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Re-Thinking Table Salt Reduction in Bread with Halophyte Plant Solutions

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Abstract: Sodium intake higher than it is physiologically necessary has been associated with some non-communicable diseases such as hypertension, cardiovascular disease, and stroke. Bread is commonly consumed and is a major source of sodium in the human diet. Among the interventions to reduce the salt content in bread, the incorporation of salty taste halophyte powder could be a promising strategy. In the present work, *Sarcocornia perennis* was incorporated as a food ingredient to substitute the salt (sodium) of white wheat bread (1.2% NaCl/0.47% sodium, flour basis). Powdered dried *S. perennis* was incorporated into bread by replacing the same amount of sodium (0.47%, flour basis) and half of the sodium concentration (0.235%, flour basis), respectively, B100 and B50 bread samples. The bread samples were analyzed to evaluate the impact of the sodium chloride replacement by *S. perennis* powder on total baking loss, specific volume, crumb color, textural properties, microbial activity, nutritional and mineral composition and sensory evaluation. The incorporation of *S. perennis* increased the specific volume but had no relevant impact on the textural properties of bread. Furthermore, the substitution of sodium chloride by *S. perennis* powder allowed a more colored (greenish and yellowish) and dark crumb leading to a lower whiteness index. Compared with control bread, the addition of *S. perennis* powder promoted a significant increase of all bread's nutrients and minerals, namely calcium, phosphor, iron, and manganese. Besides the improvement of bread quality, B100, and B50 bread samples were both sensorily well accepted and with similar scores to all the evaluated sensorial attributes. Moreover, the reduction of sodium to half (0.235% sodium (flour basis) in bread (B50) did not affect the acceptability of tasters, as compared with B100 (0.47% sodium (flour basis)). Both new bread formulation has microbiological quality as ready-to-eat product. However, taking into account greater stability over time for microbial spoilage, mainly caused by fungi and yeasts, B50 bread is more promising. The B50 bread sample is also a potential strategy to obtain a sodium reduction of 50% in bread, which could be essential to reduce the overall sodium daily intake and bring important economic and public health benefits.

Keywords: bread; salt substitution; *Sarcocornia perennis*; minerals; nutritional composition; sensory analysis

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1. Introduction

A global trend to improve the nutritional value of the human diet is to fortify traditional food products with functional ingredients using natural nutrients and antioxidants from plants [1]. Bread is one of the most popular staple foods in the daily human diet [2]. Its basic recipe consists in combining flour, water, yeast and salt. However, the generalized use of refined flour of wheat grains leads to a low nutritional quality of bread. Thus, to enhance the overall quality of bakery products through the increase of the nutritive value, attractiveness and sensory features are often added ingredients and additives such as dairy products, eggs, lecithin, inulin, spirulina, apple pomace, acerola fruit, hazelnut, walnuts, herbs, spices and various types of flour and seeds (chia, pumpkin, poppy, sunflower, flax, cumin, grapes, lentil) and others [2–12]. Furthermore, some of the ingredients, added in fresh or dried powders, are rich in compounds, namely vitamins, natural antioxidants, minerals and dietary fibers that have beneficial human health effects [13,14]. Despite these beneficial ingredients, bread and bakery products are one of the food products with the greatest contribution to dietary salt, beyond, cheeses, spreads and processed meat and fish products [15]. The salt addition strengthens the formed gluten and ensures adequate structure of bread but also enhances the palatability to satisfy consumer preferences but also increases the incidence of stroke, cardiovascular and coronary heart diseases that are associated with salt intake [16]. Thus, the salt reduction in bread could be a potentially effective strategy to reduce population salt intake to the recommended value of 5 g of salt per day allied to public health interventions in food labelling, consumer education, and the establishment of salt dietary guidelines [17,18].

One advisable strategy to enhance the nutritional value and antioxidant activity of white bread with a lower salt addition is the incorporation of salty taste halophyte plants. Halophyte plants have been a growing interest and importance in agriculture due to their productivity in soils with high salinity and low water intake environments and their high nutritional, mineral and bioactive profiles [19,20]. The possibility of cultivation under saline conditions could contribute to a sustainable agriculture, surpassing the problem of soil salinization and the scarcity of fresh water for agriculture.

The perennial succulent and edible *Sarcocornia perennis* is one of the most abundant salt marsh halophytes of European Atlantic coasts and the Mediterranean [21]. The plant is usually consumed in fresh or dried to produce "green salt" (dry powder) of high quality to be used as salt substitute [22]. The use of dried powdered halophyte plants such as Sarcocornia as a salt substitute is an innovative and promising strategy to reduce the ingestion of salt and to produce novel and functional food products such as beverages, microencapsulated oils, snacks and food additives [23,24]

S. perennis is rich in protein, fiber, fatty acids (mainly palmitic, linolenic and linoleic acid) and have a great content of minerals such as sodium (Na), potassium (K), calcium (Ca), Mg (magnesium) and iron (Fe) [19,23,25]. Moreover, the plant contains a high diversity of phenolic acids including trans-cinnamic, salicylic, coumaric, and caffeic acids and flavonoids such as luteolin and rutin and glycosylated flavonoids that confer important biological properties and health benefits related with cardiovascular system, among others [20,26,27]. In a recent study the *S. perennis* proved to be effective as a dietary substitute for regular salt in food making, providing positive effects on cardiovascular system (peripheral and central blood pressure and aortic pulse wave velocity) in young and healthy adults [27]. However, the incorporation of halophyte dried powder in white bread or bakery products is very scarcely referred in the literature.

