Universidade de Aveiro Departamento de Biologia
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MARIANA PEREIRA DA SILVA CARDOSO

Salicornia ramosissima as food: microbiological, nutritional and sensorial quality of glasswort from Ria de Aveiro

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Salicornia ramosissima como alimento: qualidade microbiológica, nutricional e sensorial de plantas da Ria de Aveiro

Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Marinha, realizada sob a orientação científica da Doutora Maria Ângela Sousa Dias Alves Cunha, Professora Auxiliar do Departamento de Biologia da Universidade de Aveiro e da Doutora Maria Helena Abreu Silva, Professora Auxiliar do Departamento de Biologia da Universidade de Aveiro.

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palavras-chave

Halófitas comestíveis, *Salicornia*, alimentos vegetais, sal-verde, segurança alimentar, valor nutricional, benefícios para a saúde

resumo

Salicornia ramosissima é uma planta halófita selvagem comestível que cresce preferencialmente em sapais e em zonas húmidas salinas. Em muitos países, o seu promissor perfil nutricional e organolético tem aumentado o seu reconhecimento como sendo um ingrediente gourmet. *S. ramosissima* é uma das espécies menos estudadas e constitui a única espécie do género *Salicornia* existente em Portugal. Assim, o presente estudo pretende contribuir para um conhecimento mais aprofundado da qualidade de *S. ramosissima* colhida na Ria de Aveiro, quanto à qualidade e estabilidade microbiológica bem como às características químicas, bromatológicas e sensoriais, tanto na forma fresca como seca (sal verde).

A carga microbiológica e a estabilidade da planta fresca e sob a forma de sal verde foram avaliadas durante 10 dias de armazenamento a 4˚C ou até 6 semanas à temperatura ambiente, respetivamente. O perfil nutricional foi avaliado com base em análise elementar e bromatológica de material fresco e seco.

Em geral, as análises microbiológicas revelaram concentrações de microrganismos abaixo dos limites recomendados aplicáveis tanto para vegetais frescos, como para ervas e especiarias, não tendo sido, do ponto de vista geral, detetadas leveduras nem patogénicos bacterianos (Escherichia coli ou Bacillus cereus).

O perfil nutricional revelou concentrações baixas de ácidos gordos e quantidades apreciáveis de água, Na e fibra. O sal verde revelou ser ainda uma boa fonte de minerais sem, no entanto, ultrapassar os limites recomendados para metais tóxicos. Com base na concentração de Na, calculou-se que a dose diária máxima compatível com o limite saudável de ingestão de Na seria de aproximadamente 7 g de sal verde ou cerca de 70 g de planta fresca.

As análises sensoriais indicam que as porções aéreas frescas incorporadas em salada suscitaram a maior aceitabilidade. A utilização de sal verde como substituto do sal tradicional foi mais satisfatória no prato de carne do que em sopa, uma vez que não foi atingido o nível de tempero (salgado) considerado satisfatório.

Os resultados suportam o incentivo ao alargamento do uso desta halófita na dieta como sendo um tempero mais saudável e abrem portas para o cultivo de *Salicornia* na Ria de Aveiro como atividade económica complementar ou na perspetiva da rentabilização de marinhas de sal atualmente abandonadas.

keywords Edible halophytes, *Salicornia*, food plants, green salt, food safety, nutritional value, health benefits

abstract *Salicornia ramosissima* is a wild edible halophyte plant that grows specially in salt marshes and salt wetlands. Due to its diverse nutritional and organoleptic profile, it is being increasingly recognized in many countries as a gourmet ingredient. Since *S. ramosissima* is one of the less studied species and it is the only species of genus *Salicornia* represented in Portugal, the present work intends to assess microbiological, mineral, bromatological and sensorial properties of fresh and dry shoots (green salt) of plants harvested in different salt marsh sites within Ria de Aveiro.

The microbiological load and stability of fresh shoots and green salt were evaluated during storage periods of 10 days at 4ºC or up to 6 months at room temperature, respectively. The nutritional profile was characterized based on elemental and bromatological analysis of fresh and dry plant material.

In general, the microbiological analysis showed that the concentration of microorganisms complied with the recommended limits for fresh vegetable and dry herbs and spices, and in general free of yeast and pathogenic bacteria (*Escherichia coli* and *Bacillus cereus*).

The nutritional profile of fresh shoots was characterized by low concentrations of fatty acids and high content in water, Na and fiber. The content of total ash in green salt and the elemental profile configure a good source of minerals, without exceeding the recommended limits for toxic metals. Considering the Na content of *S. ramosissima* determined in this study, the maximum daily dose to be consumed without exceeding healthy limit of ingestion of Na was calculated as 7 g of green salt or 70 g of fresh shoots.

The sensorial analysis indicated that the fresh shoots included in a mixed salad attracted the greatest acceptance. The use of green salt as a substitute for cooking salt showed higher acceptance in a meat dish than in a soup. In the later, the saltiness was below the satisfactory level.

The results justify that *S. ramosissima* may be more extensively used as healthy palatable food and open perspectives for the crop cultivation of this halophyte in Ria de Aveiro as a complementary economic activity or as an alternative for the reconversion of disused salt pans.

List of Figures

List of Tables

List of Abbreviations

AI	Adequate Intake
CF	Crude fiber
CFU	Colony Forming Unit
CP	Crude Protein
CVD	Cardiovascular disease
DL	Detection Limit
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DRI	Recommended Dietary Intake
DW	Dry Weight
BQL	Below Quantification Limit
EPA	Envirornmental Protection Agency
ESA	European Spice Association
FW	Fresh Weight
HDL	High-Density lipoprotein
HPLC	High Performance Liquid Chromatography
ICP-MS	Inductively Coupled Plasma- Mass Spectrometry
ISO	International Organization for Standardization
ITAU	Intituto Técnico deAlimentação Humana
MPV	Minimal Processed Vegetables
NP	Norma Portuguesa
RDA	Recommended Dietary Allowance
RNA	Ribonucleic Acid
r_s	Spearman's correlation coefficient
SF	Santiago da Fonte
TWI	Tolerable Weekly Intake

Index

1. Introduction

1.1 Current challenges to agriculture and global food supply

Since the beginning of the Agriculture Revolution, over 10.000 years ago, the human diet has experienced massive changes although changes in pool of genes related with metabolism, nutrition and taste are not expected to have changed dramatically (Simopoulos, 2008). Interestingly, many food practices from ancient uses are still largely ongoing (i.e. wild plants uses and agriculture practices) and there is even a growing interest in preserving or rediscovering traditional recipes (Guarrera et al., 2006). Furthermore, in face of the world's population growth and scarcity of freshwater supplies, researchers have been looking to the sea as a potential source of water to irrigate selected crops (Glenn et al., 1998).

Agriculture accounts for 70% of total water withdrawn for human needs, raising concern about the food supply in the future (Thomson, 2003). According to some estimates, the worldwide need for food may suffer an increase more than 70% until 2050, in order to adjust to the expected population growth (FAO, 2011). On the other hand, salinization of agriculture land represents an increasingly growing problem (Katschnig et al., 2013). Of the current 230 million ha of irrigated land, 45 million ha (20%) are affected by salt (FAO, 2008) and significant areas are becoming unusable each year. Soil salinization is a major challenge that is driving scientific research and promotes innovations on sustainable crop production (Khan and Weber, 2006).

1.2. Halophytes as biological resources

Soil salinization may represent an opportunity for halo-biotechnology. Halophyte crops and wild plants, which grow better under low, moderate or high salinities, are now regarded as food supplies or as models for the development of salt-tolerance in salt-sensitive crops, as a strategy to meet the food requirements in the future under limited fresh water resources (Flowers and Colmer, 2015). The 21st century will likely be the century of halophyte agriculture expansion for many reasons such as a response of several unacceptable conditions for most of the conventional crops.

Halophytes integrate approximately 1% of world's flora (Flowers and Colmer, 2008). Plants are considered as halophytes if they "complete their life cycle in a salt concentration of at least 200 mM NaCl under conditions similar to those that might be encountered in the natural environment" (Flowers and Colmer, 2008). That capacity of halophytes to tolerate soil salinity distinguishes them from glycophytes (i.e. *Triticum aestivum* and *Oryza sativa*). The later represent the major fraction of crops and forage species in current agriculture but are very sensitive to salinity (Panta et al., 2014).

The growth of halophytes is not compromised by saline irrigation, and they thrive in saline soils (Shabala, 2013) due to a wide range of adaptations in terms of anatomy, morphology and physiology (Flowers and Colmer, 2008). These responses may involve strategies such as the accumulation of water in succulent stems and leaves, and the compartmenting of Na^+ in the vacuoles, among others (Flowers and Colmer, 2015; Meng et al., 2018; Ventura et al., 2011). Saline stress entails ionic and osmotic pressures to which halophytes can respond with the capacity to synthesize osmoprotective compounds, thus maintaining the cellular structures through a water potential gradient (Lefèvre et al., 2009).

Ecosystem goods and services may be classified as marketed and non-marketed, and halophytes may contribute to both categories (Williams et al., 2003) (Figure 1). Halophytes, particularly the genus *Salicornia*, are also used for ornamental and/or environmental purposes. Ecological applications (non-marketed goods) are associated to their capacity to capture nutrients from the eutrophic ecosystems and to exert allelopathic effects on harmful algal blooms (Jiang et al., 2010). They also play an important role in desalinization and phytoremediation (Jiang et al., 2010; Panta et al., 2014). Furthermore, favourable outcomes of re-vegetation programs in wetlands reveal that halophytes contribute to stabilize and change soil characteristics having a positive impact in other organisms and in ecosystem health (Fogel et al., 2004). Their natural functions (i.e. nutrient removal or recycling and rehabilitation of salt-affected land) also contribute to human well-being.

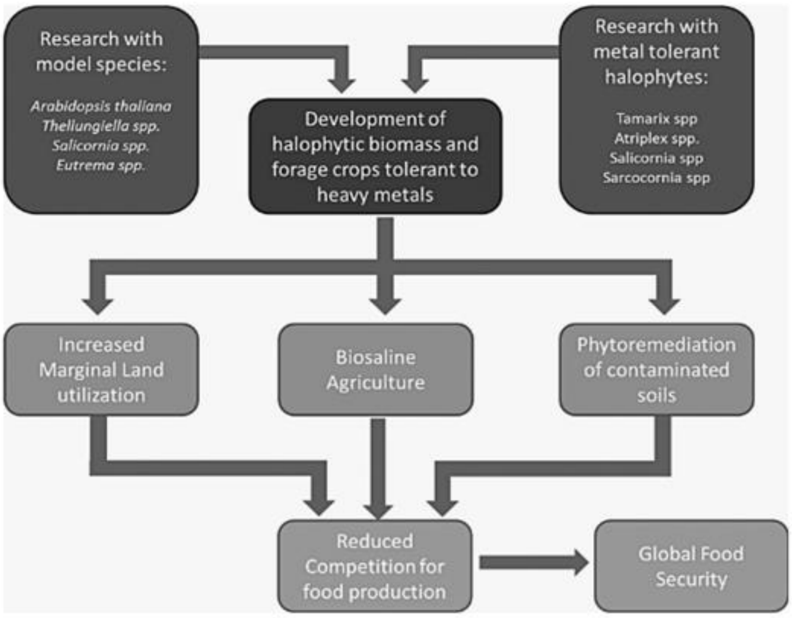

Figure 1. Potential applications and collateral advantages of halophytes on ecosystems (Van Oosten and Maggio, 2015)

Halophytes are traditionally and currently harvested for various purposes. They can be used as herbs and seasoning or salad vegetables for human consumption, forage (e.g. Atriplex barclayana, Suaeda esteroa and species of Salicornia), medicinal applications, oilseed extraction and biofuel production (Davy et al., 2001; Fedoroff et al., 2010; Guarrera et al., 2006; Panta et al., 2014; Ventura et al., 2015). From the nutritional perspective, halophytes are also be recognized as important sources of minerals (Muscolo et al., 2014; Swingle et al., 1996; Ventura and Sagi, 2013). Atriplex, Bassia, Chenepodium, Plantago, Portulaca, Salicornia, Salsola, and Suaeda are the most used edible halophytes worldwide (Tug & Yaprak, 2017).

