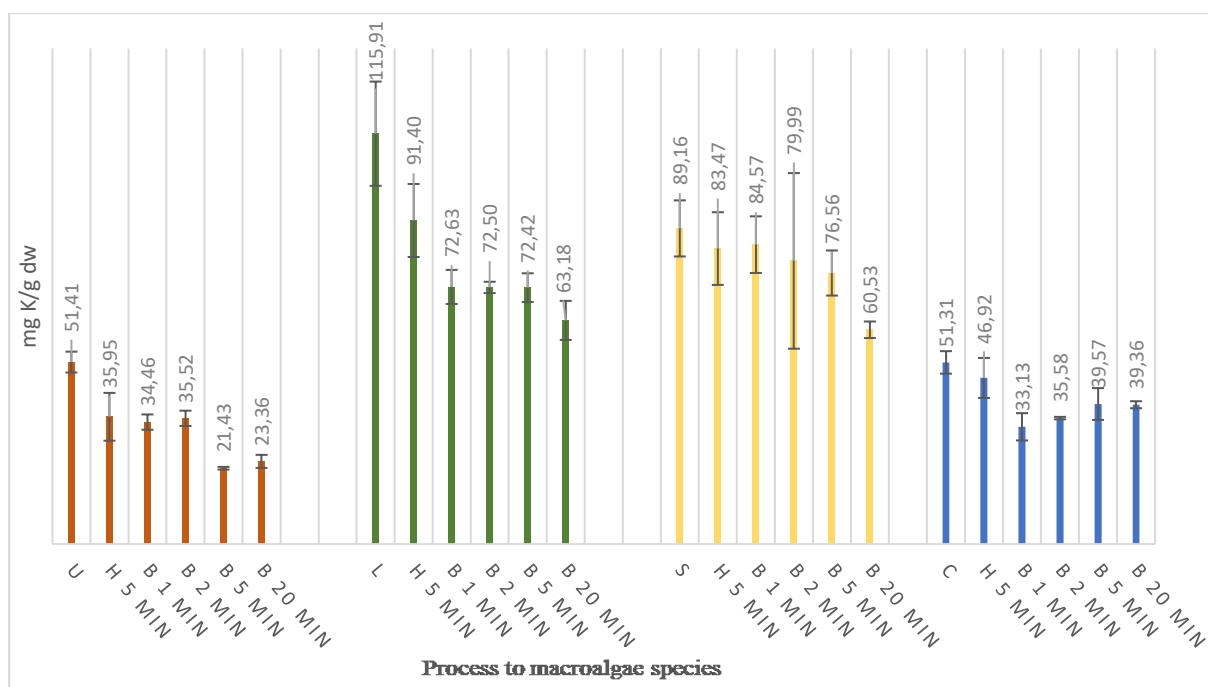


Figure 3-14- Potassium concentration (mg K/g dw) in the macroalgae *U. pinnatifida* (orange), *L. digitata* (green), *S. latissima* (yellow), and *C. crispus* (blue) after hydration and boiled for 1, 2, 5, and 20 minutes.

Processing applied to the macroalgae species: U: *U. pinnatifida* (commercialized dehydrated form); L: *L. digitata* (commercialized dehydrated form); S: *S. latissima* (commercialized dehydrated form); C: *C. crispus* (commercialized dehydrated form); H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

Figure 3-14 shows the behaviour of potassium throughout the process. *L. digitata* was the species with higher K content (115.91 mg K/g dw) as expected according to the literature review (53-113 mg K/g dw), followed by *S. latissima* with 89.16 mg K/g dw with literature reported values between 25 to 120 mg K/g dw.

*U. pinnatifida* and *C. crispus* revealed the lowest values. *U. pinnatifida* contains 51.41 mg K/g dw, similar to the values found in the literature (59.91-68.10 mg K/g dw). K levels in the *C. crispus* were 51.31 mg K/g dw, higher than the ones reviewed (13.50-31.84 mg K/g dw). Naturally, one must consider that K concentration, as the other components also vary significantly among species, due to growth conditions, development, habitat, and harvest conditions, among other factors. The values found in literature allowed the comparison between species, being the *L. digitata*, the species with the highest K level, and *C. crispus*, the one with the lowest.

The leachable ratio among species and throughout the cooking process is not very informative — the values obtained for the K that leach out are presented in Table 3-21.

Table 3-21- Potassium leachable ratio for species and throughout the process.

	Macroalgae species			
	<i>U. pinnatifida</i>	<i>L. digitata</i>	<i>S. latissima</i>	<i>C. crispus</i>
<b>Total leachable fraction (%)</b>	54.6	45.5	32.1	23.3

The leachable fraction of potassium was the lowest compared with the other components. *C. crispus* presented the smaller value, 23.3%, and *U. pinnatifida*, the higher, 54.6%.

Since *L. digitata* was the species with the highest amount of K and an intermediate value of leachable fraction (45.5%), it was possible to verify that the K levels were very consistent throughout the process.