In line with the trend to enrich bakery products with functional ingredients, Clavel-Colibrié et al. [23] concluded that crackers with 5% of powder dried *S. perennis* led to a product sensory well accepted by consumers and with an improvement of its nutritional profile, namely in terms of phenolic compounds, antioxidant activity, and minerals.

To the best or our knowledge Lopes et al. [28] suggested, for the first time, that the halophyte *Salicornia ramosissima* had potential to be used as a salt substitute in bread and Toumi et al. [29] used this halophyte powder as a functional substitute of sodium chloride in the production of wheat bread. The results evidenced that using 1.8% salt with a substitution ratio of 65.24% is the best combination to obtain doughs with high stability and better viscoelastic, extensional and fermentation properties and breads with softer and less chewy crumbs but greener than those containing only sodium chloride (NaCl).

The present work aims to evaluate the potential of another halophyte powder, *Sarcocornia perennis*, as a nutrition relevant substitute of sodium chloride in bread. In this context, control bread (CB) has 1.2% NaCl/0.47% sodium (flour basis) and B100 and B50 samples have, respectively the same amount of sodium (0.47% (flour basis) and half of the sodium concentration 0.235% (flour basis) obtained from *S. perennis*. Halophyte was incorporated as a food ingredient to substitute the salt (sodium) of white wheat bread and the physical, nutritional, mineral, sensory properties and microbiological activity were evaluated and compared with control bread (CB).

2. Materials and Methods

2.1. Dried Sarcocornia and Other Ingredients

Dried *Sarcocornia perennis* powder (Figure 1) was provided by the Salina Greens company (Tejo estuary, Alcochete, Setúbal, Portugal) in February 2022.

Figure 1. Dried *Sarcocornia perennis* powder.

Wheat flour T65 (protein 11%, lipids 1.6%; fiber 3.5%, salt: <0.1% and energy 343 kcal) from CERES (Portugal) sugar, dough conditioner (bread improver) and dry baker's yeast were the ingredients used in a local bakery (Tertúlia dos Sabores, Coimbra) to producebread.

2.2. Bread Preparation

Three bread samples were prepared according to the formulations described in Table 1. To determine the appropriate amount of water to add to the bread samples, preliminary experiments were conducted at the bakery. The level of incorporation of sodium chloride/sodium was based on flour weight. Control sample (CB) with 1.2% NaCl/0.47% sodium (flour basis) and two samples with the replacement of the initial salt (sodium) content by *S. perennis* powder: B50 (0.235% sodium, flour basis) and B100 (0.47% sodium, flour basis). To clarify, in B50 and B100 were not added sodium chloride (NaCl) and sodium concentration was, respectively, half and the same amount of sodium as the control sample (CB).

Table 1. Bread formulations to control (CB), B50 and B100 bread samples.

Ingredients	CB	B50	B100
Wheat flour T65 (g)	1000	1000	1000
Water (mL)	600	600	600
Dough Strengthener	13	13	13
Sugar (g)	11	11	11
Dry baker's yeast (g)	7	7	7
Salt (NaCl) (g)	12	-	-
S. perennis powder (g)	-	30.85	61.7

The dough was mixed by using an electric mixer (Ferneto, AEF050, Vagos, Portugal), at low-speed selection, for 6 min with the addition of the water. The velocity was increased, and the dough was kneaded for more 2 min to mix the remaining ingredients (Figure 2a). The dough rested for 5 min, was weighted, divided, rounded and molded in 30 samples of 82–84 g (Figure 2b) for each type of bread. The samples rested more 30 min at room temperature. Next, it was placed in the fridge for 16 h. Finally, the bread was cut into desired shape and baked in an industrial oven (Ramalhos MR.1128, Águeda, Portugal) with double heating (250 °C on top and 200 °C on bottom) during 8 min. The bread was cooled down at ambient temperature.

Figure 2. Automatic mixer (**a**) and rounded and molded samples (**b**).

Physical analyses were performed 4 h after of baking and some of the bread batches were dried in a convective oven at 40 °C for 2 days, crushed to powder (using an electric mill) and stored in bags using vacuum to be used for biochemical composition.

2.3. Bread Physico-Chemical Analysis

2.3.1. Total Baking Loss

The total baking loss was determined as follows:

$$\text{Total baking loss } (\%) = \frac{\text{weight of the dough for baking–weight of the bread after cooling}}{\text{weight of the dough for baking}} \times 100 \quad (1)$$

2.3.2. Specific Volume

Specific bread volume was determined using the rapeseed displacement method (AACC 44–15.02). The specific volume was calculated as the ratio between volume (cm^3) and bread weight (g).

2.3.3. Color Evaluation

The bread crumb color coordinates lightness (L*), redness/greenness (a*) and yellowness/blueness (b*) were measured using a colorimeter (Chroma Meter—CR-400, Konica Minolta, Tokyo, Japan), and were registered in the CIE Lab color space. Measurements were performed at three points using three slices from each loaf. Total color difference (TCD) was calculated as mentioned in Pires et al. [30] to quantify the overall color difference between control sample (reference) and breads with incorporation of *S. perennis* powder (B50 and B100). The whiteness index (WI) for crumb was determined as referred in Djordjević et.al. [31] and the chromaticity (C*) was determined as follows:

$$C^* = \sqrt{(a^*)^2 + \left(b^*\right)^2} \quad (2)$$

2.3.4. Texture Analysis

Bread crumb Texture Profile Analysis (TPA) was performed in a texturometer TA-XT.plus (Stable Micro Systems, Surrey, UK) equiped with a 5 kg load cell and a 25 mm diameter cylindrical probe. The bread textural properties were carried out 4 h after baking and using slices with a thickness of 20 mm. The bread samples were compressed twice to 50% of their original height at a constant speed of 1 mm/s and 5 s of waiting time. From the resulting force-time curves were analysed the following textural parameters: hardness, springiness, cohsiveness and resilience.