1.3. Salicornia as model halophyte

Salicornia L. belongs to family Chenopodiaceae and is a well-known vascular halophyte plant with a typical summer-annual cycle that occurs preferably in middle and upper marshes. *Salicornia* shows succulent and articulated stems and extremely reduced leaves (Davy et al., 2001; Kadereit et al., 2006). The development of new lateral branches corresponds to the vegetative stage, which is followed by flowering and fructification stages (Silva, 2000). In summer, during the vegetative stage, most species of *Salicornia* have green colour and lower salt content in their tissues, turning reddish and purple in autumn when maturing and accumulation higher concentrations of salt (Patel, 2016). Opposite branches in the main stem, end in a fleshy spike of variable number of fertile segments (Kadereit et al., 2006).

Salicornia genus currently includes 25 to 30 species but this is an approximation due to the fact that there is still a controversy about the number of the valid species (Davy et al., 2001; Kadereit et al., 2006). This taxonomic misunderstanding may appear mostly because of the wide phenotypic plasticity in response to abiotic conditions, as a result of genetic adaptations in populations. Molecular phylogenetic analyses confirm an ecological and morphological proximity between the halophytes *Salicornia* and *Sarcocornia* (Kadereit et al., 2006; Shepherd et al., 2005; Silva et al., 2007). However, the genus *Sarcocornia* is distinguished from all the species of *Salicornia* due to their perennial life. Unusually, in some subtropical habitats, some species of *Salicornia* can remain viable throughout the year (Davy et al., 2006). The most common species within genus *Salicornia* are *S. europaea*, *S. bigelovii*, *S. brachiata*, *S. virginica*, *S. maritima*, *S. ramosissima*, *S. herbacea* and *S. persica* (Patel, 2016). *S. ramosissima* and *S. europaea* compose the *Salicornia europaea* agg. because they are closely related species (Adam, 1981; Davy et al., 2001).

The capacity of *Salicornia* to tolerate saline stress and flooding conditions represent two of the remarkable characteristics that underlie the occurrence under wide range of climatic conditions, that include boreal, temperate and subtropical environments (Davy et al., 2001; Kadereit et al., 2007). The subfamily Salicornioideae is represented in all continents with the exception of Antarctica. *Salicornia* species occur in habitats with very different conditions in terms of diurnal and/or seasonal dynamics, tidal scour, waterlogging, salinity, soil textures, nutrient supply and other factors that underlie a significant phenotypic fluctuation (Davy et al., 2001). They can be found throughout the seashores of Europe from Artic to the Mediterranean, and also on the coastline of the Black Sea and the Caspian Sea. These plants can also grow in inland tidal mud flats, salt lake shores, clay pans or others saline and periodic flooded soils distributed around Europe (Davy et al., 2001; Hupel et al., 2011; Kadereit et al., 2006, 2007).

As in the majority of salt marsh plants, the ideal salt concentrations for growth range between 1.45 and 29 ppm. This genus is invariably correlated with saline, brackish and alkaline subtracts. However, some abiotic aspects such as humidity (the higher moisture content in seeds of *S. europaea* the greater is the germination rate) and intensity of solar irradiation, as well as the age of the plant have also an apparent influence on optimal grow under saline conditions (Davy et al., 2001; Silva et al., 2007). Tidal flooding condition, especially in salt marshes, represent a predominant factor for seed germination of *S. europaea* where the valuable ecophysiology of halophytes confer resistance from these stressors through the dormancy of seeds for example (Howes Keiffer and Ungar, 1997). Individual populations tend to be sensitive to the intertidal microtopography. Populations growing in very low marshes, at lower salinities, are more tolerant to longstanding waterlogging whereas plants in higher reaches of the salt marsh tolerate hyper-salinity in summer by the increase of evapotranspiration rates (Davy et al., 2001).

Salicornia ramosissima J. Woods is the only native species found in Portugal. It is an annual and pioneer hygro-halophytic which generally has an erected rarely prostate or decumbent stem, with a height ranging from 3 up to 40 cm long, displaying opposite reduced leaves welded together. The fleshy main stem can carry many or few articulated and succulent branches with dark-green colour although they can become yellowish green or even purple-red. In the UK, it is commonly designated as “purple glasswort” due to their characteristic colour during the autumn season. *S. ramosissima* displays a high degree of polymorphism varying widely in size, and in length, diameter and number of the articulated segments of the stems. The flowers of *S. ramosissima* are aggregated in spike-like inflorescences with 4-14 (up to 36 in rare cases) fertile articulated segments, each having two opposite three-flowered cymes (Figure 2). The flowers without differentiated perianth produce only one seed and are arranged in triangular cymes with two small basal seeds and one large central seed. Seeds are oblong, ovoid, with null or almost non-existent perisperm (Figure 2) (Ameixa et al., 2016; Castroviejo et al., 1990; Davy et al., 2001; Silva, 2000).

S. ramosissima occurs naturally throughout salt marshes, salt pans and interfluves of upper marshes being widely distributed along West of Europe and North West of Africa (Kadereit et al., 2007). It is also present in Atlantic coast of France, in inland of Germany, broadly in Iberian Peninsula, Balearic Islands, British Islands ,Serbia and Romania (Northern Transylvania) (Castroviejo et al., 1990; Davy et al., 2001; Kadereit et al., 2006; Milić et al., 2011). In Portugal, it is represented in estuarine intertidal zones and along saltpans namely of the Ria de Aveiro and Ria Formosa (Ameixa et al., 2016; Antunes et al., 2018;

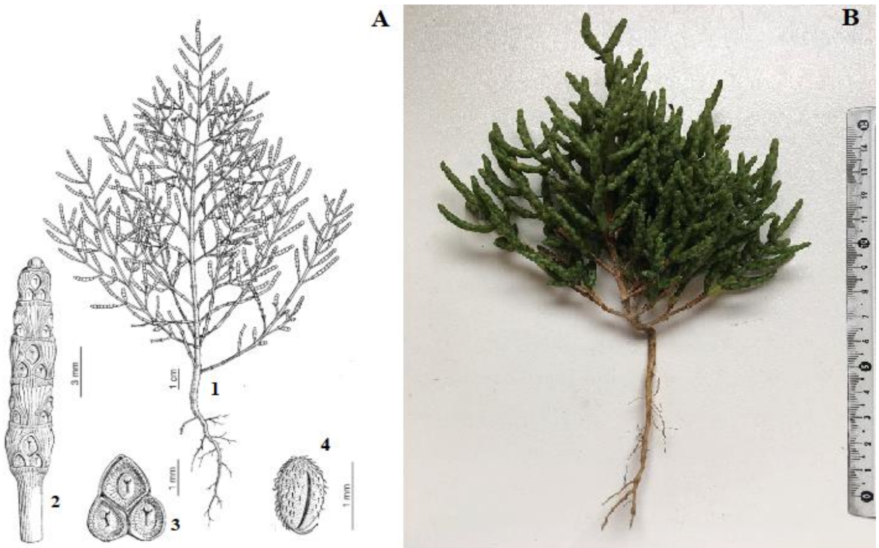

Figure 2. A) Representation of *Salicornia ramosissima*: 1- habit; 2- spike-like inflorescence; 3 - three flowered cyme; 4- seed. Adapted from (Castroviejo et al., 1990). B – Specimen collected in Ria de Aveiro

Silva, 2000) and other .

The high plasticity of *S. ramosissima* allows its growth in different type of marine substrates, ranging from gravel to mud and clay (Figure 3). It reveals a pioneer character, being frequently the first higher plant in the colonization process of salt marshes and mud habitats creating conditions for the settlement of other higher plant species. This represents an important feature in terms of management and conservation of estuarine habitats (Adam, 1981; Davy et al., 2001; Silva et al., 2007).

Figure 3. Salicornia ramosissima growing in a permanently flooded salt pan (A) and in a sandy mound separating adjacent salt pans (B) in Ria de Aveiro

1.4. Applications of *Salicornia* species

Since 2011, the International Center for Biosaline Agriculture (ICBA) has been focusing on *Salicornia* genotypes for different application such as biofuel (from seeds), vegetable and forage, with the purpose of identifying, and promoting their sustainable use and attaining economic viability. Favourable agriculture procedures and a careful selection of plants may contribute to the regain for agriculture, of land otherwise worthless thus generating local economic profit (ICBA, 2015). The wide tolerance to adverse environmental conditions, including soil type, salinization and waterlogging, make *Salicornia* species promising salt-crop candidates (Glenn et al., 1998; Isca et al., 2014a). Around 130 million ha of coastal area on a global scale were estimated to be suitable for *Salicornia* spp. crop cultivation (Bassam, 2010). Crop productivity, market prices, organoleptic and nutritional properties, as well as nutraceutical effects and functional secondary metabolites (e.g. osmolytes) are some of the motives for the growing interest for crop cultivation of halophytes (Ventura et al., 2015, 2011). *S. bigelovii*, *S. europaea* and *S. ramosissima* have been valuably marketed as fresh products, in Europe and USA, (Boer, 2008; Martínez-garcia, 2010; Ventura et al., 2011).

Salicornia sp. have been historically and traditionally included as a food source, mostly as a fresh or pickled vegetable, being already recognized as a cuisine specialty (Davy et al., 2001; Raposo et al., 2009). However, only recently has the demand for this plant experienced a notorious increase. Such interest is particularly due to the use of *Salicornia* as food, either in the fresh form, as salad green or as a seasoning, in a dry and ground ("green-salt"). In the Iberian Peninsula and France, *S. ramosissima* is used in human diet as a gourmet food., and consumed as a green salad or as pickles) (Davy et al., 2001; Ferreira et al., 2018; Isca et al., 2014b). Because the fresh green shoots of *S. ramosissima* have a natural salty flavour and crunchy texture, they have been increasingly used in fresh salads (plants in vegetative stage) and included in several recipes as a salt substituting seasoning (Chevalier, 1922). Combined aqueous extracts from 13 plants including *S. herbacea* showed approximately less 43% of sodium than the traditional sea salt, and still conferring the same satisfying palatability for saltiness (Lee, 2011). Therefore, using powdered *S. herbacea* as seasoning is regarded as a good strategy for reducing the concentration of added Na in food (Shin and Lee, 2013). This would contribute to control de risks of hypertension in consumers (Zhang et al., 2015) while improving flavour and texture (Kim et al., 2014; Lim et al., 2013). The use of powdered *S. ramosissima* material as a healthier alternative to salt and functional element in food has also been successfully demonstrated (Antunes et al., 2018; Lopes et al., 2017).

Nutritionally, *S. ramosissima* is a source of Mn and Na, among other essential minerals, as well as antioxidants, phenolic compounds (Barreira et al., 2017) and flavonoids which are related to some biological activities and nutraceutical properties. The photoprotective role against UV light, triggered the research on biomedical application of *S. ramosissima* extracts (Surget et al., 2015). Studies on antioxidant effects conducted in mice models showed a therapeutic role particularly on male reproductive dysfunction (Ferreira et al., 2018). In early development stages, *S. pusilla* is rich in ascorbic acid (Davy et al., 2001), vitamin A, carotenoids, and chlorophyll that are valuable dietary supplements (Lu et al., 2010).

The lipidic profile of *S. ramosissima* includes fatty acids, alcohols, sterols and aromatic acids that are considered as beneficial for human health (Isca et al., 2014). *S. ramosissima* contains less fibre and protein than other Salicorniaceae, like *Arthrocnemum macrostachyum,* but even so, it is considered a fibre-rich food (Barreira et al., 2017). The content of oil and protein in *S. bigelovii* oilseeds is similar to soybean and safflower and has been used for the extraction of vegetable oil (Glenn et al., 1999; Martínez-garcia, 2010) and also as fodder (El Shaer, 2010; Ventura et al., 2015) and fish feed in aquaculture systems (Kitto, 2004). *S. bigelovii* oilseeds are also being explored for the sustainable production of biofuels (Hendricks et al., 2011; Martínez-garcia, 2010).