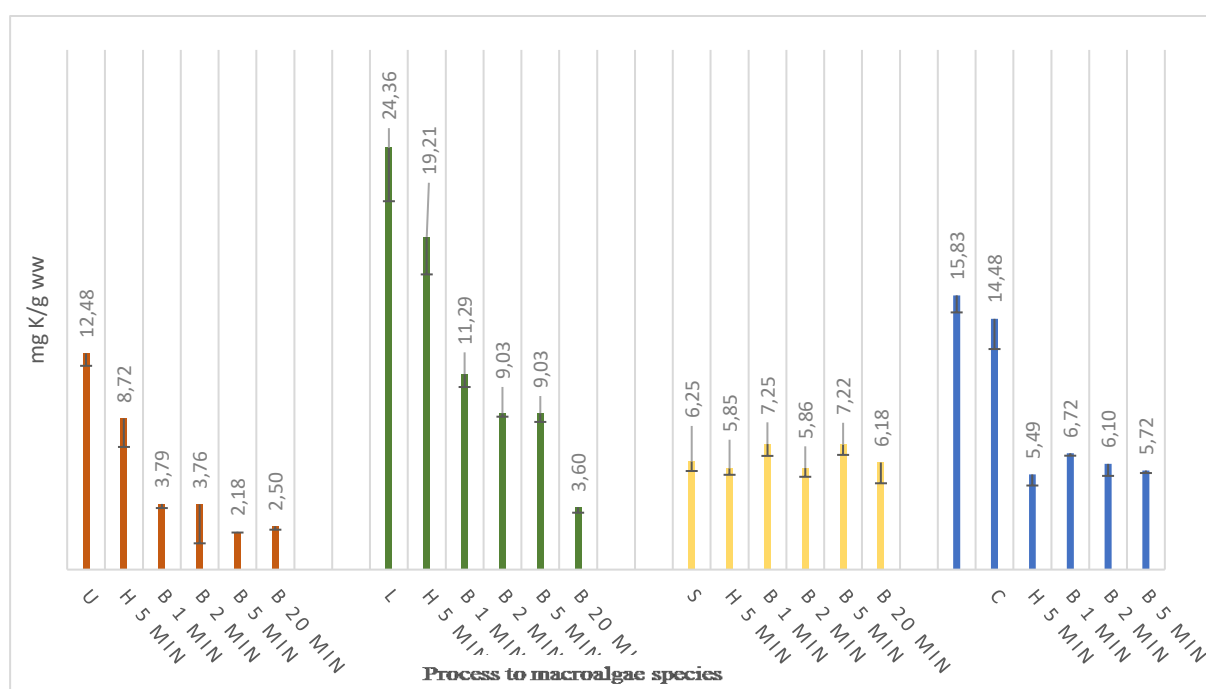


Figure 3-15- Potassium concentration (mg K/g ww) in the macroalgae *U. pinnatifida* (orange), *L. digitata* (green), *S. latissima* (yellow), and *C. crispus* (blue) after hydration and boiled for 1, 2, 5, and 20 minutes.

Processing applied to the macroalgae species: U: *U. pinnatifida* (commercialized dehydrated form); L: *L. digitata* (commercialized dehydrated form); S: *S. latissima* (commercialized dehydrated form); C: *C. crispus* (commercialized dehydrated form); H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

The National Academies of Sciences and Engineering for Dietary Reference Intakes of Sodium and Potassium recommend a daily intake of potassium divided by age groups. The values are presented in Table 3-22.

Table 3-22- Summary of Dietary Reference Values for potassium (g/day) (National Academies of Sciences, Engineering 2019).

Population	Adequate intake (g K/day)
0-6 months	0.40
7-12 months	0.86
1-3 yr	2.0
4-8 yr	2.3
9-13 yr (males)	2.5
14-18 yr (males)	3.0
9-18 yr (females)	2.3
≥19 yr (males)	3.4
≥19 yr (females)	2.6

The updated values are, in general, lower than the pre-existent ones. However, a great amount of K is needed to ensure an adequate intake. The total mass of macroalgae necessary to ensure an adequate intake of K considering an adult was calculated (male/female) (see Table 3-23). The obtained values are merely indicative since there are other daily intake sources of K.

Table 3-23- Portion of macroalgae necessary (g ww) to ensure an adequate intake of potassium.

Process	AI (g macroalgae/day)			
	<i>U. pinnatifida</i>	<i>L. digitata</i>	<i>S. latissima</i>	<i>C. crispus</i>
Dehydrated sample	272.5/ 208.4	139.6/106.7	544.3/416.2	214.8/164.3
Rehydrated 5 min	390.1/298.3	177.0/135.4	581.4/444.6	234.9/179.6
Boiled 1 min	897.0/ 686.0	301.2/230.3	469.1/358.7	619.1/473.4
Boiled 2 min	904.4/ 691.6	376.7/288.0	580.0/443.6	506.0/387.0
Boiled 5 min	1561/1193	376.5/722.6	470.8/360.0	557.1/426.0
Boiled 20 min	1360/1040	944.9/722.6	549.9/420.5	594.6/454.7

Table 3-24 represents the Na/K ratio to each species.

Table 3-24-Sodium/Potassium ratio for the macroalgae species *C. crispus*, *U. pinnatifida*, *L. digitata* and *S. latissima*.

Process	Mean Na/K			
	<i>U. pinnatifida</i>	<i>L. digitata</i>	<i>S. latissima</i>	<i>C. crispus</i>
<b>DF</b>	1.03	0.16	0.36	0.34
<b>H 5 min</b>	1.32	0.24	0.25	0.39
<b>B 1 min</b>	1.34	0.29	0.46	0.64
<b>B 2 min</b>	1.12	0.29	0.32	0.58
<b>B 5 min</b>	2.16	0.30	0.50	0.49
<b>B 20 min</b>	1.71	0.32	0.68	0.53

DF: dehydrated form of *C. crispus*, *U. pinnatifida*, *L. digitata* and *S. latissima*.