2.4. Microbiological Analysis

Bread samples were prepared by mashing and mixing in peptone water. Subsamples were diluted decimally, and 0.1 mL aliquots were spread plated on nutrient agar (NA), MacConkey agar (MCA), and potato dextrose agar (PDA) for the enumeration of aerobic viable bacteria, coliforms and fungi, respectively. The NA and MCA plates were incubated at 37 °C for 24–48 h while PDA plates were incubated at room temperature (°C) for 3 days. The colonies were then counted and expressed as colony forming units per gram (cfu/g) of samples. The evaluation was carried out on the 2nd and 4th day after cooking, during the storage time, bread samples were placed in a cloth bag (a) and in a paper bag (b) at room temperature.

2.5. Proximate Composition of S. perennis and Bread

The proximate composition of *S. perennis* powder and bread samples was analysed following the Association of Official Analytical Chemists (AOAC, 1997) methodologies [32]. Moisture content (method 930.04 and 930.22), ashes (method 930.05 and 930.22), total lipids (method 930.09 and 935.38), crude protein (method 978.04 and 950.37) using a nitrogen conversion factor of 6.25 to *S. perennis* and 5.7 to bread samples, crude fiber (method 930.10 and 950.37), total dietary fiber (985.29) and 991.42 method to insoluble dietary fiber, with a Total Dietary Fiber Assay Kit (Megaezyme, Ireland). Soluble dietary fiber was determined by the difference between total dietary fiber and insoluble dietary fiber. The carbohydrates' content was determined from the difference between 100 and the sum of the percentages of moisture, ashes, total lipids, crude protein and dietary fiber contents. The Regulation (EU) No. 1169/2011 of the European Parliament and of the Council of 25 October 2011 was used for the calculation of the energy values (expressed in kcal/100 g and kJ/100 g) [33].

With the ashes, the mineral content was analyzed through dry mineralization (method 975.03). The ashes were wetted with 10 drops of distilled water and 3–4 mL of HNO_3 (33% (v/v) and the HNO_3 was evaporated on a hot plate set at 100–120 °C. The crucibles were returned to the muffle and incinerated for another 1 h at 500 °C. The ashes were dissolved in 10 mL of HCl (20% (v/v) on a hot plate set at 100–120 °C around 30 min and transferred and filtered to a 50 mL volumetric flask, added 10 mL of $LaCl_3$ (5%), and the volume adjusted with distilled water. After dilutions with water (1:100 and 1:200) the analysis was carried out with flame atomic absorption spectrometry (FAAS) (PerkinElmer PinAAcle 900 T, Waltham, MA, USA) equipped with the cathode corresponding to each element (sodium, potassium, magnesium, manganese, calcium, copper, iron and zinc). The phosphorus content was determined by spectrophotometry (method 948.09) with a PG instrument T80 + UV/VIS spectrophotometer (UK).

2.6. Sensory Analysis

An untrained sensory analysis panel (n = 67, age: 17–64, gender: 44 female, 20 male, 3 without response) was recruited from Coimbra Agriculture School (ESAC) in order to evaluate possible changes in the organoleptic characteristics of bread with incorporation of *S. perennis* powder (B50 and B100), as well as the control sample (CB). To assess the preference for a given product the tasters used a Product Preference Test. Panelists were placed randomly at room temperature and water was served to clean their palates between samples. The bread samples were evaluated to attributes aspect, color, aroma, texture, flavor and global assessment using one hedonic scale with nine levels (1 (dislike extremely) to 9 (like extremely)).

2.7. Statistical Analysis

All experiments were performed in triplicate, except for specific volume, which was performed in duplicate. To analyze possible differences between the properties evaluated in the bread samples, one way analysis of variance was used (ANOVA), complemented with Tukey post-hoc test, to compare between the different samples. The analyses were performed using SPSS version 28 (from IBM), and a level of significance of 5% was used in all statistical tests.

3. Results and Discussion

3.1. Proximate Composition of S. perennis

The nutritional and mineral composition of *Sarcocornia perennis* is presented in Table 2.

Table 2. Proximate composition of dried *S. perennis* (powder).

Biochemical Compounds (g/100 g)	
Moisture content	6.42 ± 0.05
Total lipids	0.85 ± 0.03
Protein	11.28 ± 0.08
Crude fiber	9.84 ± 0.06
Total dietary fiber	23.18 ± 0.05
Insoluble dietary fiber	19.99 ± 0.03
Soluble dietary fiber	3.19 ± 0.03
Carbohydrates	14.79 ± 0.10
Total ash	33.84 ± 0.03
Minerals (mg/100 g)	
Sodium (Na)	7614.12 ± 49.78
Potassium (K)	1116.57 ± 11.84
Calcium (Ca)	275.81 ± 6.32
Magnesium (Mg)	510.80 ± 8.94
Phosphor (P)	144.22 ± 1.96
Iron (Fe)	11.54 ± 050
Copper (Cu)	1.09 ± 0.09
Zinc (Zn)	2.98 ± 0.08
Manganese (Mn)	2.97 ± 0.07

Like most vegetables, *Sarcocornia* species, namely *S. perennis* collected in Tejo estuary, are low in protein and lipids. However, *Sarcocornia* species collected in Southern Portugal have a slightly higher content of lipids (1.20–2.25%) and lower content of protein (6.9–8.10%) [19]. The different protein content can be associated with the stress response to natural stress conditions, such as temperature, salinity and UV radiation that contribute to protein synthesis [34]. In addition, *S. perennis* is rich in dietary fiber, from which 86% is the insoluble portion of dietary fibre, corresponding to compounds such as lignin, cellulose and hemicellulose. Similarly, the neutral detergent fiber (NDF) obtained to Sarcocornia species of Southern Portugal is in the range of 20.8–34.1% [19]. The high levels of insoluble dietary fibre and the low caloric content of this halophyte (158.03 ± 0.05 kcal/100 g dry weight) can promote important health benefits including a reduced risk of cardiovascular diseases and weight loss.