In addition to its value as food, fodder or oilseeds, *Salicornia* species, particularly *S. herbacea,* are sought for other applications related to human and animal well-being and health (Im et al., 2007). *S. herbacea* has been long used in traditional medicine in Oriental countries for the relief of conditions like atherosclerosis, hypertension, diabetes, obesity, hyperlipidaemia and tumours (Park et al., 2006; Rhee et al., 2009). Anti-thrombus, anti-inflammatory, immunomodulatory and antidiabetic activities have also been demonstrated (Han and Kim, 2003; Jang et al., 2007; Lee et al., 2005),). It is also a promising skin rejuvenating agent because of antioxidative and significant skin whitening effects (Han and Kim, 2003; Jang et al., 2007; Julião, 2013; Sung et al., 2009). *S. herbacea* has been used in broiler industry as animal probiotic, reducing the fatty acid content of the basal diet of broilers and increasing the protein content in meat (Sarker et al., 2010).

1.5. Food safety issues on dietary halophytes

As the recognition of the economic and nutritional value of halophytes increases, there is also an increasing public concern on the quality of these plants as minimal processed vegetable (MPV) items as well as on their risk as vehicles for toxic compounds such heavy elements or food-borne pathogens (Johnston et al., 2006; Ragaert et al., 2007; Spink and Moyer, 2011).

The fact that some halophytes have the capacity to sequester or accumulate toxic elements makes them efficient phytoremediation agents (Manousaki and Kalogerakis, 2011) and in some cases, promising dietary sources of minerals. However, it also enhances the need for the careful assessment of the concentration of toxic elements, particularly metals, in the edible parts of plants harvested or cropped in contaminated areas (Patel, 2016). High concentrations of toxic metals and metalloids have been reported. *Salicornia* acts as a phytoextractive engine and hyper-accumulates Zn. Concentration of 5.2 µg/gdw of Cr, 1.5 µg/gdw of Pb and 2.3 µg/gdw of Ni have been reported for *Salicornia ramosissima* collected at Ria Formosa. However, the concentration of heavy metals in edible portions are still below the harmful legislated limits (Barreira et al., 2017). Although *S. ramosissima* is considered a Cd- accumulator (Pérez-Romero et al., 2016), some toxic elements like Cd are mostly confined to the roots, suggesting the existence of biological mechanisms that preclude the translocation aerial parts of the plant (Pedro et al., 2013).

Having the consumption of halophyte as fresh/raw vegetables or MPV in perspective, the impact of microbial contaminants in terms of food safety and product stability gain relevance (Raposo et al., 2009). Fresh-cut vegetables are particularly susceptible to spoilage that can cause deterioration and loss of nutritional value. Microbial growth of a large variety of bacteria, moulds and yeasts, considered typical of vegetables, is facilitated in minimal processed food (Fröder et al., 2007). The concentration of mesophilic aerobic bacteria after minimal processing of fresh-cut vegetables commonly varies between 10^3 and 10^6 CFU g^{-1} and frequently moulds are present in smaller numbers than mesophilic bacteria (from 1.0×10^2 to 4.0×10^4 CFU g^{-1}). Yeasts may remain longer and their growth is more persistent in minimal processed vegetables, ranging between 1.0×10^2 and 4.0×10^8 (Nguyen-the and Carlin, 1994; Tournas, 2005). Enteric bacteria, like *Salmonella* and *Escherichia coli,* as well as spore forming Gram positive bacteria, like *Bacillus cereus,* are often associated with outbreaks (Olsen et al., 2000) because they remain in the form of biofilms on vegetables even after washing and sanitizing (Allende et al., 2006; Nguyen-the and Carlin, 1994). Low temperature and controlled atmosphere storage delay microbial growth while preserving as much as possible the physical and chemical compounds of the fresh or MPV (Ragaert et al., 2007). There is, however, an urgent need for information about the microbiological risk associated with the use of halophytes as MPV, seasoning or pickles. One study by Raposo *et al.,* (2009) in which the microbiological quality of fresh shoots of *S. ramosissima* from a salt marsh of South Portugal was analysed after 4 weeks of storage, concluded that the concentration of total microorganisms, total coliforms, yeasts and moulds was still below the limits recommended considered for fresh vegetable.

The market of *Salicornia*-based food products is still developing. In Portugal, dehydrated shoots and branches of *S. ramosissima* are sold mainly as a traditional/regional biological product (green-salt). The production through small-scale farming and manual processing operations may entail elevated risk of microbiological spoilage (Vitullo et al., 2011). Storage conditions around 6 ºC are considered ideal for the preservation of organoleptic properties of powdered plants and to assure microbiological stability for more than 3 months (Antunes et al., 2018).

The recognition of the value of *Salicornia* as a food product and the broadening of its use in food still needs to be supported by information on its microbiological quality, nutritional value and stability during storage. It is also important to ascertain the variability between harvesting sites so that crop production of *Salicornia* can indeed be regarded as a relevant activity in the context of local economy. The objective of this work was to contribute to the demonstration that *Salicornia* harvested or cultivated in Ria de Aveiro meets the nutritional and microbiological safety requirements to be used as substitute of marine salt or as a fresh salad vegetable and also to highlight other properties that can widen the spectrum of applications of this plant species.

2. Material and methods

2.1. Sampling collection

Aerial parts of *S. ramosissima* plants were collected in three different intertidal sites of Aveiro lagoon (Portugal) herein after referred to as Bôco (site B), Pontinha (site P) and Santiago (site S), corresponding to saltmarshes (B), active (S) and former (P) saltpans. In site B, sampling was conducted in October 2017 during the fructification stage and in June 2018, corresponding to the vegetative stage. In sites P and S, plant material was collected only in June 2018, during the vegetative stage of the plant. Besides this samples, fresh plants were collected in September 2018 in vegetative stage near to Troncalhada (T) saltpans. Plant material was packaged in polyethylene bags and transported to the laboratory in isotherm boxes.

2.2. Preparation and storage of fresh shoots

Samples collected in June from B, P and S, and plants collected in September in T, all of them in the vegetative stage, were treated separately according to procedure used for commercial purposes. The aerial parts were washed with tap water. Stems were removed, and shoots were aseptically cut in small pieces with a disinfected scissor. Plants from B, P and S were stored in air-tight sterile polyethylene bags and stored at 4ºC for 10 days. Plants from T, after minimal processing of washing and cutting were immediately used for the purpose.

2.3. Preparation and storage of green-salt

Fresh shoots of plants from B in fructification stage were prepared similarly to the procedures described above and stored at 4 ºC for approximately 2 days before further uses. Also, fresh shoots sampled in vegetative stage from all sites (B, P, S) were prepared from the same washing, cutting and storage procedures. Samples from each site was separated into petri plates, each containing approximately 10g of fresh material and dried at 70 ±0.5 °C (APT Line ™ ED E2 Binder) until constant weight was achieved (approximately 5 days). In order to obtain the green-salt, the dried material was grinded, and divided into 15 g sub-samples and packed in air-tight glass jars. All the samples were kept at room temperature in a dark shelf to mimic storage conditions of commercial samples.

2.4. Analyses

Analyses were conducted with plants collected from different sites in vegetative and fructification stages. A summary of the types of samples used in each category of analyses is presented in table 1.

Table 1. Experimental design of samples collected and evaluated in this study

Samples	Sampling site	Phonological stage	Collecting time	Analyses
B	Bôco	Fructification	October 2017	Microbiological Bromatological
		Vegetative	June 2018	Microbiological Bromatological Elemental
P	Pontinha	Vegetative	June 2018	Microbiological Bromatological Elemental
S	Santiago	Vegetative	June 2018	Microbiological Bromatological Elemental
T	Marinha da Troncalhada	Vegetative	September 2018	Sensorial

2.4.1. Microbiological analyses of fresh shoots

Microbiological analyses of the fresh shoots of plants in vegetative stage from B, P and S (suitable to be consumed as salad greens) were conducted at the sampling day and after 10 days of storage at 4 ºC. Samples were divided into 3 sub-samples of 10 g each and they were aseptically weighed and homogenised with 90 mL Ringer solution (Merck) in a household blender (Moulinex) for 3 min. The homogenate was taken as a 10^{-1} dilution (w/v) of the sample. Coarse debris was separated by centrifugation, for 5 min at 4000 rpm (TDL 40 B centrifuge). The supernatant was serially diluted in Ringer solution. Enumeration of total aerobic mesophilic bacteria, total coliforms, *E. coli*, *B. cereus*, yeasts and moulds was conducted according to Portuguese Standards of Food Microbiology and International Organization for Standardization, described just below.

Total aerobic mesophilic bacteria were quantified according to NP 1409 (1987). Three replicate aliquots of each dilution were pour-plated in Plate Count Agar (Liofilchem). Cultures were aerobically incubated at 30 °C for 72 h.

Total coliforms and *E. col*i were enumerated according to (NP 1410, 1984) and ISO (NP, 2014). Three replicate aliquots of each dilution were pour-plated in Chromocult® Coliform Agar (Merck). Cultures were incubated at 37 °C for 24h and typical colonies were enumerated in the most adequate dilution.

Enumeration of *Bacillus cereus* was carried out based on ISO 7932 (2004). Triplicate aliquots were spread-platted on Mannitol Yolk Polymyxin Agar (OXOID). Cultures were incubated at 30 ºC for 18- 24 hours. Typical colonies were enumerated in the most adequate dilution.

Yeasts and moulds were analysed according to (NP 3277-1, 1987). Five replicate aliquots of 200 µl were spread-plated on Dicloran Rose Bengal Chloramphenicol Agar (Merck). Cultures were aerobically incubated at 25°C for 5 days. Colonies of yeast and molds were counted separately.

For each parameter, colonies were counted in the most adequate dilution. The values were averaged, corrected for the dilution factor and aliquot volume and expressed as colony forming units per gram fresh weigh (CFU/ gfw^{-1}).

2.4.2. Microbiological analyses of green-salt

Microbiological quality of stored samples of green-salt obtained from plants in fructification stage (collected at site B in October 2017) was assessed by monthly analyses during a period spanning 6months of storage. In samples prepared from plants in vegetative stage (collect at sites B, P and SF in June 2018), analyses were conducted only during a storage period of 2 months.

For the preparation of suspensions, 3 subsamples of 2 g of each type of green-salt were aseptically homogenized with 18 ml of Ringer solution (Orbital shakers PSU-10i & PSU-20i Grant-bio) at 450 rpm for 90 min. The homogenate was taken as a 10^{-1} dilution (w/v) of the sample. Coarse debris was separated by centrifugation, for 5 min at 4000 rpm (TDL 40 B centrifuge). The supernatant was serially diluted in Ringer solution. Aerobic bacteria, total coliforms and *E. coli*, *Bacillus cereus,* yeasts and moulds were analysed as described in section 2.2.1.

2.5. Elemental analysis of green salt

Mineral elements were determined in green-salt (only that prepared from plants in vegetative stage) stored for approx. 1 month. Extracts were prepared by acid digestion following the EPA Method 3050B (Environmental Protection Agency, 1996). Approximately 0.5 g of dried ground material was transferred to digestion vessels (Digitubes, SCP Science). Three ml of 69% HNO_3 (PlasmaPURE Plus) and 2 ml of 30% H_2O_2 (Labkem) was added to each vessel and the mixture was homogenized. The tubes were covered with a watch glass and placed into a graphite digestion block (Digiprep MS, SCP Science). Samples were heated at 50 ± 0.5ºC for 15 min and reheated at 80 ± 0.5 ºC 15 min. The extracts were evaporated covered with a vapor recovery device at room temperature to dryness over 1-2 days and cooled. The dry extract was diluted in 25 ml with Milli-Q water and stored at 4 ºC until analysis. Negative (blanks without plant material) and positive (*Fucus vesiculosus* Bladderwrack ERM® certified reference material, Merck) controls were subjected to the same extraction procedure.