Processing: H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

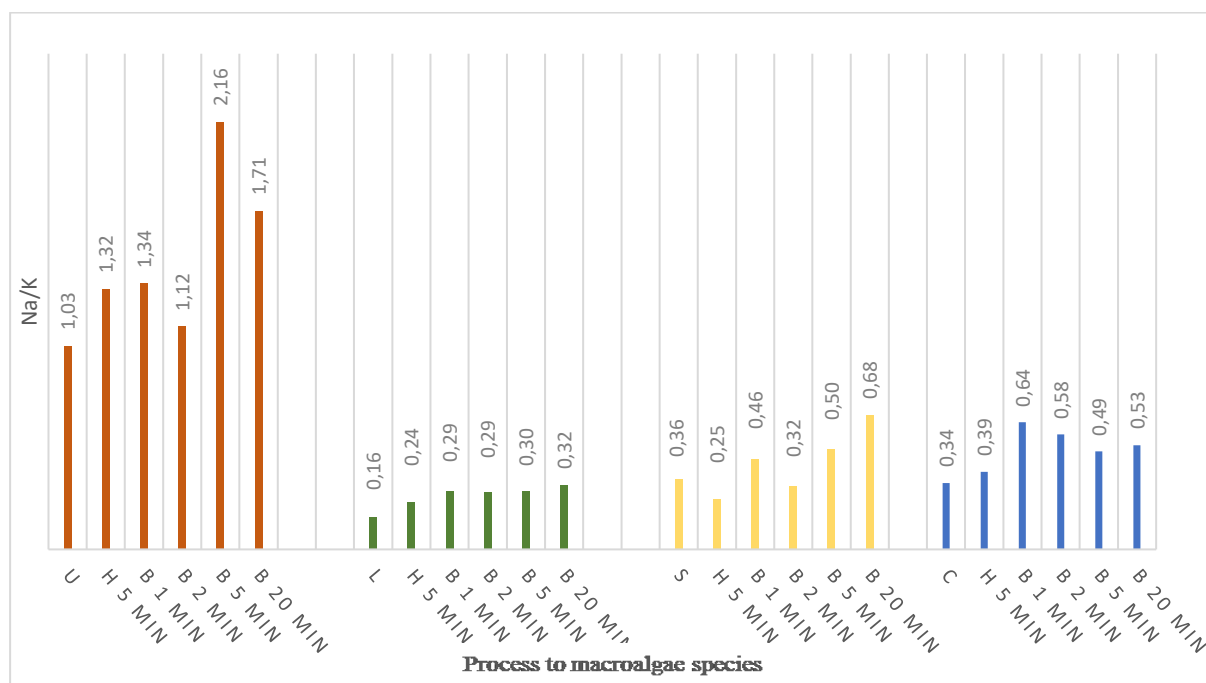


Figure 3-16- Sodium/potassium ratio in the macroalgae *U. pinnatifida* (orange), *L. digitata* (green), *S. latissima* (yellow) and *C. crispus* (blue) after hydration and boiled for 1, 2, 5 and 20 minutes.

Processing applied to the macroalgae species: U: *U. pinnatifida* (commercialized dehydrated form); L: *L. digitata* (commercialized dehydrated form); S: *S. latissima* (commercialized dehydrated form); C: *C. crispus* (commercialized dehydrated form); H 5 min: hydration 5 min; B 1 min: boiled 1 min; B 2 min: boiled 2 min; B 5 min: boiled 5 min; B 20 min: boiled 20 min

As described previously, the low Na/K ratio is an important macroalgae characteristic. Several authors have highlighted that the consumption of macroalgae could contribute to higher intakes of K, helping to balance the dietary Na/K ratio (Circuncis et al. 2018). The obtained values were in accordance with the literature: *ca.* 0.1-1.8 for red species and 0.3-1.5 for brown species (Circuncis et al. 2018). In the species under study, *L. digitata* was the species with the lowest

ratio, and *U. pinnatifida* exhibited the highest. Reports from WHO suggest that achieving the guidelines for both the Na and K intakes would yield a Na/K molar ratio of approximately 1.00 (WHO 2012). A high Na/K ratio is an indicator of a higher Na intake comparatively to K, which is what happens in the most traditional food sources. Macroalgae, on the contrary, have a composition of higher K values when compared with Na.

### 3.2. Canned product proposal

After quantifying the selected minerals in the characterized macroalgae, some innovative canned products were prepared. The choices for the preliminary tests are represented in Table 2-1 (chapter 2). Table 3-25 presents the results obtained for each canned sample and are represented considering:

S1: Canned mackerel with salt treatment

S2: Canned mackerel without salt treatment

S3: Canned mackerel with dehydrated *S. latissima*

S4: Canned mackerel with hydrated *U. pinnatifida*

S5: Canned mackerel without salt treatment and hydrated *U. pinnatifida*

Table 3-25- Results obtained for each canned sample in dry weight presented as min-max mg/g dw (n=6).