Generically, the ash levels (33.84 ± 0.03% dw) of this *S. perennis* collected in Tejo estuary (Alcochete, Portugal) are higher than those observed for *S. perennis* sampled in Southern Portugal (23.3%) [25], but lower than the same halophyte collected from salt pans in Figueira da Foz (43.62%) [22] which may be related with the mineral composition of the soils from which the halophytes were collected.

S. perennis is a valuable source of essential minerals, having the more relevant sodium and potassium and in a lower extension of magnesium, calcium and phosphor. Sodium is a vital mineral in maintaining electronic fluid balance, but its excessive consumption is associated with cardiovascular diseases and hypertension. However, the high levels of potassium, also essential to an adequate fluid balance, might contribute to reducing the harmful effects of sodium excess since improves the excretion of sodium from the body. Sodium, potassium and magnesium were also the macro-mineral prevalent in *S. perennis* species collected in Southern Portugal [25]. Iron, copper, zinc and manganese are essential micronutrients that play essential roles in chloroplast reactions, protein synthesis, and hormone growth, among others [35]. The levels of these minerals are like those found in edible *Sarcocornia perennis* plant collected in Southern Portugal [25]. Even though the concentrations of these micronutrients in halophyte powder are low, the intake of 10 g of *S. perennis* powder provides 8.2%, 10.9%, 3.0%, 14.8% of the daily value recommended values to iron, copper, zin and manganese, respectively [33].

3.2. Bread Characterization

3.2.1. Baking Loss and Specific Volume

The dough mass of each sample was manually divided and shaped into equal-sized pieces of dough (82–84 g) and after baking were obtained the breads of Figure 3.

CB B50 B100

Figure 3. Samples breads: CB—control bread (1.2% NaCl/0.47% sodium, flour basis); B50—0.235% sodium (flour basis) obtained from *S. perennis* powder and B100—0.47% sodium (flour basis) obtained from *S. perennis* powder.

The weight of control (CB), B50 and B100 bread samples was 71.2 g, 73.4 g, and 76.42 g, respectively (Table 3). The increase of the bread weight with the addition of *S. perennis* powder, particularly to B100 bread, can be related to the increase of fiber content in loaves of bread that typically enables the absorption of more water than the main components of flour. Similar behavior was observed to total baking loss that represents the water evaporation during baking. There were no significant differences between the bread samples CB and B50. However, the percentage of water loss during the baking of B100 sample was lower, which allows for a higher weight of this bread. The hygroscopic nature of *S. perennis* powder allowed a high retention of water during bread baking. Similar findings were found in common wheat bread with sodium chloride substitution with *Salicornia ramosissima* powder which is a halophyte species similar morphology with *Sarcocornia* spp. [29].

Table 3. Physical characteristics of control (CB), B50 and B100 bread samples.

	CB	**B50**	**B100**
Total baking loss (%)	13.5	14.1	9.5
Weight (g)	71.2 ± 1.8	73.4 ± 1.8	76.42 ± 2.0
Specific volume (cm^3/g)	3.24	3.50	3.66

Regarding bread's specific volume, it was possible to perceive that the values increased from control (3.24 cm^3/g) to samples that incorporated *S. perennis*, having B100 the higher volume per weight unit (3.66 cm^3/g). Toumi et al. [29] found a specific volume of 2.8 cm^3/g for bread prepared with 1.8% NaCl.

The added salt hinders yeast activity, which results in lower carbon dioxide (CO_2) production but, on the other hand, induces changes in molecular conformations and gluten structure that increase the ability to retain CO_2. The final impact of sodium chloride on specific volume, which is one of the most important visual characteristics of bread depends on the proper balance of these two factors, coupled with the used equipment, type and quality of the ingredients, and the technique of dough preparation. Even this, comparing the specific volume of bread with 1.2% NaCl (this study) and 1.8% NaCl [29] it was observed that this parameter decreased with the increase in salt (NaCl) amount, as observed by Toumi et al. [29].

The incorporation of *S. perennis* powder in bread, instead of NaCl, developed higher well-aerated loaves. This tendency could be explained by the increase of fibre which forms a less tenacious gluten structure with a subsequent increase of air incorporation ability in loaves during baking. Similar findings were found for the specific volume of wheat flour doughs with the addition of *S. ramosissima* powder, where the breads prepared exclusively with Salicornia powder presented the highest specific volume values (about 3.2 cm^3/g) at all levels of salt amount (0.6, 1.2 and 1.8%) [29].

Other prominent strategies used to reduce the salt content in bread are the salt replacers such as potassium chloride (KCl), magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$) or mixtures [36]. Discrimination testing revealed that all breads where 50% NaCl was systematically substituted by different combinations of salts were identified as different (dough properties, bread quality, appearance and sensory perception) from the control, apart from the 50% KCl and the 40% KCl with 10% $MgCl_2$ breads [37]. However, in rheological tests, Marco et al. [38] observed that KCl significantly decreased protein interaction, possibly weakening the gluten network, but this effect was not observed in the specific volume of the final product.