Elemental analysis was performed at the GEOBIOTEC Laboratory (University of Aveiro) in an Inductively Coupled Plasma -Mass Spectroscopy (ICP-MS, Agilent Technologies 7700 Series). A total of 27 elements were quantified (Li, Na, Mg, P, K, Ca, Zn, Fe, Mn, Be, Al, V, Cr, Co, Ni, Cu, As, Ag, Cd, Sn, Sb, Ba, W, Ti, Mo, Pb and Th). Determinations were conducted in duplicate and the concentrations were calculated by interpolation with calibration curves constructed with standard solutions.

2.6. Bromatological analysis of fresh and green salt

Bromatological analysis of fresh shoots and green salt (material collected in June 2018 corresponding to vegetative stage) were outsourced (Biogerm, SA) in collaboration with ITAU (Instituto Técnico de

Alimentação Humana). Green salt corresponding to site B was not sufficient for analysis. Therefore, material from this site was analysed only as fresh shoots. Analyses were conducted according to the internal standard procedures of the external service provider.

Moisture and total ash content were determined by thermogravimetry (IT-DLQ-31). Total nitrogen was determined by the Kjeldahl method and a factor of 6.25 was used to estimate the crude protein content (AOAC, 2000; ISO, 2005). Crude fat was determined after Soxhlet extraction AOAC 1990 (ISO, 2015) and lipids were analysed by HPLC. Total dietary fibre was determined using a Fibertec™ 1023 Dietary Fiber Analysis System (Prosky et al., 1985) and carbohydrate content was calculated by difference (AOAC, 2006).

2.7. Sensory analysis

2.7.1. Sensory analysis of fresh shoots

In order to evaluate the acceptance of *S. ramosissima* (green salt) as a substitute for salt, a free-choice sensory profile of this plant was designed according to ISO (2016) (Annex I). The samples from T were evaluated by an untrained panel of 35 participants, comprising professors, students and staff from the academic community of University of Aveiro. All the participants were lightly elucidated on the general objective of the sensory analysis. Individual portions of a green salad were prepared containing equal proportions of green lettuce leaves, purple cabbage, sweet corn and fresh shoots of *Salicornia ramosissima*, and finally dressed with olive oil and vinegar, without any addition of salt. Sensory attributes were assigned to a typical preference and acceptability consumer test. Participants used a 5-point hedonic scale to evaluate 3 sensorial attributes: saltiness, overall impression and intention of purchaseion.

2.7.2. Sensory analysis of green salt

The quality tests of green salt were also performed taking into account the same free- choice sensory profile described by ISO (2016)(Annex II). In the survey, 3 sensorial descriptors were proposed to the panelists: saltiness and overall impression, as parameters of acceptability and intention of purchase as a preference test where the same structured hedonic scale for the analysis of fresh shoots was applied. The sensorial analysis of green salt was achieved with the   ITAU collaboration, and thus it was performed in a canteen provided by ITAU food sector. Samples of green salt used in this sensorial trial were provided by Horta dos Peixinhos company. The sensorial test included a panel of 70 untrained participants, including company staff members. A common vegetable soup containing broccoli, white cabbage, potato and onion, exclusively dressed with olive oil and green salt (0.5 g/soup) was prepared and served in individual portions for participants. In parallel, green salt was also tested as a salt substitute (0.6 g/garnish) in a grilled pork meal, and used in the seasoning with lemon juice. The grilled pork meal was accomplished with white rice.

The average and standard deviation values were calculated for each sensorial attribute.

2.8. Statistical analyses

The univariate statistical model was used to determine significant variances between groups in microbiological analysis. The Tukey multiple comparison test, at a significant level of 0.05, was performed to define homogenous groups according to the effect of sampling sites and storage time on microbiological parameters. Data from elemental analysis were obtained by one-way ANOVA for a significance of 0.05. When significant differences in the concentration of minerals between sampling sites were demonstrated, Tukey's test was applied to define homogenous groups from the mean values of elements. A statistical correlation analysis using Spearman's test were applied to estimate Spearman's rank correlation. All the statistics were conducted using SPSS Statistics 24 software for windows. Mean values and standard deviation (S.D.) of microbiological, elemental and sensorial data were reported.

3. Results and discussion

3.1. Microbiological analysis

3.1.1. Microbiological quality and stability of fresh shoots

The results of the microbiological analysis of fresh shoots (vegetative stage) collected from B, P and S are represented in Figures 4 and 5, respectively. Total coliforms, *Escherichia coli* and *Bacillus cereus* were not detected. Considering that fresh shoots are most commonly incorporated in salads, the results for aerobic mesophilic bacteria and fungi (yeasts and molds) were compared with the criteria for microbiological quality of raw vegetables (Table 2).

Table 2. Recommended microbiological limits for raw vegetables

Microbiological parameters	Limits (CFU/gdw)[1] Satisfaction	Acceptance	Reference
Aerobic mesophilic bacteria	$\leq 10^4$	$\leq 10^6$	(INSA, 2018)
Molds	$\leq 10^2$	$\leq 10^3$	

[1] Values are recommended specifications of CFC/g for different types of raw vegetables

The concentration of aerobic mesophilic bacteria (Figure 4) ranged from 6.6× 10^2 (site S) to 7.5 × 10^5 CFU/gfw (site B) without significant differences between sampling sites (ANOVA, p>0.05). In fresh samples, the acceptance limit was not exceeded. In samples collected from sites B and S, there was a significant increase (ANOVA, p<0.05) in the concentration of aerobic mesophilic bacteria after 10 days of storage and the satisfaction limit was exceed for all sampling sites. However, all samples were still

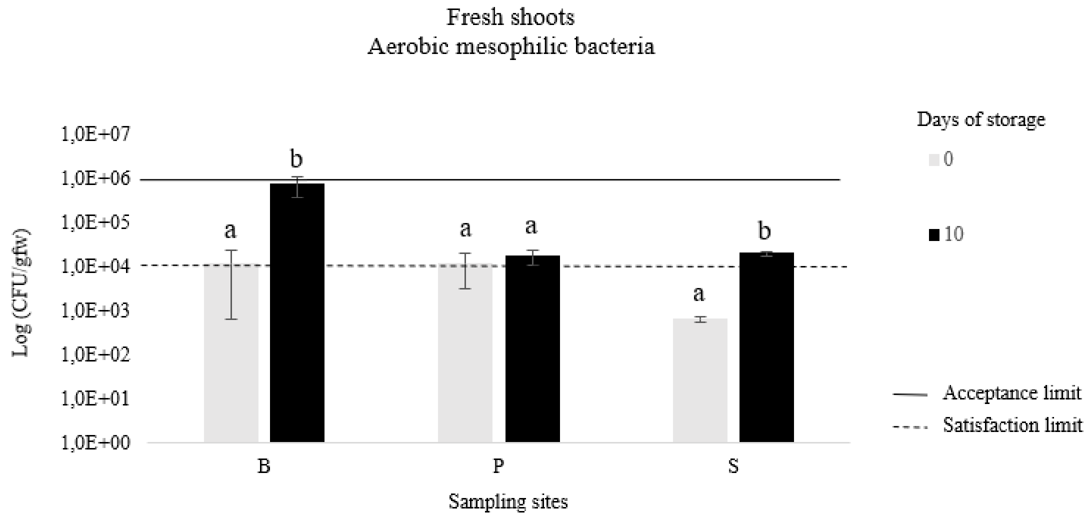

Figure 4. Concentration of aerobic mesophilic bacteria in fresh shoots of vegetative-stage *Salicornia ramosissima* collected from site B, P and S and in material stored at 4˚C for 10 days. Values correspond to the average of 3 sub-samples and error bars represent the standard deviation. The solid line represents the maximum satisfaction limit and the dashed line represents the maximum acceptance limit for aerobic mesophilic bacteria according to INSA (2018). Letters indicate homogeneous groups defined by post-hoc Tuckey test.

classified as satisfactory.

The concentrations of fungi, before and after 10 days of storage, is represented in Figure 5. Yeasts were not detected so counts correspond to filamentous fungi (molds) only. In fresh material, molds were only detected in samples collected from site P (6.7 CFU/gfw). After storage, molds were detected in all samples (9.2 × 10 CFU/gfw - 1.4 × 10^2 CFU/gfw). The highest value corresponded to samples from site B (1.4 × 10^2 CFU/gfw) but at the end of the storage period, differences between sites were not statistically significant (ANOVA, p>0.05). Before storage, all samples met the satisfaction limit but after storage, but this limit was exceeded in stored material from sites B and S. The acceptability limit was never exceeded.

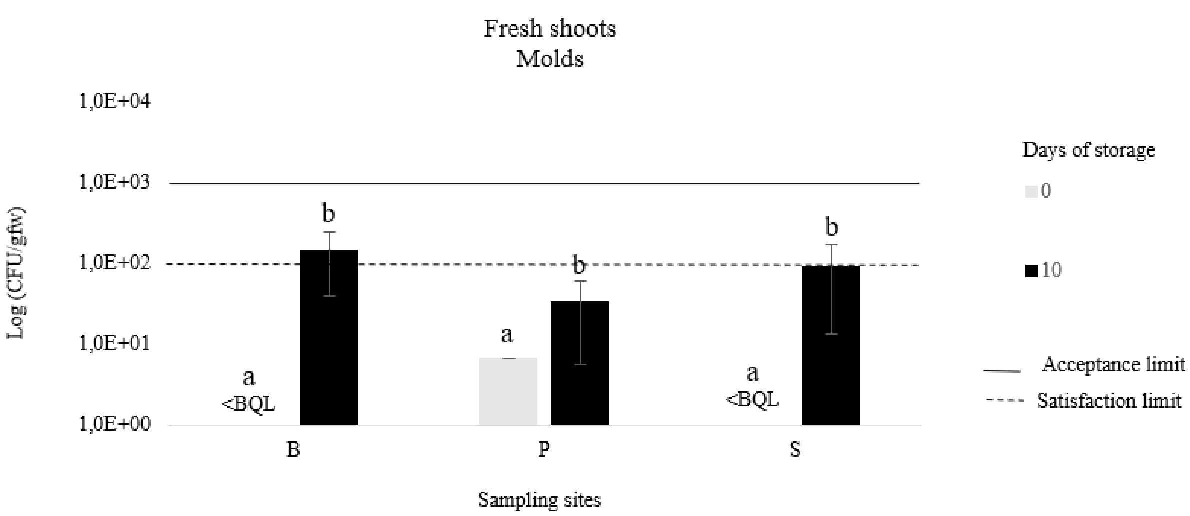

Figure 5. Concentration of moulds in fresh shoots of vegetative-stage *Salicornia ramosissima* collected from site B, P and S and in material stored at 4˚C for 10 days. Values correspond to the average of 3 sub-samples and error bars represent the standard deviation. The solid line represents the maximum satisfaction limit and the dashed line represents the maximum acceptance limit for molds according to INSA (2018). Letters indicate homogeneous groups defined by post-hoc Tuckey test. BQL – below quantification limit (<1 UFC/gdw).

Using the guidelines for raw vegetables (e.g. lettuce, purple cabbage, among others) for the evaluation of the microbial quality of fresh shoots of *S. ramosissima* as salad greens, the results indicate an overall satisfactory quality of freshly harvested material from Ria de Aveiro. The grey mold *Botrytis cinerea* that causes rot in fruits and vegetables has been detected in *S. bigelovii* as a phytopathogen, causing infections in branches and steams (Rueda Puente et al., 2014) which demonstrated that halophytes can be colonized by fungi. High levels of yeasts and molds in minimal processed vegetables may cause deterioration and turn the tissue of plants soft and slimy (Oliveira et al., 2010). However, in the present study fungi were virtually absent which agrees with literature reports that fungi are less abundant in vegetables than mesophilic bacteria (Nguyen-the and Carlin, 1994).