Samples	Macrominerals		Microminerals		
	mg/g dw		µg/g dw		
	Na	K	I	Se	As
<b>Refined olive oil</b>					
S1	<LOD	<LOD	8.56-9.80	<LOD	<LOD
S2	<LOD	<LOD	3.34	<LOD	<LOD
S3	<LOD	<LOD	10.76-17.56	<LOD	<LOD
S4	<LOD	<LOD	6.84-16.13	<LOD	<LOD
S5	<LOD	<LOD	2.1-17.2	<LOD	<LOD
<b>Macroalgae</b>					
S3	0.31-0.39	0.79	192.2-246.4	0.34-0.36	3.73-4.11
S4	1.14-1.32	1.89-2.11	47.5-71.1	0.49-0.56	6.63-9.07
S5	1.06-1.34	1.50-1.58	59.5-92.8	0.13-0.19	5.38-5.73
<b>Canned mackerel</b>					
S1	0.22-0.40	1.05-1.20	3.72-9.60	0.97-1.00	2.86-3.43
S2	0.20	1.15	6.08	1.11	3.21
S3	0.49-0.55	1.40-1.41	214.9-334.5	1.10	3.47-3.83
S4	0.46-0.50	1.14-1.18	10.4-19.5	0.99-1.04	2.86-3.50
S5	0.37	1.08-1.23	11.9-25.4	0.90-0.91	2.60-3.04

S1: Canned mackerel with salt treatment; S2: Canned mackerel without salt treatment; S3: Canned mackerel with dehydrated *S. latissima*; S4: Canned mackerel with hydrated *U. pinnatifida*; S5: Canned mackerel without salt treatment and hydrated *U. pinnatifida*

During sterilization in the autoclave, diffusion occurs between the water present in the fish and the coating oil (olive oil). This effect was demonstrated by the inverse correlation between fat and moisture content (Arias, Castrillón, and Navarro 1991). If canning is carried out in oil, fish muscle also loses proteins, minerals, and vitamins (the water loss can vary between 9-28%). In this work, diffusion was confirmed by the transfer of iodine to olive oil; as described, iodine previously in organic form is protein-bound iodine, both in macroalgae and fish (Hou et al. 1997; Aubourg 2001; Leloup and Fontaine 1951). Na and K could exist in the olive oil due to the same leachable principle, although mainly present in its inorganic form; however, the LOD of this method is superior to the one used in the iodine quantification.

Samples with macroalgae (S3, S4, and S5), showed a higher content of iodine in the olive oil when compared with the fish samples, probably due to a similar transfer of organic-bound iodine from seaweeds. Water content in macroalgae is also highest, and a more significant transfer between algae and olive oil could happen.

In conclusion, the values found for the utilized macroalgae agreed with the previous tests. *S. latissima* was the species in which the I was the highest, and *U. pinnatifida* was the species with higher K and As content. Both algae can be utilized for different purposes.

For the same fish samples, the values are presented in wet weight, considering a mean humidity value of 63.7 g water (value found in “*Tabela de Composição Alimentar e Nutrição*”, INSARJ 2017). Table 3-26 presents these values for the canned mackerel in olive oil.

Table 3-26- Macro and microminerals (min-max mg/g ww and min-max µg/g ww, respectively) in canned mackerel.

Samples	Macrominerals		Microminerals		
	mg/g ww		µg/g ww		
	Na	K	I	Se	As
<b>Canned mackerel</b>					
<b>S1</b>	0.08-0.145	0.381-0.436	1.35-3.49	0.352-0.363	1.04-1.25
<b>S2</b>	0.149	0.417	2.47	0.403	1.17
<b>S3</b>	0.178-0.20	0.508-0.512	78.01-121.42	0.400	1.26-1.39
<b>S4</b>	0.167-0.182	0.414-0.428	3.75-7.08	0.359-0.378	1.04-1.27
<b>S5</b>	0.134	0.392-0.446	4.32-9.22	0.327-0.330	0.944-1.10

Studies that evaluate the mineral content of canned mackerel are scarce. However, some values were found in literature and compared. The values found are described in Table 3-27, Na, K, Se, and As levels were found for canned mackerel samples, I was found for fresh fish.

Table 3-27- Values found in literature for macrominerals (Na, K) and microminerals (Se, I, As) in mackerel samples (canned or fresh).

		References
<b>Canned mackerel in olive oil</b>		
<b>Na (mg/g ww)</b>	6.20	(Bandarra et al. 2004; INSARJ 2017)
<b>K (mg/g ww)</b>	3.80	(Park et al. 2018)
<b>Se (µg/g ww)</b>	0.420	(Olmedo et al. 2013)
<b>As (µg/g ww)</b>	0.052	
<b>Fresh mackerel fish</b>		
<b>I (µg/g ww)</b>	0.46-0.57	(Julshamn, Dahl, and Eckhoff 2001)

Na values do not vary significantly with the fish immersion in brine (S1: 0.08-0.145 mg Na/g ww; S2: 0.146 mg Na/g ww). When compared with the literature, Na levels were lowest in this study, as reported in Table 3-27.

The Na values in fish increased slightly after macroalgae addition (S3:0.11-0.13 mg Na/g ww, S4:0.11-0.12 mg Na/g ww and S5: 0.09 mg Na/g ww).

The fish samples without macroalgae revealed the highest levels of K (S1: 0.381-0.436 mg K/g ww, S2: 0.417 mg K/g ww). These values increased slightly when macroalgae were added, so there was a percentage of K transferred between macroalgae and fish, mainly in sample S3, when using the algae without processing.