3.2.2. Color

The color of bread is a crucial characteristic that influences the consumers' choices. Figure 4 illustrates the color coordinates of control bread (CB) with NaCl and breads B50 and B100, with *S. perennis* powder. Regarding bread color, the substitution of sodium chloride by *S. perennis* powder allowed a more colored and darker crumb with a lower L* parameter and a higher greener (a* negative) and yellow (b* positive) parameters. Comparing B50 with B100, it is also observed that the increase of halophyte powder addition turned the bread color more greenish and yellowish. The increase of crumb color intensity can be attributed to the higher addition of natural pigments such as carotenoids and chlorophyll present in *S. perennis* powder. Moreover, the higher yellow appearance with the increase of *S. perennis* addition can be explained by the degradation of pigments during baking, producing brown-colored compounds. This finding is in accordance with the results obtained by Clavel-Coibrié el al. [23] with the addition of the same halophyte powder in snacks. The whiteness index of bread control was 69.7 and decreased from 55.2 (B50) to 49.2 (B100) with the increased halophyte powder addition.

Moreover, the chromaticity (C*) also increased from the control bread (14.22) to bread with the addition of *S. perennis*, being 27.04 and 32.86 to B50 and B100, respectively. The overall assessment of the change in colour, measured as total color difference (TCD), with the addition of *S. perennis* was 15.6 to B50 and 22.2 to B100. Considering these values, it was possible to observe that the addition of Sarcocornia powder introduced a perceptible change in the color of the bread.

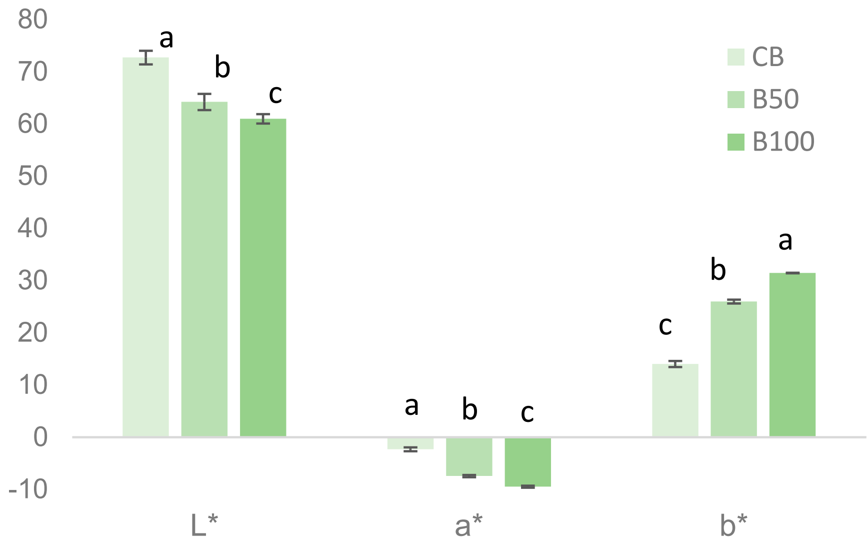

Figure 4. Color parameters lightness (L*), greenness (a*), yellowness (b*) of bread without *S. perennis*. CB—control bread (1.2% NaCl/0.47% sodium, flour basis); B50—0.235% sodium (flour basis) obtained from *S. perennis* powder and B100—0.47% sodium (flour basis) obtained from *S. perennis* powder. Sample breads with different letters on the same color parameter are significantly different (one-way ANOVA, $p < 0.05$).

3.2.3. Texture

As color, bread texture is a determinant attribute to define the quality of bread and the acceptance of this product. The hardness of control, B50 and B100 bread samples is represented in Figure 5. For the three samples, no significant difference was observed in comparison with the control bread. This finding agreed with the results of Clavel-Coibrié et al. [23] where no significant difference of hardness was observed in crackers with incorporation up to 10% of *S. perennis* in comparison with the control cracker. However, Toumi et al. [29] referred that the hardness value decreased in bread samples prepared with the high level of Salicornia powder (1.8%), as compared with the hardness of bread with 1.8% sodium chloride. This different behavior may be due to the presence of other factors, such as the amount of powder halophyte, baking time, temperature.

