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Macroalgal biomass as an additional ingredient of bread

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Abstract

Algae with nutritional value have great potential for production of algal biomass for addition in products. Bread is one of the most consumed foods and one of the major sources of calories in diets in many countries, it has therefore been the target of many enrichment studies. In this work, conventional breads added with 2.5, 5.0 and 7.5% of *Cladophora* spp. and *Ulva* spp. macroalgal biomass were developed and evaluated. In the breads with macroalgal biomass, the nutrient composition, caloric value, technological and sensory evaluation were determined. The addition of macroalgal biomass resulted in protein content increase, ranging from 16 to 18% ($w.w^{-1}$), fibers increase from 1 to 2% ($w.w^{-1}$) and a decrease of lipids from 17 to 12% ($w.w^{-1}$).The sensory and technological characteristics changed slightly in relation to the control bread. The results show the potential use macroalgal biomass in the formulation of conventional bread.

Introduction

The food industry consumes large amounts of algae known to provide a wide variety of nutrients. The characteristics of these algae combined with the great potential for cultivation have drawn the attention of industries to their exploration (Rocha *et al*., 2007). Biomass is also a source of a wide variety of compounds beneficial to humans, such as proteins, vitamins, β-carotene, metals (Fleurence, 1999), fibers, antioxidants, polyunsaturated fatty acids and polysaccharides (Raymundo *et al*., 2004). Due to this biomass composition, seaweeds are increasingly sought after as food in western countries.

Algae are easily obtained on moist land or aquatic surfaces, freshwater or saltwater media and are an important source of essential compounds for human nutrition (Rocha *et al*., 2007). They are capable of occupying any medium that can offer them enough light and moisture whether temporary or permanent. Therefore, they are found in fresh water, sea water, on moist soil or even on snow. Despite the apparent simplicity of these organisms, some algae have internal systems that are only found in superior vegetables (Fleurence, 1999).

Macroalgal biomass can be produced in culture tanks under controlled conditions, or collected from natural environments. In natural environments the mass occurrence of algae, also known as flowering, has negative impacts on aquatic life due to deoxygenation of water and fish deaths, besides the unsightly and undesirable odor when in decomposition (Ereno, 2010).

Cladophora spp. is green, aquatic filamentous algae found on the shores of lakes and rivers. This seaweed grows attached to rocks, underwater plants and other solid surfaces. With the agitation of the currents, it is possible for this macroalgae can go out and live in rivers. In Thailand, the macroalgae *Cladophora* spp. is known as “Kai” and is abundant in the rivers of the north (Traichaiyaporn *et al*., 2010). The local people who live around these rivers use them for their own consumption and for sale in the neighboring regions. *Ulva* spp. represents an important biomass still little used (Lahaye *et al*., 1997). Humans use some of the Ulva species as food since they are rich in fiber, proteins (Lamare and Wing, 2001), vitamins, β-carotene and metals (Fleurence, 1999), in Chile the *Ulva* is utilized as food and for biogas production (Grunewald, 2003).

Bread is one of the most consumed foods and is a major source of calories in diets in many countries. Therefore, it has been the subject of many enrichment studies involving addition of new ingredients or flours to the bread. The supplementation of bread with protein using fish pulp, is a viable alternative for providing all the essential amino acids, making this product easily acceptable, and capable of reaching the low-income public (Centenaro *et al*., 2007).

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Whey is a low cost protein source, with applications in the food industry. In bread, the serum can improve the aroma, flavor and texture, besides improving nutrients. Large bakeries may adopt the study of bread enrichment with this nutritionally rich raw material, replacing water (Souza and Bezerra, 2005).

A variety of ingredients can be added in the preparation of special breads to give the consumer the option of a new and affordable product. Developing a bread formulation with macroalgae tends to minimize the negative impacts of algae, such as the flowering phenomenon. Thus, this paper proposes to develop and evaluate conventional bread, adding *Cladophora* spp. and *Ulva* spp. macroalgal biomass in its formulation.

Material and Methods

The macroalgae, *Ulva* spp. and *Cladophora* spp., collected in the Patos Lagoon estuary (RS, Brazil), were used as ingredients in formulation of bread consisting of a biomass from the mixture of the two macroalgae genus. This was prepared in the Laboratory of Coastal Vegetal Ecology (ECOVECO). The macroalgae were washed with distilled water for the removal of impurities and subsequent identification of the genus, they were then dried at 45°C for 48 h and then ground. The biomass was characterized and stored at -10°C. Characterization involved analysis of moisture, ash, protein, fat and fiber according to the procedures recommended and described by (AOAC, 2000). Carbohydrates were calculated by difference. To calculate the caloric value, the coefficients of protein (4.0), carbohydrate (4.0) and lipid (9.0 $kcal.g^{-1}$) were used (Watt and Merrill, 1963).

Macroalgae added bread was developed as shown in Figure 1.