Total coliforms and *E. coli* were included in this experiment because their presence in fresh food is interpreted as indication of pre-harvesting contamination or deficient hygienic conditions during storage or preparation (Castro-Rosas et al., 2012; Miskimin et al., 1976). *Bacillus cereus* is not a frequent contaminant of vegetables but it has been reported in particular cultivation conditions (Harmon, 1987) and the endospores can remain viable for long periods in mixed salads (Holtzapffel and Mossel, 1968). However, these indicators or pathogens were not detected confirming the good microbiological quality of fresh *Salicornia* and the low microbiological risk associated with Ria de Aveiro as harvesting or potential crop cultivation area.

Microbiological quality decreased during storage but after 10 days, but the acceptability criteria were still met. After storage, the concentration of molds was generally significantly higher (<2 log increase) than in freshly harvested material but and in terms of bacteria, a significant increase (~ 2 log) was only observed in material from sites B and S. A study conducted by Raposo et al., (2009) evaluating the microbial loads (aerobic mesophilic bacteria, total coliforms, yeasts and moulds) of fresh *S. ramosissima* from Ria Formosa during a 4-weeks study also concluded that after this storage periods, material was still acceptable in terms of microbiological quality. Comparing the effect of storage for a corresponding 10-day period, samples from Ria de Aveiro had better microbiological quality, and in addition, yeasts and fecal indicators were still below the quantification limits. This may be related with lower environmental levels of microbiological contamination of the harvesting sites in relation to Ria Formosa.

Although the microbiological quality of refrigerated fruits and vegetables is still mostly determined by the microbiological quality at the moment of harvesting, refrigeration during transport and storage is still the most common strategy of preserving properties and extending shelf life (Brackett, 1994; Wiley and Yildiz, 2017). In the present study, microbiological loads were still in compliance with the guidelines for fresh vegetables with indicated that this period can be accepted as within and acceptable shelf-life. However, a “by eye” evaluation indicated that some properties like color, texture and succulence may have been negatively affected and therefore, a formal sensorial is necessary to a more rigorous definition of the shelf-life fresh *Salicornia* shoots.

Overall, our results demonstrate of the good microbiological quality and microbiological stability during refrigerated storage of fresh shoots of *S. ramosissima* and argue in favor of the use of this halophyte as a salad green.

3.1.2. Microbiological quality and stability of green salt

3.1.2.1. Green salt prepared from plants in fructification stage

The results of the microbiological analysis of samples of green salt (material prepared from plants in early fructification stage collected at B and stored for 6 months) are represented in Figures 6 to 8.
Considering that green salt is mostly used as seasoning, the results were interpreted in the light of the criteria for microbiological quality for herbs and spices (Table 3).

The concentration of aerobic mesophilic bacteria varied between 3.3 and 4.7 $\times 10^3$ CFU/g dw. The maximum value observed was after 3 months of storage and herein there was a significant difference in relation to the initial value (ANOVA, $p<0.05$). The lowest concentration was observed at the end of the storage period (6 months) and this value showed to be significantly different in comparison to the first month of the experiment (ANOVA, $p<0.05$) (Figure 6).

Table 3. Recommended microbiological limits for herbs and spices

	Limits (CFU/gdw)[1]		
Microbiological parameters	Satisfaction	Acceptance	Reference
Aerobic mesophilic bacteria	$< 10^4$	$<10^7$	(Ribeiro, 1974; Stannard, 1997)
Yeasts and molds	$<10^3$	$<10^4$	(Muggeridge and Clay, 2001)
Total coliforms	$<10^4$	N.A.	(ICMSF, 2008)
E. coli	<10	$<10^3$	(Muggeridge and Clay, 2001)
Bacillus cereus	$<10^2$	$<10^4$	(Stannard, 1997)

[1] Values are recommended specifications of CFC/g for different types of herbs and spices

The concentration of aerobic mesophilic bacteria varied between 3.3 and 4.7 ×103 CFU/g dw (Figure 6). The maximum value observed was after 3 months of storage and from then on there was a significant decrease in relation to the initial concentration (ANOVA, $p<0.05$). The lowest concentration was observed at the end of the storage period.

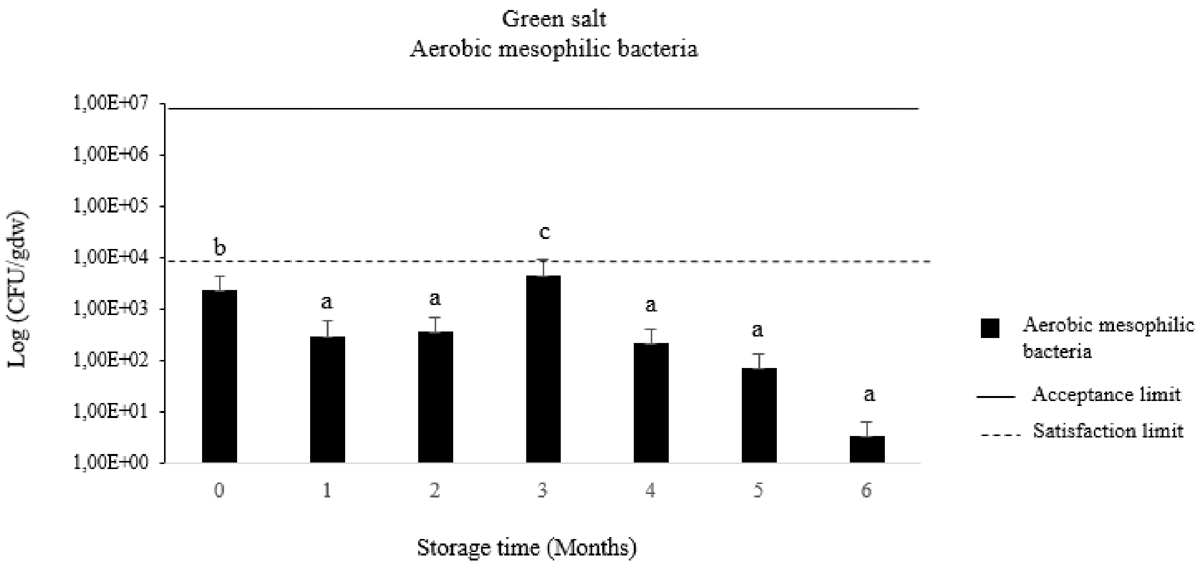

Figure 6. Variation of the concentration of mesophilic bacteria in green salt obtained from *Salicornia ramosissima* in fructification stage collected from site B and stored at room temperature for 6 months. Values correspond to the average of 3 sub-samples and error bars represent the standard deviation. Solid line represents the maximum satisfaction limit according to Ribeiro (1974) and the dashed line represents the maximum acceptance limit according to Stannard (1997). Letters indicate homogeneous groups defined by post-hoc Tuckey test.

Bacillus cereus (2.3 ×10 CFU/g dw) were only detected in the analyses conducted after 3 months of storage and even in this case, below the satisfaction limit (Table 3).

The concentration of total coliforms varied between 0 and 1.3×10^3 CFU/gdw during the first 2 months of storage. Surprisingly, in the analyses corresponding to the 3rd month of storage, an abnormally high value (1.3×10^3 CFU/gdw) was detected. Considering that this matches in time the highest concentration of mesophilic bacteria and the only positive detection of *Bacillus cereus* but also that the concentrations determined in the 3 different sub-samples were not significantly different (data not shown), results were interpreted as resulting from bacterial contamination during processing of the samples for analysis (Figure 7) rather than from accidental contamination of the glass jars used for packaging (ANOVA, $p<0.05$). From the 3rd month of storage on, total coliforms were no longer detectable. *Escherichia coli* were not detected in any of the samples analyzed.

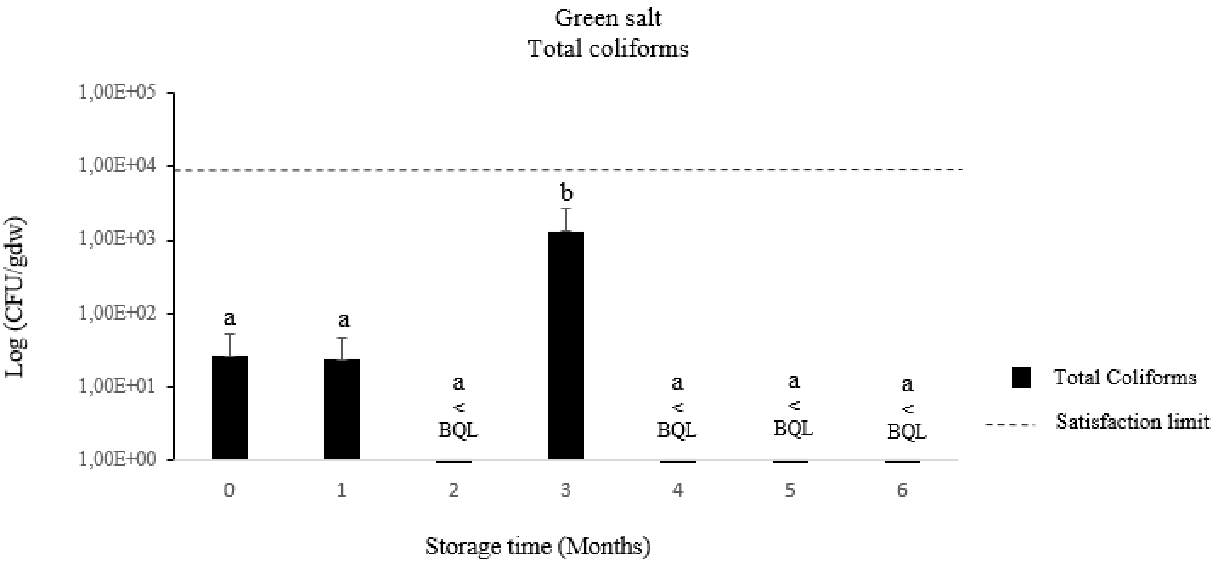

Figure 7. Variation of the concentration of total coliforms in green salt obtained from *Salicornia ramosissima* in fructification stage collected from site B and stored at room temperature for 6 months. Values correspond to the average of 3 sub-samples and error bars represent the standard deviation. The dashed line represents the maximum satisfaction limit according to ICMSF (2008). Letters indicate homogeneous groups defined by post-hoc Tuckey test. BQL – below quantification limit (<1 UFC/gdw).

Green salt was free of yeasts. All colonies corresponded to molds. The concentration of molds varied between 0 and 1.6×10^2 CFU/gdw (Figure 8). In general, there was a decrease in the concentration of molds during storage and values were always below the satisfaction limit.

Microbiological quality of green salt in compliance with guidelines for the microbiological quality of herbs and spices (Table 3) indicating that the product was stable during 6 months storage at room temperature. However, the comparison of the results obtained in this study with other published reports, indicates that the microbial load of green salt may depend on the provenance of the plants used to prepare it. A study conducted with green salt produced with *S. ramosissima* harvested in Ria Formosa, and stored for 6 months of storage at different temperatures (6ºC and ~ 23ºC) reported higher initial concentrations of aerobic mesophilic and an increase of the microbial load during storage, to values that exceeded the acceptance limit (Antunes et al., 2018). Differences in the natural microbial communities associated with each particular harvesting site and in the physical conditions and practices during dehydration and grinding may also contribute to differences in the microbiological quality of green salt, like other dried herbs (Vitullo et al., 2011).

In the present work, yeasts were not detected but molds were in general, present. The growth of yeasts and moulds is strongly dependent on the water activity (a_w) and usually drying procedures reduce the incidence of yeasts which explains why yeasts were not detected in green. However, the spores of some groups of xerophilic molds (e.g. *Aspergillus*) can germinate under low a_w. *Aspergillus flavus* and *A. ochraceus*, are common in spices, and since they produce carcinogenic and immunosuppressive mycotoxins, their presence in high concentrations represents a significant risk (Beuchat, 1983; Kovács, 2004). The concentration of molds decreased during storage. This was also observed for other dried herbs, like basil, and it is caused by antibacterial metabolites present in the plant (Montes-Belmont and Carvajal, 1998). Although there is still no evidence of antifungal activity of *S. ramosissima* the results justify further testing.