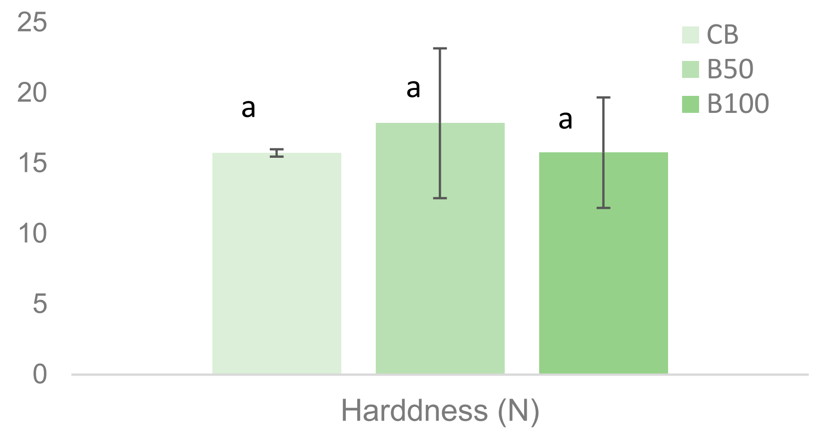

Figure 5. *Cont.*

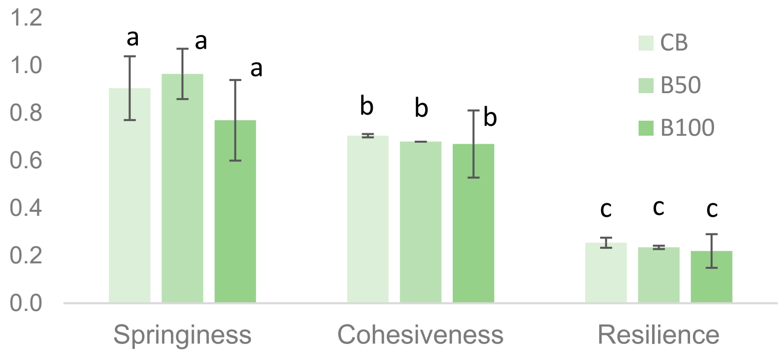

Figure 5. Hardness, springiness, cohesiveness and resilience of bread: CB—control bread (1.2% NaCl/0.47% sodium, flour basis); B50—0.235% sodium (flour basis) obtained from *S. perennis* powder and B100—0.47% sodium (flour basis) obtained from *S. perennis* powder. Sample breads with different letters on the same texture parameter are significantly different (one-way ANOVA, $p < 0.05$).

Springiness, cohesiveness and resilience of bread samples were not affected by the replacement of sodium chloride by *S. perennis* powder (Figure 4). Similarly, Toumi et al. [29] observed that these texture parameters were not affected by salt amount or NaCl/Salicornia ratio in bread preparation.

However, NaCl reduction with chloride potassium resulted in bread with higher firmness [38].

3.2.4. Proximate Composition

Comparing with control bread sample, the addition of *S. perennis* powder promoted the increase of all nutrients of bread, as present in Table 4, except for carbohydrates.

Table 4. Moisture content (g 100 g^{-1} WB) and proximate composition of control (CB), B50 and B100 bread samples (g 100 g^{-1} DB).

	CB	**B50**	**B100**
Moisture	34.06 ± 0.07 [c]	34.75 ± 0.10 [b]	36.19 ± 0.10 [a]
Ash	2.56 ± 0.01 [b]	2.28 ± 0.02 [c]	3.16 ± 0.03 [a]
Fat	0.11 ± 0.01 [b]	0.44 ± 0.03 [a]	0.38 ± 0.05 [a]
Protein	11.13 ± 0.18 [b]	11.19 ± 0.02 [b]	11.48 ± 0.05 [a]
Total Fibre	2.15 ± 0.14 [c]	4.40 ± 0.12 [b]	6.83 ± 0.15 [a]
Fibre insoluble	0.25 ± 0.08 [b]	0.54 ± 0.06 [b]	1.05 ± 0.12 [a]
Fibre soluble	1.91 ± 0.06 [c]	3.85 ± 0.18 [b]	5.78 ± 0.03 [a]
Carbohydrates	84.00 ± 0.35	81.70 ± 0.11	78.17 ± 0.23
Energy (kcal/100 g)	386.06 ± 0.21 [a]	384.25 ± 0.01 [b]	375.42 ± 0.16 [c]
Energy (J/100 g)	1616.35 ± 0.86 [a]	1608.75 ± 0.02 [b]	1571.84 ± 0.69 [c]

Different lowercase letters correspond to significant differences ($p < 0.05$) between bread samples.

Regarding moisture content, the results showed an increase in moisture, particularly from B50 to B100 bread samples. As referred in Section 3.2.1, the increase of bread water content in B50 and B100 samples can be due to the higher retention of water during bread baking with the addition of the hygroscopic powder. The addition of dried halophyte powder increased the ash content of breads due to the minerals of the halophyte powder. Regarding the fat content, the incorporation of Sarcocornia powder on breads increases this nutrient but no significant difference was observed between B50 and B100.In terms of protein, similar values were observed for the breads with the incorporation of halophyte powder and the control bread. In terms of total fibre, insoluble and soluble fiber the content B50 and B100 breads increased by two and three folds, respectively. These higher values result from the higher fibre content of halophyte, compared to wheat flour. Indeed, the B50 and B100 breads can be claimed as a source of fibre since the products contains more than 3 g fibre per 100 g. In addition, the B100 bread can be almost considered a product high in fibre because its content is near of 6 g of fibre per 100 g [39]. The content of fiber in bread is an important aspect both in the dough behaviour as well as for its well-known health benefits. The carbohydrate content of control, B50 and B100 remained relatively constant as well the caloric value of all samples, ranging from 386.06 to 375.42 kcal/100 g.

3.2.5. Mineral Composition

The mineral composition of control, B50 and B100 breads is presented in Table 5. The control (CB) and B100 samples were prepared with the same amount of sodium (0.47%, flour basis) but obtained from different sources, respectively, sodium chloride and *S. perennis* powder and B50 was prepared with half of sodium (0.235%, flour basis), obtained from *S. perennis* powder. As observed in Table 5, the content of sodium in control (CB) and B100 bread was similar while the value was reduced to around half (339.01 mg/100 g) to B50.

Table 5. Mineral composition (mg 100 g^{-1} DB) of control (CB), B50 and B100 bread samples.

Mineral	CB	B50	B100
Na	613.40 ± 11.01^{a}	339.01 ± 5.38^{b}	643.65 ± 14.08^{a}
K	152.43 ± 6.71	195.63 ± 2.54	243.71 ± 2.36
Ca	393.27 ± 15.29^{b}	401.09 ± 1.90^{b}	444.11 ± 4.96^{a}
P	370.04 ± 7.53^{b}	$379.53 \pm 5.97^{a,b}$	397.66 ± 1.71^{a}
Mg	40.28 ± 4.75^{b}	48.80 ± 1.18^{b}	68.81 ± 5.03^{a}
Fe	3.26 ± 0.02^{b}	4.87 ± 0.23^{a}	5.52 ± 0.27^{a}
Zn	0.89 ± 0.01^{b}	0.99 ± 0.02^{a}	1.00 ± 0.02^{a}
Cu	0.33 ± 0.01^{b}	0.43 ± 0.02^{a}	0.37 ± 0.01^{b}
Mn	0.92 ± 0.02^{b}	$1.01 \pm 0.05^{a,b}$	1.10 ± 0.05^{a}

Different lowercase letters correspond to significant differences ($p < 0.05$) between bread samples.