Besides the antioxidation properties of Se, this element also presents significant effects against induced neurotoxicity by inorganic mercury, usually present in fishes like tuna (Aschner and Costa 2017). In this work, Se measured in mackerel was slightly lower (S1: 0.352-0.363  $\mu\text{g Se/g ww}$  and S2: 0.403  $\mu\text{g Se/g ww}$ ) when compared with other studies (Table III-28). Se levels in fish do not vary significantly with macroalgae addition.

Organic iodine present in perciforms (mackerel order) varies between 5 to 29% as protein-bound iodine. Nevertheless, iodine in fish varies with environmental growth conditions, age, sex, and species (Leloup and Fontaine 1960; Huang and Hickman 1968). The obtained iodine levels in mackerel (S1:1.35-3.49  $\mu\text{g I/g ww}$  and S2: 2.47  $\mu\text{g I/g ww}$ ) were superior to those found in the literature (0.46-0.57  $\mu\text{g I/g dw}$ ) but considering the several factors described previously the results could not be compared.

As expected, fish samples with macroalgae (S3, S4, and S5) revealed an increase of iodine level. In particular, S3 revealed a high iodine transfer between algae and fish, which could be justified by the used of macroalgae in its dehydrated form and by the high iodine content found in this species. (S3: 78.01-121.42  $\mu\text{g I/g ww}$ ).

Values of arsenic in fish (S1: 1.04-1.25  $\mu\text{g As/g ww}$  and S2: 1.07  $\mu\text{g As/g ww}$ ) were much higher than the observed values in literature (0.052  $\mu\text{g As/g ww}$ ). This result could be justified by the utilized sample of mackerel. Arsenic can be accumulated by marine organisms through exposure present in water and sediments or the food chain (Olmedo et al. 2013).

Arsenic levels were similar between mackerel and samples with *U. pinnatifida* addition (S4 and S5). However, with *S. latissima* (S3), the arsenic in fish increases slightly (9-22%). This could be justified by the high As levels found in the *S. latissima*.

Considering the recommended daily allowance (RDA) for an adult, the contribution of each sample (considering 90 g dw canned product) was calculated for Se, I, and K (min-max) (Table 3-28).

Table 3-28- Contribution of canned products to recommended daily allowances (\*).

Samples	Macrominerals		Microminerals	
	%		%	
	K		I	Se
	Male	Female		
S1	1.84-2.10	2.76-3.15	310-580	87.3-90
S2	0.35-2.01	0.53-3.02	315	99.9
S3	2.41-2.43	3.62-3.64	9158-14215.01	96.9-97.0
S4	2.04-2.13	3.06-3.19	647-1.23 x10 <sup>-3</sup>	84.5-88.8
S5	1.99-2.24	2.99-3.36	823-1.78x10 <sup>-3</sup>	71.1-72.6

(\*) RDI's: Iodine (150 µg/day); Selenium (70 µg/day); Potassium (2400 mg/day female; 3600 mg/day male);

The contribution of macroalgae to K RDA is the lowest, as it was envisaged due to the elevated value of the recommended allowance. The actual worldwide panorama of low consumption of K is problematic, as described previously. This product associated with a balanced diet rich in K could increase the intake of this mineral and balance the Na/K ratio.

The iodine levels were very high, mainly in the canned fish with the species *S. latissima*. Some future work is needed to reduce the amount applied or even to modify the process (after rehydration, for example). With the samples S1 and S2, it was possible to analyse the iodine quantity in the mackerel, which was higher than expected. Iodine levels in fish vary with the environment of growth (Leloup and Fontaine 1960), which may promote high variability.

Samples S4 and S5 presented a high amount of iodine, which could be justified by the used amount of *U. pinnatifida* (4 and 10 g, respectively).

This product ensures *ca.* 100% of the RDI of Se. As previously described, an alternative source of Se is needed, besides meat products, therefore these canned products can be considered as a viable alternative.

### 3.2.1. Sensory analysis

As described in chapter 2, a sensory analysis of the samples displayed in Figure 3-17 was made by a panel of volunteers. The results of the sensory analysis were treated, and the results are presented below.



Figure 3-17- Samples 1,2,3,4 and 5 of produced canned products.

S1: Canned mackerel with salt treatment; S2: Canned mackerel without salt treatment; S3: Canned mackerel with dehydrated *S. latissima*; S4: Canned mackerel with hydrated *U. pinnatifida*; S5: Canned mackerel without salt treatment and hydrated *U. pinnatifida*

Sample 1 with brine and without macroalgae: was good in general (right colour, a pleasing aroma, and pleasant look) however, the texture was slightly dry, which can be explained by the brine concentration. Shear force on the fillet is greater for high brine concentration than low brine concentration due to the lower moisture content, which conducts a firmer fillet texture (Jirrinandana et al. 2002). However, the taste was considered excellent and very similar to a canned mackerel already found in the market.

Sample 2 without brine or macroalgae: was good in general (right colour, pleasing aroma and, pleasant look), and when compared to S1, the texture was improved, and the taste was good, even without sodium chloride addition.

Sample 3 mackerel with brine and *S. latissima*: was the sample with the most appealing characteristics, with both colours of macroalgae and fish considered very satisfactory. The smell was more ‘sea-like,’ but this was not revealed as an inconvenient to the consumer. The texture of this sample was better, more soft and pleasant. In general, the texture and taste were classified as excellent.