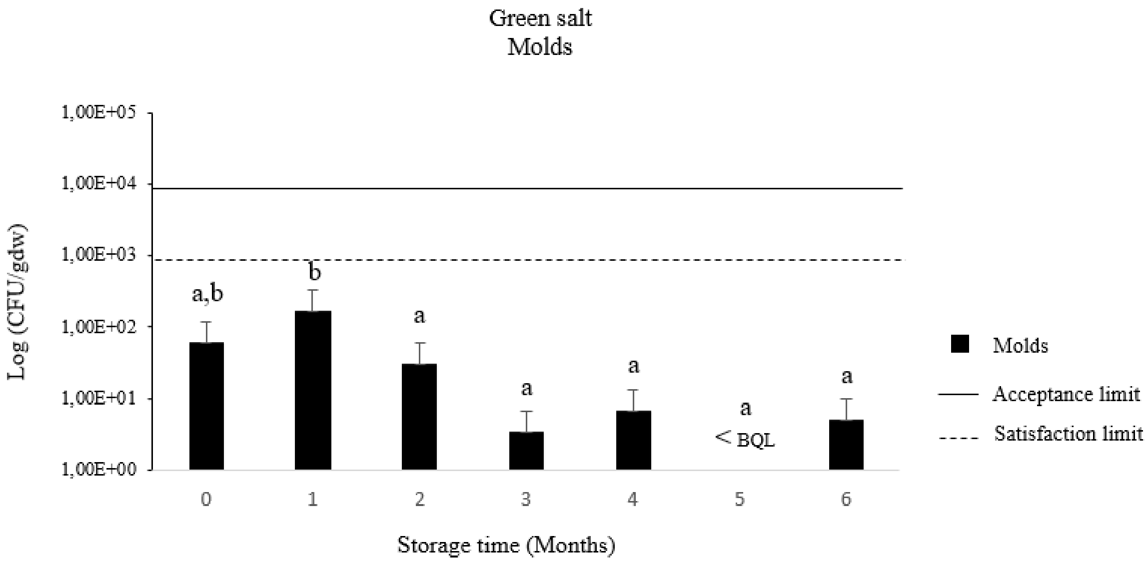

Figure 8. Variation of the concentration of molds in green salt obtained from *Salicornia ramosissima* in fructification stage collected from site B and stored at room temperature for 6 months. Values correspond to the average of 3 sub-samples and error bars represent the standard deviation. Solid line represents the maximum satisfactory limits and dashed line means the maximum acceptable limits according to Muggeridge et al. (2001). Letters indicate homogeneous groups defined by post-hoc Tuckey test.BQL – below quantification limit (<1 UFC/gdw).

Escherichia coli, fecal indicator bacteria associated with the risk of enteric disease, were not detected. Fecal contamination of spices or dried herbs, and especially the presence of *E. coli,* is considered rare because of the sensitivity of these microorganisms to low water activity (Julseth, 1974). In the case of green salt, the presence of *E. coli* would most likely be related to post-harvesting cross-contamination, considering the relatively low survival of fecal bacteria saline environments and salt marshes (Myers and Ambrose, 2015).

Bacillus cereus were found only in one the analysis, with a low concentration. *B. cereus* is not common in estuarine sediments, although species of *Bacillus* have been reported in polluted systems (Mahler et al., 1986). However, *B. cereus* was already detected in the rhizosphere of *S. ramosissima* (Szymańska et al., 2016) and because it is potentially tolerant to elevated salinity, its use as a plant-growth promoting bacteria for the attenuation of salinity stress in crop plants has been proposed (Chakraborty et al., 2011). In this work, the occurrence of *B. cereus* was fortuitous. Considering that spore-forming pathogenic bacteria such as *Bacillus cereus* is several times related with spoilage of fresh vegetables and its arising is mostly due to improper or careless handling in terms of hygienic conditions (Beuchat, 1996; Fröder et al., 2007), this may represent a reasonable explanation for the unique occurrence of *B. cereus* in the 3rd month of experiment. Thus, the results suggest that the microbiological load of samples conforms well to the specifications for herbs and spices during 6 months of storage, for all the microbiological parameters.

3.2.1.2. Green salt prepared from plants in vegetative stage

The results of microbiological analysis of green salt prepared with plants harvested in vegetative stage at sites B, P and S, and stored for 2 months are represented in Figures 9 and 10.

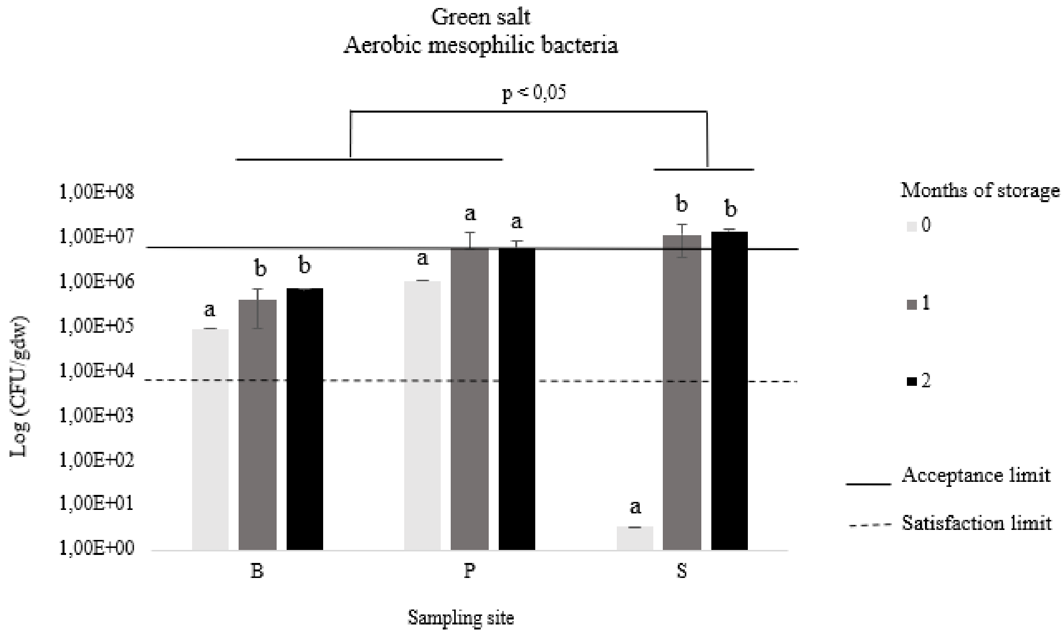

Figure 9. Variation of the concentration of aerobic mesophilic bacteria in green salt obtained from *Salicornia ramosissima* in vegetative stage collected from sites B, P and S and stored at room temperature for 2 months. Values correspond to the average of 3 sub-samples and error bars represent the standard deviation. The dashed line represents the maximum satisfaction limit according to Ribeiro (1974) and solid line represents the maximum acceptance limit according to Stannard (1997). Letters indicate homogeneous groups (storage time) defined by post-hoc Tuckey. The significant differences between sampling sites are indicated above the chart.

The concentration of aerobic mesophilic bacteria varied from 3.3 to 1.4×10^7 CFU/g dw and both the lowest and the highest value correspond to samples from site S. The initial concentration of aerobic mesophilic bacteria in samples prepared with plants from sites B and P exceeded the satisfaction limit. Samples from site S had the lowest initial concentration, well below the satisfaction limit, but there was a sharp increase during the first month of storage. From the first month of storage on, the concentration of aerobic mesophilic bacteria is green salt prepared with material from site P was very close to the acceptance limit and this limit was actually in sea salt prepared with material from site S. These results are similar to those obtained by Antunes et al. (2018) for green salt prepared with *S. ramosissima* from Ria Formosa, in similar storage condition.

Total coliforms, *E. coli, B. cereus,* yeasts were not detected in green salt prepared with plants in fructification stage.

At the beginning of storage, molds were only detected in samples from sites B and P (3.3 and 13 CFU/g dw, respectively) and the values were well below the satisfaction limit. During storage, the concentrations decreased and after 2 months of storage, molds were no longer detected in any of the samples.

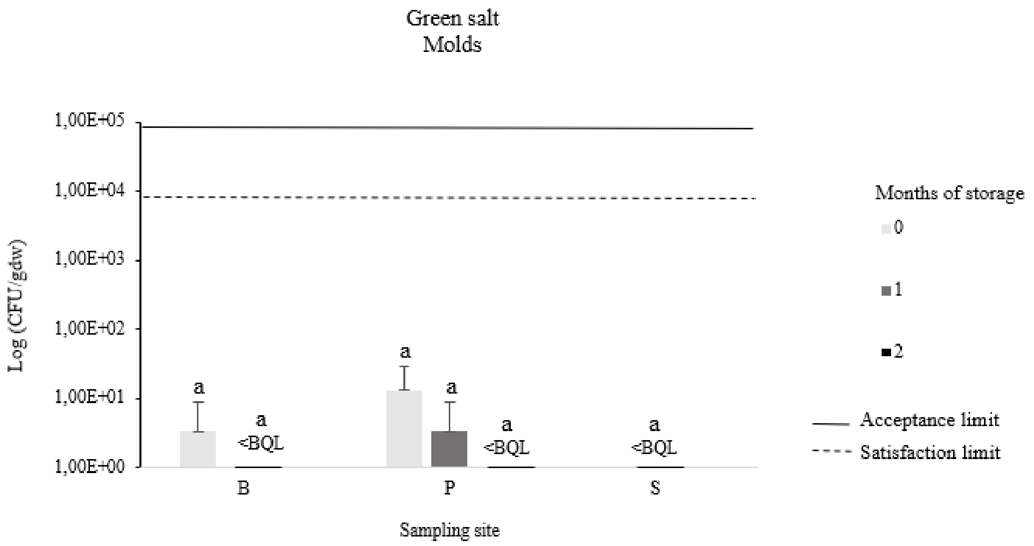

Figure 10. Variation of the concentration of molds in green salt obtained from *Salicornia ramosissima* in vegetative stage collected from sites B, P and S and stored at room temperature for 2 months. Values correspond to the average of 3 sub-samples and error bars represent the standard deviation. The dashed line represents the maximum satisfaction limit and solid line represents the maximum acceptance limit according to Muggeridge et al. (2001). Letters indicate homogeneous groups (storage time) defined by post-hoc Tuckey.

An overall analysis of the results indicates that phenological stage of the plant, harvesting site and storage time significantly affected the microbiological quality of green salt. In general, samples prepared from plants in vegetative stage showed much higher loads of aerobic mesophilic bacteria in relation to samples prepared from material in fructification stage. These differences were not observed in the other descriptors of microbiological quality. Such differences, indicating seasonality in the microbiological load of plant material, may be related with the composition and water content (succulence) of the shoots in different stages that may promote the colonization with more dense microbial communities during the vegetative stage.

Considering that aerobic mesophilic bacteria are indicators of hygienic conditions in harvest and pos-harvest practices (Tournas, 2005) and also taking into account that, in this study, we tried to reproduce procedures used in the preparation of commercial green salt, this results indicate that green salt produced from more mature plants represents a lower risk to the consumer. Therefore, the common practice of using plant in vegetative stage as salad greens and the less succulent shoots of plants in fructification stage for green salt is an interesting strategy of expanding the harvesting period, increase profitability and reducing microbiological risk.

3.2. Elemental analysis of *Salicornia ramosissima*

The mineral and metal contents in *S. ramosissima* (vegetative stage) harvested in sites B, P and S, are presented in Table 4. Na was the most abundant element, ranging from 203.2 ± 7.22 to 215.9 ± 1.46 mg/g without significant differences between sampling sites. Mg, K, Ca and Fe were also detected. In terms of major elements, the only significant difference between sites corresponds to a significantly higher concentration of K in plants from site S. Mn, Zn and Al, were the most represented metals, with significant differences between harvesting sites for the former two. The highest concentrations of Mn (59.7 ± 2.66 µg/g) and Zn (44. 1 ± 5.36 µg/g) were detected in plants from sites S and P, respectively. The concentration of Cu varied between 10.3 ± 0.87 and 6.2± 1.16 µg/g without significant differences between harvesting sites. Toxic metals, like Cu, Cr, Cd, Pb and Co, were detected in very low concentrations (<1 µg/g). As, Be, Ni, V, W, Ti, Bi, Th and Ag were also analyzed but the concentrations were below the limit of detection of the equipment.