In addition, the incorporation of halophyte powder in bread increased their potassium content, which, along with the sodium, regulates the fluid balance in the human body. The ratio of sodium to potassium intake is a greater risk factor for hypertension and cardiovascular diseases than either electrolyte alone. Higher intakes of potassium can attenuate the adverse effects of sodium on blood pressure [40]. Thus, the consumption of bread with incorporation of *S. perennis* powder allows a balanced of potassium and sodium electrolytes in human body, reducing the risk of cardiovascular disorders, such as high blood pressure. Moreover, the sodium and potassium present in bread are from natural source and not added as salts.

Regarding the other minerals, the addition of halophyte powder led to a significant increase of this macro and micronutrients, particularly in B100 bread sample (Table 5). According to regulation [33] the bread samples can be considered products with a significant amount of calcium, phosphor, iron, and manganese since minerals, per 100 g, are higher than 15% of recommended daily allowances. In addition, the incorporation of *S. perennis* powder can also contribute to increasing the antioxidant activity of bread, maintaining (B100) or even reducing to half (B50) the sodium content, as compared with control bread (CB). Clavel-Coibrié et al. [23] referred that, compared with control, the incorporation of 5% of *S. perennis* into cracker led to a significant increase in total phenolic content and antioxidant activity. In fact, halophyte Sarcocornia species usually have a high phenolic content as part of their antioxidant defense mechanism. The main compounds of *Sarcocornia ambigua* are phenolic acids such as ferulic, caffeic, vanillic and p-coumaric and flavonols (kaempferol and galangin) [41]. *S. perennis* collected in Portugal, Southern and Tejo estuary have a high phenolic content (ranged from 15.79 mg GAE/g, dw and 20.5 mg GAE/g, dw) and high antioxidant capacity (58.49 mg Trolox/g, dw) [23,25]. Thus, the incorporation of the halophyte in bread can promote health benefits not only due to the reduction of salt but also to the increase of its bioactive compounds.

3.2.6. Microbial Counts

The major problem associated with the microbial contamination in bakery products is the economic losses mainly due mould spoilage, another concern is the possibility of mycotoxins production that may pose serious health risks to humans [42]. As shown in Table 6, the mean bacteria count of the bread samples tested ranged from 2.6×10^2 to 2.4×10^3 Cfu/g on day 2. International microbiological standards recommended units of bacterial counts for dry and ready to eat foods are $<10^4$ Cfu/g for total viable bacteria, in our study our counts indicate a good hygienic status of the bread [43].

Table 6. Microbiological analysis: Cfu/g (colony forming units per gram) to control (CB), B50 and B100 bread samples.

		TVBC-2d	YMC-2d	CC-2d	TVBC-4d	YMC-4d	CC-4d
(a)	CB	2.9×10^2	7×10^1	NG	3.5×10^3	1.2×10^3	NG
	B50	3×10^2	9×10^1	NG	6.8×10^3	1.4×10^3	NG
	B100	1.9×10^3	1.6×10^3	NG	7.36×10^4	1.84×10^4	NG
(b)	CB	9.7×10^2	7.4×10^2	NG	4.06×10^4	1.32×10^4	NG
	B50	2.6×10^2	1.9×10^2	NG	5.28×10^4	4.1×10^4	NG
	B100	2.4×10^3	2.4×10^3	NG	8.9×10^4	6.2×10^4	NG

NG: no growth detected (a): bread samples were stored in a cloth bag; (b): bread samples were stored in a paper bag; TVBC-2d: Total viable bacteria counts after 2 days; YMC-2d: Yeasts and molds counts after 2 days; CC-2d: Coliforms counts after 2 days; TVBC-4d: Total viable bacteria counts after 4 days; YMC-4d: Yeasts and molds counts after 4 days; CC-4d: Coliforms counts after 4 days.

Concentrations of total yeast and mould counts also showed similar pattern comparing B50 and Control. Higher concentrations were verified in B100 with greater impact if storage is done in a paper bag (6.24×10^4 Cfu/g). Several factors condition the development of deteriorating microorganisms, having the greatest impact, water activity, concentration of salts and other compounds that can act as preservatives [44]. In this sense the greater amount of fibre and the hygroscopic nature of *S. perennis* powder allowed a greater water retention promoting the development of microorganisms in B100. However, in B50 the impact of water activity is minor, and it is still compensated by the prevalence of some minerals such as sodium, potassium and magnesium and phenolic compounds with antioxidant activity [45]. Some studies showed different salts as sodium chloride (NaCl), potassium chloride (KCl), magnesium chloride ($MgCl_2$) acts as preservative in bread, led to the slowest colony growth rates and delayed lag phase of the microorganisms like coliforms as *Escherichia coli* or moulds as *Penicillium* sp. and *Aspergillus* sp. [46]. Antioxidant compounds used in bakery products have a high antimicrobial activity against *Staphylococcus aureus*, *Salmonella typhimurium* and inhibited growth and toxin production of *Aspergillus* sp. and *Bacillus* species.

3.2.7. Sensory Analysis

Figure 6 shows the results of the sensory evaluation of three bread samples (CB, B50 and B100): aspect, color, aroma, texture, flavor and overall acceptance; all expressed in the scale from 1 (dislike extremely) to 9 (like extremely).