Sample 4 mackerel with brine and *U. pinnatifida*: The aspect was less attractive than the sample S3 since the *U. pinnatifida* its darker and was include in more significant portions. The smell was, as expected, more likely to seafood, because of the highest amount of macroalgae. In

general, the fish texture was better than the sample S1 as the S3; however, the macroalgae texture was less appreciated.

Sample 5 mackerel without brine and with *U. pinnatifida*: The fish in this sample presented a 'greenish' coloration due to the high amount of macroalgae applied in the product (*ca.* 10g), so the aspect was classified as less pleasant.

In general, all the volunteers consider that immersion in brine before cooking was not necessary for these canned products; the fish texture improved with the macroalgae incorporation as well as the general taste.

All the volunteers agreed that S3 was the sample more pleasant and tasteful.

# CHAPTER 4

## 4. CONCLUSIONS AND FUTURE RESEARCH

This thesis had the purpose of studying the impact of macroalgae processing (boiling and rehydration) mainly in the levels of iodine but also in other minerals (As, Se, Na, and K).

The primary aim of this work was the development of a new and accessible food product rich in iodine and also Se in order to fill the gap that the current European diet has. To achieve this main objective, a canned mackerel enriched with seaweeds was developed.

Besides being an excellent source of most minerals, the analysed algae can compensate for the frequently low content of minerals in food plants grown on soils, from which modern agricultural methods have depleted minerals.

Some macroalgae, with the highest iodine values, can be cooked (boiled) to ensure safe consumption, as for macroalgae species *S. latissima* and *L. digitata*. Data about the processing of macroalgae is not commonly described in the literature.

Besides iodine, Se dietary recommended intake can be assured by the daily consumption of macroalgae. In this study, the species which revealed the highest Se level was *C. crispus*, followed by *L. digitata*. Also, both are a great source of other minerals, in particular, iodine. So, the introduction of this seaweed (after some processing steps) in a regular daily diet, ensure the safe intake of these minerals that are not found in land plants.

K is an essential mineral, as described previously, and the macroalgae are a more abundant source of K that, when consumed, allows an increase in K ingestion, promoting a healthy balance between Na and K.

The leachable fraction of minerals in macroalgae has been poorly characterized, and this study showed the importance of its determination. Most of the macroalgae revealed significant levels of leachable fractions of minerals, which need to be taken into account when dietary recommendations are made. The potential toxic elements, As and Na, were analysed to ensure the products and macroalgae are safe to consume. The leachable fraction of these minerals

suggested that the rehydration period recommended (in the package) it is enough to decrease the toxic elements levels substantially.

The first proposed canned food needs to be further optimized. There was an elevated risk associated with the high iodine level; therefore, some changes can be made to increase the quality of the product. The results allowed to conclude that macroalgae toxic mineral levels decrease significantly after the applied processing steps. The introduction of another step (processing of seaweeds before canning) can be challenging to apply in the industry (particularly boiling), but rehydration or intensive wash can. This additional step can lead to a safer and more interesting product. Still, more canned samples (changing the recipes, quantities, and applications) must be made, in order to validate the obtained results.

Human nutrition is an essential field study nowadays. With the decrease in organic farming and with the intensive agroindustry, essential minerals that the human body absorbed in the past from soil-based plants are now in decrease. It is crucial to search for new mineral sources. Macroalgae are the plants with the highest levels of minerals, so if introduced in regular European food habits, some mineral deficits of utmost importance such as iodine and selenium could be reduced.

Considering that macroalgae ingested regularly are the best natural source of iodine and that they represent an easy and nontoxic way to meet the daily requirement of this element in the diet, new studies should be made to the introduction of macroalgae in already regular eaten food products to prevent iodine deficiency.

Canned products are a practical food application, with a long shelf-life. Some products with macroalgae already exist in the market. However, concerning canned products, only a few exist.

To continue the work developed in this project, some alternative food products can be made, for example, with macroalgae extracts instead of the macroalgae. The previous use of seaweed-based extracts has been tested by several authors, mainly to increase food functionalities that are associated with antioxidant capacity (Neto et al. 2018). Additionally, water-soluble mineral content is transferred to the extracts, as well. Other alternatives are processing the macroalgae before incorporation or change the quantity applied to decrease some mineral levels. There are countless alternatives to use macroalgae benefits in food development that need clearly to be further explored.

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# APPENDICES

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## A. Sensory Sheet

Table A- 1- Sensory analysis sheet.

Ficha de Análise Sensorial			
Nome: _____		Data: ___/___/____	
Escala de análise 1. Não gosto mesmo nada 2. Não gosto muito 3. Não gosto 4. Não gosto nem desgosto 5. Gosto 6. Gosto muito			
Amostra	Características	Classificação	Comentários
1	Aparência		
	Coloração (peixe e alga)		
	Aroma (peixe e alga)		
	Sabor		
	Textura		
2	Aparência		
	Coloração (peixe e alga)		
	Aroma (peixe e alga)		
	Sabor		
	Textura		
3	Aparência		
	Coloração (peixe e alga)		
	Aroma (peixe e alga)		
	Sabor		
	Textura		
4	Aparência		
	Coloração (peixe e alga)		
	Aroma (peixe e alga)		
	Sabor		
	Textura		
5	Aparência		
	Coloração (peixe e alga)		
	Aroma (peixe e alga)		
	Sabor		
	Textura		

## B. ICP-MS calibration curve parameters

Table B- 1- Example of an ICP-MS calibration curve and respective parameters.