As previously referred, halophytes can sequester and accumulate inorganic solutes and minerals ions in the cell vacuoles (Davy et al., 2001) and are, therefore, interesting source of dietary nutrients, particularly minerals. The results indicate the important role of Na^+ cation as first-line osmolyte in *S. ramosissima* (Ventura and Sagi, 2013).

Table 4. Mineral content (in relation to dry weight) of the aerial parts of *Salicornia ramosissima* harvested in 3 different sites of Ria de Aveiro. Values represent the average of 3 analytical replicates.

Element	B	P	S
Na (mg/g)	210.6 ± 22.58 ^{a}	203.2 ± 7.22 ^{a}	215.9 ± 1.46 ^{a}
Mg (mg/g)	18.2 ± 2.81^{a}	15.1 ± 0.37^{a}	19.3 ± 1.23^{a}
K (mg/g)	10.6 ± 0.43 $^{a, b}$	9.3 ± 2.46 ^{a}	13.2 ± 0.96 ^{b}
Ca (mg/g)	6.6 ± 2.76 ^{a}	6.8 ± 3.49 ^{a}	9.5 ± 0.26 ^{a}
Fe (mg/g)	0.08 ± 0.05 ^{a}	0.1 ± 0.02 ^{a}	0.1 ± 0.05 ^{a}
Mn (µg/g)	44.9 ± 21.92 $^{a, b}$	22.8 ± 5.15 ^{a}	59.7 ± 2.66 ^{b}
Zn (µg/g)	20.4 ± 2.00 ^{a}	44. 1 ± 5.36 ^{c}	34.3 ± 2.30 ^{b}
Li (µg/g)	2.2± 0.12 ^{a}	3.6 ± 0.72 $^{a, b}$	4.6 ± 0.74 ^{b}
P (µg/g)	1.5 ± 1.89 ^{a}	1.75 ± 0.33 ^{a}	1.8 ± 0.08 ^{a}
Al (µg/g)	20.2 ± 3.67 ^{a}	47.9 ± 5.41 ^{a}	40.2 ± 19.33 ^{a}
Cu (µg/g)	6.6 ± 1.79 ^{a}	6.2 ± 1.16 ^{a}	10.3 ± 0.87 ^{a}
Cr (µg/g)	0.6 ± 0.03 ^{a}	1.0 ± 0.49 ^{a}	0.7 ± 0.09 ^{a}
Cd (µg/g)	0.7 ± 0.01^{b}	0.2 ± 0.00^{a}	0.9 ± 0.03^{c}
Pb (µg/g)	0.3 ± 0.34 ^{a}	0.8 ± 0.23 ^{b}	0.6 ± 0.08 ^{b}
Mo (µg/g)	0.3 ± 0.34 ^{a}	0.8 ± 0.23 ^{b}	0.6 ± 0.08 ^{b}
Co (µg/g)	0.2 ± 0.76 ^{a}	0.2 ± 0.07 ^{a}	0.1 ± 0.02 ^{a}
Sn (µg/g)	0.1 ± 0.01 ^{a}	0.3 ± 0.23 ^{a}	0.2 ± 0.09 ^{a}
Ba (µg/g)	0.5 ± 0.05 ^{a}	0.6 ± 0.02 ^{a}	0.5 ± 0.05 ^{a}
Sb (µg/g)	0.04 ± 0.02 ^{a}	0.05 ± 0.06 ^{a}	0.03 ± 0.02 ^{a}

Letters (a-c) represent Tuckey post-hoc homogeneous groups (sampling sites).

As previously referred, halophytes can sequester and accumulate inorganic solutes and minerals ions in the cell vacuoles (Davy et al., 2001) and are, therefore, interesting dietary source of minerals. This process has been demonstrated in S. *europaea* shoots in which elements like K, Mg and Ca accumulate in vacuoles remain within the cells of the edible portions of plant (Ozawa et al., 2010) and may explain the appreciable content of several essential elements in shoots and seeds with great potentiality as a mineral-rich food (Del Gobbo et al., 2013; Hanif et al., 2006; Ventura and Sagi, 2013). As far as we know, the present study provides the most characterization of the elemental composition (a total of 26 elements) of *S. ramosissima* from Ria de Aveiro. The results obtained correspond to an almost double concentration of Na, in relation to what was previously reported for *S. ramosissima* from Ria Formosa, higher concentrations of K, Ca, and Mg but lower concentrations of Fe, Mn and Zn (Barreira et al., 2017). This may indicate that *S. ramosissima* from Ria de Aveiro can provide a more pronounced salty taste although representing a poorer source of some micronutrients like Fe.

Based on the results we estimated the necessary amount of green salt to meet the daily adequate intake of Na, and the correspondent percentage of each mineral was also estimated for the consume of approximately 7 g of green salt a day. The Dietary references intakes which include the Adequate intake and the Recommended dietary allowance are represented in Table 5.

Table 5. Dietary Reference Intakes (DRIs) represented by the RDA and AI for some studied elements and the comparison with the correspondent % of RDA and AI when consuming of 7 g of green salt a day (Institute of Medicine, 2011).

Element	Dietary Reference Intakes (DRIs)	% DRIs correspondent to 7 g of green salt
Na	1500 mg/day [1]	100 [1]
Mg	240 mg/day [2]	52.2 [2]
K	4700 mg/day [1]	1.7 [1]
Ca	1000 mg/day [2]	5.4 [2]
Fe	8 mg/day [2]	10 [2]
Mn	2.3 mg/day [1]	13 [1]
Zn	11 mg/day [2]	2.1 [2]
P	700 mg/day [2]	0.002 [2]
Cu	900 µg/day [2]	6.1 [2]
Cr	35 µg/day [1]	15.0 [1]
Mo	45 µg/day [2]	9.1 [2]

[1] AI: Adequate Intake
[2] RDA: Recommended Dietary Allowance

Toxic metals, that can represent a problem in terms of edible vegetables, were found in low concentrations. Aluminum content of dry shoots from *S. ramosissima* was relatively high (20.2 to 47.9 µg/g) comparing with other trace elements but still within the range reported for other plants, like lettuce, tea, herbs and spices (Greger, 1992; Pennington, 1988). Due to the characteristic cumulative effect of aluminum on human body, EFSA (2008) established a Tolerable Weekly Intake (TWI) limit of 1mg/kg.bw.week that however, would not be exceeded by a daily consumption of 10 g green salt per day

(24 mg/60 kg/week). Accordingly, the daily intake of other metals and metalloids would not be exceeded with the daily consumption of 10 g of green salt.

Our findings suggest that the regular use of green salt as seasoning, rather than the traditional cooking salt (maximum of approximately 10 g/day) may contribute to a mineral-balanced diet.

3.3. Bromatological analysis of *Salicornia ramosissima*

3.3.1. Estimate composition of fresh shoots

The results of the analysis of moisture, protein, total fat acids, total sugars, fiber, carbohydrate, total ash and energy in fresh shoots are represented in Table 5. Fresh shoots contain 25-91% water, 1.4-2.0 % protein, 0.1-0.3% total fat, 0.1-2.8 % carbohydrates, < 0.5 % sugars, 2.9-3.3 % fiber and 4.5-6.4% ash.

Table 6. Approximate composition (per 100 g fresh weight) of fresh shoots of *Salicornia ramosissima* in vegetative stage harvested in Ria de Aveiro.

Contents	Harvesting site		
	B	P	S
Moisture	91	85	90
Protein (N×6.25)	1.4	2.0	1.8
Total fat	0.1	0.3	0.1
Carbohydrates	0.1	2.6	0.8
Total sugars	< 0.5	<0.5	< 0.5
Fibre	3.2	3.3	2.9
Total ash	4.5	6.4	4.5
Energy (KJ/Kcal/ 100g)	56/14	117/ 28	72/ 17

The high water amount may indicate that plants were harvested at maximum freshness (Zafar and Sidhu, 2018). Such results were similar to those obtained by Barreira et al., (2017) for the *S. ramosissima* from Ria Formosa. The protein content was slightly higher than reported for *S. bigelovii* Torr. (Lu et al., 2010) and within the range reported for spices and herbs (APN, 2018) which are not considered as protein-rich food. *S. ramosissima* has a low fatty acid content (< 1%) (Zafar and Sidhu, 2018) which is within the range reported by Lu et.al., (2010) for *S. bigelovii* Torr. Fiber is one of the main components. A dose of 100 g of fresh shoots of *S. ramosissima* may represent an intake of 50 % of the daily guidance amount for this macronutrient and thus decreasing considerably the risk of cardiovascular and coronary heart diseases (Marlett et al., 2002; Threapleton et al., 2013). In vegetables, fiber content is influenced by the stage of development as well as by the growth conditions (Zafar and Sidhu, 2018). This information may represent a reasonable explanation for a higher crude fiber content in comparison to the values commonly reported for *S. bigelovii* Torr. the later occurs in subtropical coastal deserts (Lu et al., 2010) while *S. ramosissima* is better represented in Mediterranean environments (Rubio-Casal et al., 2003). The concentration of carbohydrates and sugars was low. Therefore *S. ramosissima* represents a low-carbohydrates intake, which in addition to the high content in water and fiber and the low fatty-acid content represents obvious health benefits (USDA, 2009). Values of total ash were also higher than those reported for *S. bigelovii* Torr. (Lu et al., 2010). and similar to *S. herbacea* L., that has is recognized as rich in various minerals (Min et al., 2002). The adaptations of *S. ramosissima* to saline soils and sediments underlie the potential to accumulate essential minerals (Van Oosten and Maggio, 2015; Barreira et al., 2017).

These results confirm that *S. ramosissima* shoots harvested from salt pans and salt marshes of Ria de Aveiro (Portugal) can be used as a nutritionally interesting vegetable, representing a good source of water, minerals, and fiber and suitable to be included in low-sugar and low-fat diets.

3.3.2. Estimate composition of green salt

The results of the analysis of moisture, protein, total fat acids, total sugars, fiber, carbohydrate, total ash and energy in green salt are represented in Table 6. Green salt from site B was insufficient for analysis. Green salt contains 2.3-2.5% water, 10.9-12 % protein, 0.5-0.7% fatty acids, 12-18 % carbohydrates, < 0.5 % sugars, 26 % fiber and 44-47% ash.

Table 7. Approximate composition (per 100 g dry weight) of green salt prepared with *Salicornia ramosissima* in vegetative stage harvested in Ria de Aveiro.

Contents	Harvesting site	
	P	S
Moisture	2.5	2.3
Protein (N×6.25)	12.0	10.9
Total fat	0.5	0.7
Carbohydrates	< 0.5	18
Total sugars	26	< 0.5
Fibre	12	26
Total ash	47	44
Energy (KJ/Kcal/ 100g)	642/ 154	688/185

The water content was lower than for other retailed spices like oregano, bay leaf, basil and thyme (Banerjee and Sarkar, 2003; Sospedra et al., 2010; USDA, 2018) which may contribute to the microbiological stability of the product. The European Spice Association (ESA) recommends maximum water contents of 12 and 10 % for herbs and spices, respetively (ESA, 2014). Green salt complies with these limits. The protein content was similar to other halophytes like *Arthrocnemum macrostachyum* (Barreira et al., 2017), *Atriplex barclayana (*Díaz et.al., 2013), and *S. bigelovii Torr*. (O'Leary et al., 1985), but approximately double in relation to green salt from S. *ramosissima* harvested in Ria Formosa (Barreira et al., 2017). An even higher protein content in dry matter (22.10 g/100g) was reported for a close species, *S. herbacaea* indicating that green salt may represent a good source of protein.

Total fat of green salt was lower than some traditional herbs and spices, like dried basil for example (USDA, 2018) and therefore it represents an interesting alternative as seasoning or in marinades in low-fat diets (Tapsell et al., 2006).