Globally, the control bread was the preferred one concerning the sensory attributes, which is in line with the novelty of the bread samples with Sarcocornia powder [23]. Similar trend was observed to crakers with the incorporation of this dried halophyte. However, the texture score to control bread is very similar to the scores to B50 and B100 bread with the incorporation of Sarcocornia powder which is in accordance with the texture parameters. As concerns B50 and B100 bread samples, the scores are similar to all the evalauted sensorial attributes and their acceptance was good. One interesting point is that the reduction of sodium to half (0.235% sodium (flour basis) in bread (B50) did not affect the acceptability of tasters, as compared with B100 (0.47% sodium (flour basis)). One strategy to increase bread palatability with the *S. perennis* incorparation could be the gradual reduction of NaCl and the increase of the halophyte powder to acustomize the consumers to the changed taste, whereby the new level of powder remains unnoticed by most consumers.

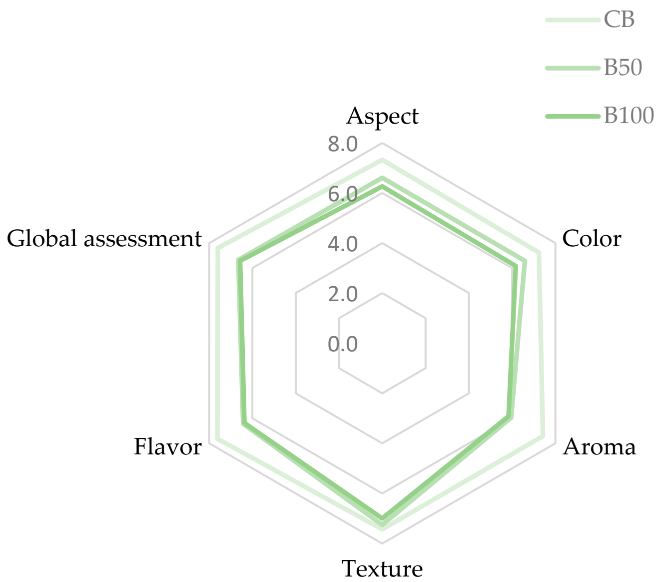

Figure 6. Responses of the sensory analysis panel tasters (n = 67) to CB—control bread (1.2% NaCl/0.47% sodium, flour basis); B50—0.235% sodium (flour basis) obtained from *S. perennis* powder and B100—0.47% sodium (flour basis) obtained from *S. perennis* powder.

Gorman et al. [47] proposed the inclusion of brown seaweed (4% substitution for flour) as an ingredient that could help reduce the salt content. The seaweed bread made with 10% and 20% salt reduction was not significantly different than the control and was acceptable to consumers and associated with soft, chewy and having no aftertaste. However, the increase in bread salt reduction (up to 50%) was associated with negative flavour and textural attributes. Furthermore, the saltiness perception also decreased, and the formulations were not well-liked by consumers.

Likewise, the use of potassium chloride as sodium choride replacement allowed to heterogeneity in consumer hedonic reaction, mainly associated with differences in texture perception. Moreover, NaCl replacement equal to or higher than 40% caused an increase in bitterness, off-flavor, and metallic flavor [48]. However, Marco et al. [38] considered that it was possible to replace up to 50% of NaCl with KCl without reducing quality and consumer acceptability. Extending past purely KCl being used to replace NaCl, Sinesio et al. [49] and Raffo et al. [50] observed that NaCl replacement with PanSalt® (NaCl 57%, other salts and minor ingredients) at 1.5% or 3% failed to maintain the saltiness level of bread and turned it sweeter compared to the NaCl counterparts. However, the overall flavour intensity of bread with 1.5% PanSalt® did not differ from 1.5% NaCl bread and no undesirable flavours were perceived. Moreover, the overall liking did not differ between the 1.5% NaCl and 1.5% PanSalt® breads but the higher value of PanSalt® produced a perceptible increase in bitter taste and aftertaste in the crust. These finding emphasize that replacing NaCl with KCl-based replacers could impact in the overall flavour perception rather than the perception of salty taste.

Moreover, the bread salt-reduction claims influence sensory expectations and perception. Thus, it is critical to determine the quantity of NaCl replacement without affecting the product's characteristics and sensory attributes and to familiarize the consumers with the changed taste, regarless of the sodium reduction strategy used to replace salt.

4. Conclusions

Healthier bread was successfully developed with the incorporation of *S. perennis* as a salt (NaCl) substitute. Powder dried *S. perennis* was incorporated to replace the same amount of bread sodium (0.47%, flour basis) and half of the sodium concentration (0.235%, flour basis). The *S. perennis* powder revealed to be a valuable ingredient for the development of bread with lower sodium and improved nutritional, mineral and functional properties. Furthermore, the substitution of salt with halophyte powder did not exhibit a noteworthy influence on the handling of dough.

Bread with halophyte powder is a good source of nutrients, especially fibre, and with a significant amount (higher than 15% of recommended daily allowances) of calcium, phosphor, iron, and manganese per 100 g. Moreover, the consumption of this bread allows a balanced ratio of potassium and sodium electrolytes in the human body. Besides the improvement of bread quality, bread samples with 0.47% Na and 0.235% Na (flour basis) were both sensorily well accepted. Besides this, the bread with 0.235% Na (flour basis) is a potential strategy to obtain a sodium reduction of 50% in bread, reducing the overall sodium daily intake and bringing important health benefits. Since this bread has a guarantee of quality in microbiological terms and taking into account its composition allows controlling the growth and development of microorganisms capable of deteriorating these products, it can avoid health problems associated with the production of mycotoxins, as well as not lead to economic losses in this industry.

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