Element	Calibration curve parameters	R <sup>2</sup>	LOD (µg/L)
Iodine	$y = 24955x - 83074$	0.998	0.62
Arsenic	$y = 13685x - 1090.3$	1.000	0.22
Selenium	$y = 1311.6x + 183.06$	1.000	0.57

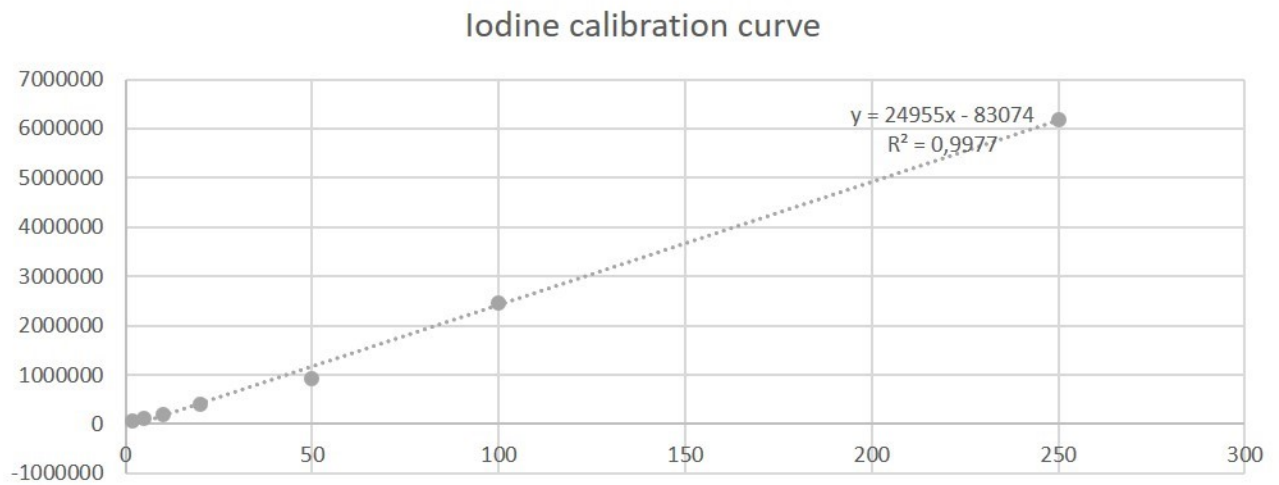


Figure B- 1-I calibration curve (14-06-2019).

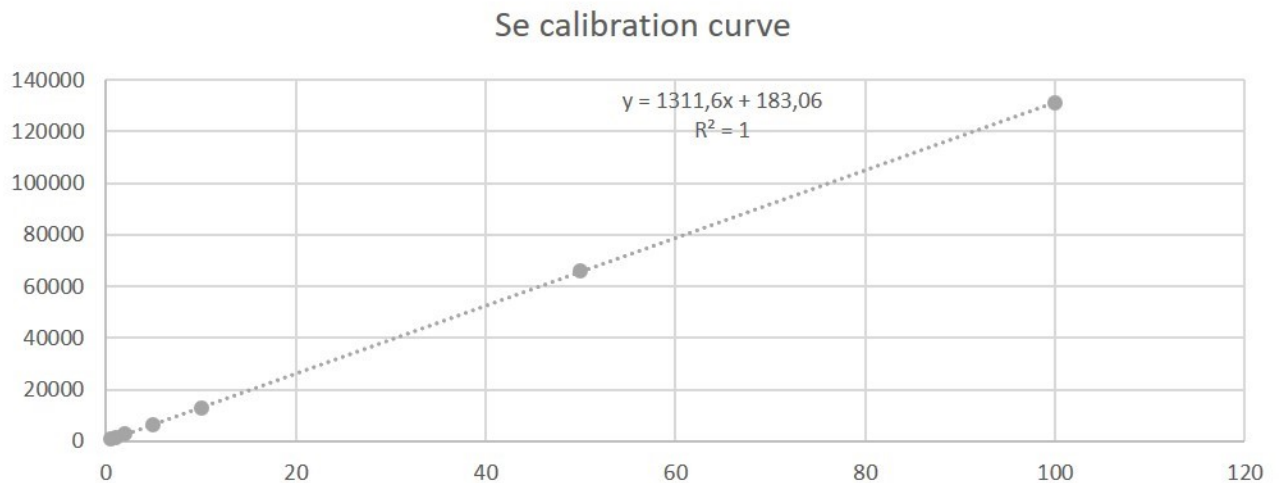


Figure B- 2- Se calibration curve (14-06-2019).