Dietary fiber corresponds to the faction of the edible portions of plants than is not so easily digested as other macronutrients. The high fiber content of green salt is related to the somewhat woody structure of *S. ramosissima*. From the point of the view of digestibility and palatability, the high content of fibers may not be a very appealing trait, by dietary fiber content has positive physiological effects being essential for the normal maintenance of the gut function (NHMRC, 2006). Carbohydrates in food correspond to small sugars and starches and represent sources of energy (Institute of Medicine, 2005). Green salt has good content in carbohydrates (12-18 g/100gdw), although lower than other spices and herbs (Achinewhu et.al., 1995) but the fraction corresponding to sugars is very small (< 0.5 g/100gdw) indication that it contains essentially polymeric carbohydrates, like starch.

Differences in ash content reflect environmental conditions (e.g. salinity and drought) and differences in the bioavailability of minerals in soil (Pytlakowska et al., 2012) which influence the uptake of minerals by plants (Hu and Schmidhalter, 2005). Total ash content in green salt obtained from *S. ramosissima* harvested in Ria de Aveiro (44-47 g/100gdw) was higher than the average of ~30 g/100g reported for this species in a study involving plants harvested in different Portuguese estuaries (Barreira et.al., 2017). Our results are within the range reported for *S. herbacaea* and other halophytes (Ventura et al., 2011) and slightly lower than in *S. bigelovii* (Díaz, Benes & Grattan (2013).

The results indicate that green salt produced from *S. ramosissima* harvested in Ria de Aveiro has a macro and micronutrient composition comparable to other herbs and spices, with a lower fatty acid and higher fiber content.

3.4. Sensory evaluation

3.4.1. Sensory evaluation of fresh shoots

Results of the sensory evaluation of fresh shoots of *S. ramosissima* by a panel of 35 untrained participants are represented in Figure 11. In a hedonistic scale of 1 to 5, the mean scores were 3.57 ± 0.66 for saltiness, 3.63 ± 0.65 for overall impression and 3.66 ± 0.60 for intention of purchase. The results indicate a good overall acceptance of fresh shoots in salad and the average opinion is centred on a "slightly salty" assessment for saltiness, the "good" evaluation for overall impression and "probably buy" rating on the intention of purchase. Saltiness was not significantly correlated with overall impression or intention of purchase (Table 6). This may indicate that other characteristics, like colour, succulence and crispness, may contribute for an overall pleasant perception of shoots in mixed salads. It may also be an indication of public awareness on relation of dietary salt cardiovascular disease that makes saltiness a less valued attribute (Lopes et al., 2017).

Table 8. Spearman's correlation coefficient and significant values between sensorial descriptors for salad (n=35)

Sensorial descriptors	Saltiness	Overall impression	Intention of purchase
Saltiness			
Overall impression	-0.275		
Intention of purchase	-0.116	0.483*	

* Correlation is significant at the 0.01 level;

This assumption seems to be in agreement with those referred by Ventura et.al., (2011) where besides the salty taste, sensorial and nutritional argued in favour of *Salicornia*-containing food. The fact that most participants knew about green salt but were unfamiliar with *S. ramosissima* as a salad green and even so were not reluctant to try it, may indicate that emotional factors like curiosity and an interest for new flavours may have contributed to the overall novel shoots, as a fresh vegetable and even more as a salt substitute, was not familiar to the positive overall impression and consequent intention of purchase.

3.4.2. Sensorial evaluation of green salt

Results of the sensory evaluation by a panel of untrained participants of green salt used as seasoning in soup or meat pork dish are represented in Figure 11. In a hedonistic scale of 1 to 5, the mean scores for the meat pork dish were 2.37 ± 0.78 for saltiness, 3.69 ± 0.67 for overall impression and 3.15 ± 0.91 for intention of purchase. The sensorial evaluation by at least 50 panellists is considered to provide a good perception of a possible consumer acceptability (Zoecklein, 1991). The results obtained in this study, although configuring a general opinion of "low salty taste" to "reasonable" present more positive perspectives for overall impression and intention of purchase. Aromatic properties of *S. ramosissima*, like other herbs, may enhance the global palatability of the meat pork dish and underlie the intention to purchase, since this last parameter was significantly correlated with saltiness and overall impression (Table 9).

Table 9. Spearman's correlation coefficient and significant values between sensorial descriptors for meat (n=70)

Sensorial descriptors	Saltiness	Overall impression	Intention of purchase
Saltiness			
Overall impression	0.176		
Intention of purchase	0.272*	0.657**	

** Correlation is significant at the 0.01 level.
* Correlation is significant at the 0.05 level.

The sensorial evaluation of green salt included in soup (Figure 11) produced mean scores of 1.86 ± 0.86 for saltiness, 3.06 ± 0.83 for overall impression and 2.94 ± 1.08 for intention of purchase.

Green salt has been proposed as a substitute of cooking salt in the assumption that since other mineral present in the also contribute to the salty flavour (Lee, 2011), the same sensory effect can be provided without exceeding the recommended maximum doses of 2000 mg Na/day (WHO, 2018). However, the score of 1.86 for saltiness corresponds to poor palatability when green salt was used in soup in the same proportion as cooking salt (0.5g/200ml), as often proposed as a strategy to reduce Na in food (Lima, 2018). Considering the Na in green salt (~200 mg/gdw), the amount of Na added to a soup bowl was approximately 100 mg which roughly half the amount of Na (195 mg) corresponding to 0.5 g NaCl. As a continuation of this work, other concentrations of green salt must be tested in soup to acjhieve a good balance between Na intake and palatability.

The overall impression of the green-salt seasoned soup was strongly correlated with saltiness (Table 10). It may indicate that in soup, the low saltiness may have been determinant in the overall opinion and intention of purchase of green salt deliverd in soup, in relation to the other uses of S. *ramosissima* tested in this study.

Table 10. **Spearman's correlation coefficient and significant values between sensorial descriptors for soup (n=70)**

Sensorial descriptors	Saltiness	Overall impression	Intention of purchase
Saltiness			
Overall impression	0.597**		
Intention of purchase	0.239*	0.002	

** Correlation is significant at the 0.01 level;
* Correlation is significant at the 0.05 level.

3.4.3. Overall overview of the sensory appreciation of *S. ramosissima*

A summary of the sensory evaluation of *S. ramosissima* used in food under different forms and for different purposes is represented in Figure 11. The evaluation was based on the 3 sensorial descriptors (saltiness, overall impression and intention of purchase) for all presentations of the plant, and ranked in a 1-5 hedonistic scale. Fresh shoots present the highest scores for all the parameters represent therefore the most interesting form of the plant for the consumer. The use of green salt as seasoning was better accepted in meat than in soup.

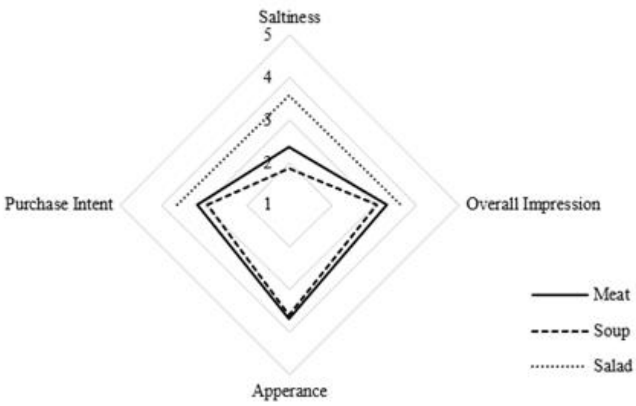

Figure 11. Spider diagram of sensorial profile of fresh shoots of Salicornia ramosissima included in a mixed salad, soup or meat where saltiness, overall impression and purchase intent were evaluated by a panel of 35 participants. The hedonistic scale of 1-5 corresponded to the following scores:
Saltiness: 1- tasteless; 2 - low saltiness; 3 - reasonable; 4 - tasteful; 5 - too salty.
Overall impression/Appearance: 1 - dislike very much; 2 - dislike; 3 - reasonable; 4 - like; 5 - like very much.
Intention of purchase: 1- Definitely not, 2 - probably not; 3 - maybe yes or not, 4 - probably yes, 5 - definitively yes.

4. Conclusions

S. ramosissima represents an important natural resource not only because of its ecological role and bioremediation potential but also as a valuable food item.

This study demonstrates that fresh shoots consumed as salad greens are the more appreciated than green salt. Fresh shoots are safe in terms of microbiological loads and concentration of toxic metals, are stable for 10 days when stored at refrigerating temperatures and meet the requirements of low-salt diets while representing good sources of minerals of water and dietary fiber.

Green salt was stable for 6 months when stored at room temperature and was better accepted as seasoning when used in pork meat. In soup, it failed to provide the same sensation of saltiness that an equivalent dose of cooking salt.

In either forms, *S. ramosissima* was well accepted and aroused significant intention of purchase among the participants in the sensory evaluation test.

The results of this work provide the basis for the expansion of the use of *S. ramosissima* in a healthier diet and open perspectives for the crop cultivation of this halophyte as alternative activity that can stimulate an economically and environmentally sustainable reconversion of deactivated saltpans in Ria the Aveiro.

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6. Annex

6.1. Annex I

19 Outubro 2018

Análise sensorial - Teste subjetivo de aceitação

O presente questionário tem por objetivo avaliar a *Salicornia ramosissima* desidratada (sal verde) pelos provadores/ consumidores destinando-se unicamente para fins académicos e como tal a sua avaliação fará parte de um banco de dados confidenciais e será de grande relevância para a pesquisa em questão.

Agradecemos que coloque uma cruz (X) nos campos em branco de acordo com a sua apreciação individual relativa ao produto testado utilizando como base a escala hedónica (1-5) apresentada para cada descritor sensorial.

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Dissertação de mestrado em Biologia Marinha

Idade: _____ Sexo: ( ) F ( ) M

Descritores sensoriais	1	2	3	4	5
Sabor salgado[a]					
1 Muito Pouco 2 Pouco 3 Razoável (q.b.) 4 Ligeiramente salgado (apetitoso) 5 Muito salgado					
Escala Sabor global[a]					
1 Mau 2 Mediocre 3 Razoável 4 Bom 5 Muito bom					
Intenção de compra[b]					
1 Decididamente não compraria 2 Provavelmente não compraria 3 Talvez sim/ Talvez não 4 Provavelmente compraria 5 Decididamente compraria					

[a] Escala hedónica- Expressa o gostar ou desgostar de um produto; [b] Escala de atitude- Expressa a frequência de consumo.

<u>Principais aplicações dos testes de aceitação:</u>

- ✓ Desenvolvimento de novos produtos;
- ✓ Otimizar e melhorar a qualidade de produtos já existentes.

6.2. Annex II

QUESTIONÁRIO DE AVALIAÇÃO DE SATISFAÇÃO DOS CONSUMIDORES

Análise sensorial – Teste subjetivo de Aceitação da Salicórnia Desidratada

19 outubro 2018

Sexo: F ☐ M ☐

Idade: ________ anos

O presente questionário tem por objetivo avaliar a aceitabilidade da *Salicornia ramosissima* desidratada (sal verde) pelos consumidores, destinando-se unicamente para fins académicos e como tal a sua avaliação fará parte de um banco de dados confidenciais e será de grande relevância para a pesquisa em questão.

Agradecemos que classifique as variáveis, de acordo com a sua perceção, colocando uma cruz (X) nos campos em branco.

Sabor salgado[a]	**1** Muito Pouco	**2** Pouco	**3** Razoável	**4** Ligeiramente salgado	**5** Muito salgado
Paladar	**1** Mau	**2** Medíocre	**3** Razoável	**4** Bom	**5** Muito bom
Apresentação (cor, aspeto, textura,…)	**1** Mau	**2** Medíocre	**3** Razoável	**4** Bom	**5** Muito bom
Intenção de compra[b]	**1** Decididamente não compraria	**2** Provavelmente não compraria	**3** Talvez sim/ talvez não	**4** Provavelmente compraria	**5** Decididamente compraria

[a] Escala hedónica- Expressa o gostar ou desgostar de um produto; [b] Escala de atitude- Expressa a frequência de consumo.

Observações:

Obrigado pela sua colaboração.