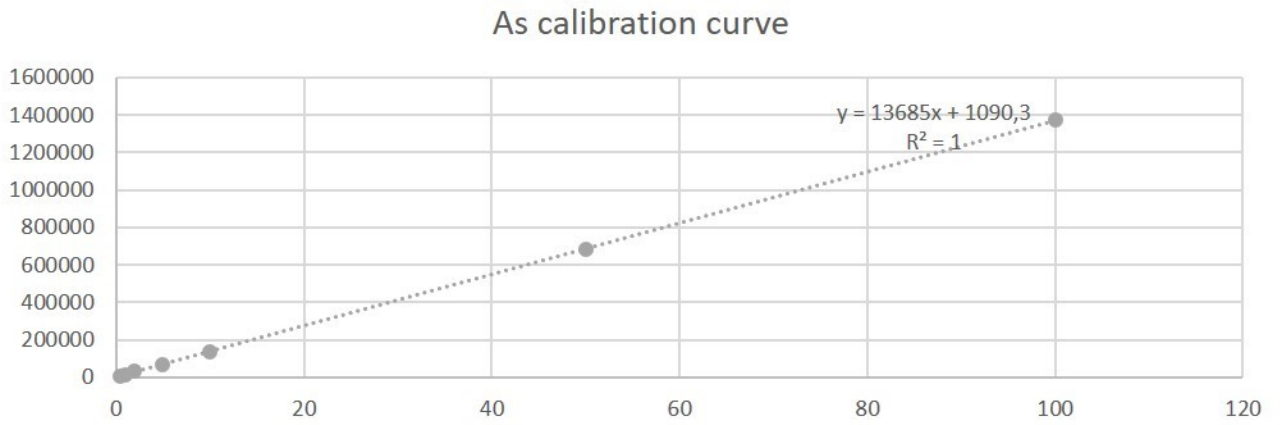


Figure B- 3- As calibration curve (14-06-2019).

### C. HR-CS-FAAS calibration curve parameters

Table C- 1- Example of a HR-AAS-MS calibration curve and respective parameters.

Element	Calibration curve parameters	R <sup>2</sup>	LOD (mg/L)	LOQ (mg/L)
Potassium	$y = 0.119x - 2.32e-06$	0.999	1.1	3.6
Sodium	$y = 0.102x + 0.00796$	0.998	0.3	1.0

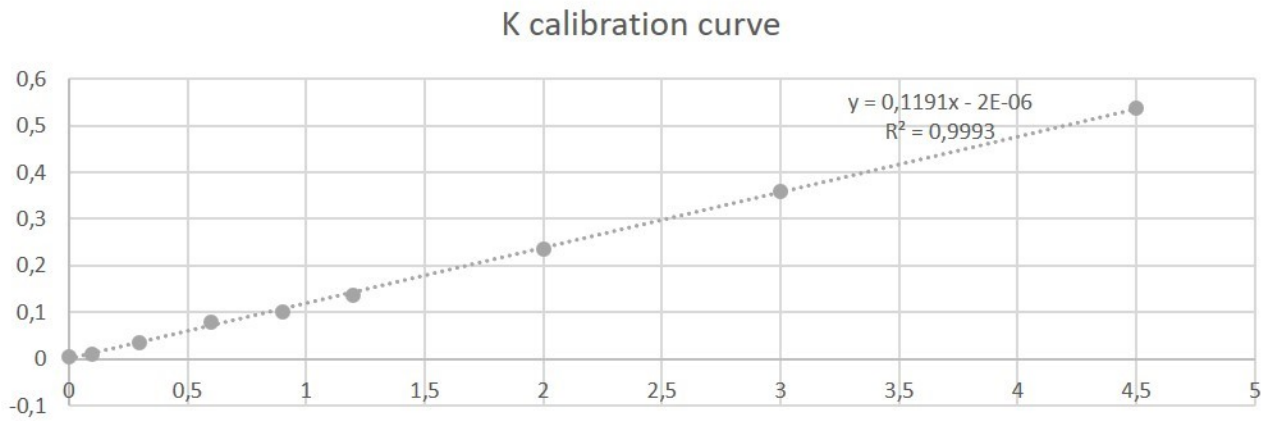


Figure C- 1- K calibration curve (20-05-2019).

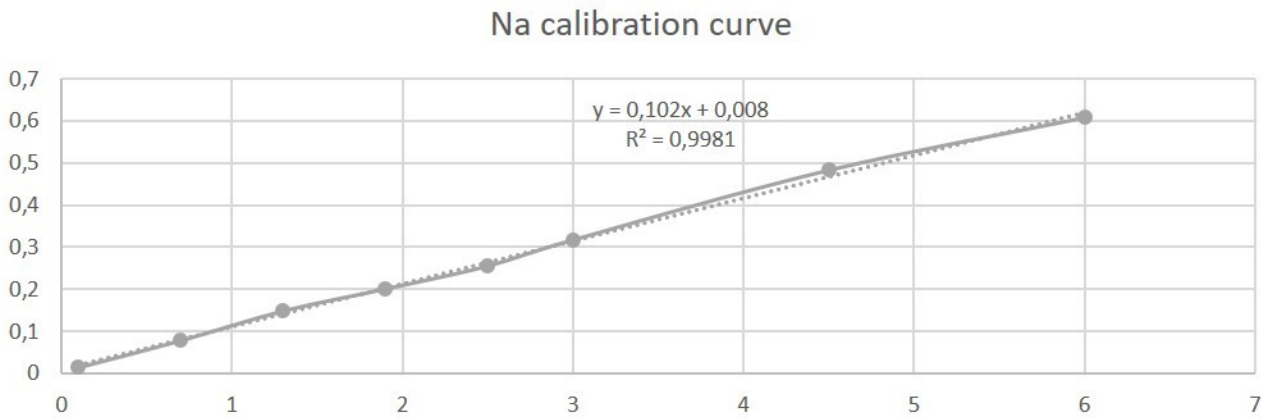


Figure C- 2- Na calibration curve (6-12-2018).