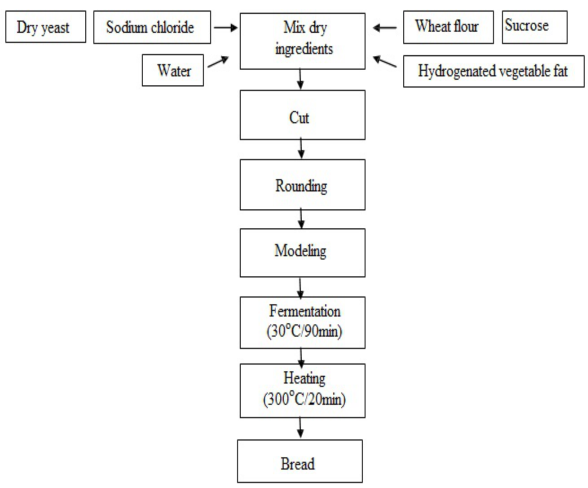

Figure 1. Flowchart of the bread making process

The formulation used for the production of bread consisted of 100 g of wheat flour, 5 g of sucrose, 3 g of dry yeast, 6 g of hydrogenated vegetable fat, 2 g of sodium chloride, 90 mg ascorbic acid and 57 g of water for all treatments. The biomass concentrations used were 0, 2.5, 5 and 7.5% (based on wheat flour) as adapted from El-Dash (El-Dash, 1978).

According to the tests, breads with different concentrations of biomass were prepared, and the physical, chemical, sensory and technological analyses were carried out to determine consumer acceptance.

Technological evaluation of the bread was made from the specific volume and notes from technological characteristics attributed according EL-DASH's worksheet (El-Dash, 1978). This assessment was based on a total of 75 points, due to the fact that aroma and flavor were not evaluated. For subsequent analysis of the results, the final sum of the scores was converted to a scale of 100 points. The specific volume ($mL.g^{-1}$) was obtained by the ratio between the apparent volume (mL) determined by displacement of millet seeds (Pizzinato and Campagnolli, 1993) and mass (g) after baking.

Hardness in fresh bread and hardness 24 hours after baking was evaluated according to the methodology of AACC (2000). The experiment was performed in triplicate, following the space color system L* a* b*, defined by the CIE (International Commission on Illumination) in 1976. The values of L* (lightness), a* and b* (chromaticity coordinates), Chroma (C*) and hab (hue angle), referred to as CIELChcolor system according to Minolta (1993) (Equation 1 and 2) were evaluted.

$$Chroma(C*) = ((a^*)^2 + (b^*)^2)^{1/2} \quad (1)$$

$$h_{ab} = tan^{-1}(b^*|a^*) \quad (2)$$

The attributes evaluated in the sensory analysis of appearance were color, odor and consistency. The sensory evaluation methodology was based on the classification proposed by the NBR 12994 (ABNT, 1994). Sensory tests (sorting), objective methods used in sensory analysis of foods with the effects of the opinions of the individuals on the preference and attitude of the consumer using the evaluation form, were used.

The results were compared using analysis of variance (ANOVA) and the average results were compared by the Tukey test, with $p < 0.05$ and $p < 0.2$. All analyses were performed in triplicate.

Results and Discussion

Table 1 shows the composition of the macroalgal biomass applied on bread.

Table 1. Characterization of the biomass of macroalgae applied in the formulation of the breads

Determinations	Values(1)
Moisture (%)	7.0 ± 0.19
Ash (%) d.b	26.3 ± 1.04
Protein (%) d.b	8.7 ± 2.42
Fat (%) d.b	1.1 ± 0.28
Fiber (%) d.b	3.9 ± 0.62
Carbohydrates (%) d.b	60.0 ± 2.87
caloric value (Kcal.100g^{-1})	168.9 ± 3.52
Digestibility (%)	45.0 ± 1.36
Carotenoids (μg.g^{-1})	378.58 ± 0.023

(1)Values are means ± SD of analyses performed in triplicate. d.b: dry basis

This composition is important because, according to Schwochow and Zanbon (2007) the macroalgae composition may vary by time of year that it is collected. A comparison can be carried out with native Brazilian macroalgae that could be considered for implementation in food such as *Chondracanthus teedei* with levels of protein (14.66%), ash (28.68%), fibers (2.21%), lipids (1.82%) and humidity (86.73%) and *Kappaphycus alvarezii* with levels of protein (8.50%), ash (58.25%), fibers (7.30%), lipids (0.39%) and humidity (87.11%) (Bastos *et al.*, 2012). Despite of the variability, it is verified that the biomass in this study yields acceptable values that can be implemented in food since its protein content is higher than *Kappaphycus alvarezii* and its fat content is lower than *Chondracanthus teedei* while the fiber content is higher.

The lipid content in algae is low, ranging from 1-5%,*Ulva lactuca* also shows high levels of omega 3 and 6.The mineral fraction of some algae represent 36% of total dry weight. The green algae, including *Ulva* and *Cladophora*, have 10-30% protein content. (Patarra, 2008). The fiber content varies with the algal species analyzed, with an average of 30 - 40% dry weight (Tabarsa *et al*., 2012).

In Figure 2 shows the proximal composition of control breads and macroalgal biomass enriched bread. The increase in the concentration of macroalgal biomass in the bread resulted in a significant protein increase from 16.5 to 18.5% (w.w-1). Prabhasankar *et al.* (2009), developed wakame noodles with macroalgae, aiming at increasing the nutritional qualities with macroalgae in the human diet. They mixed the dried wakame in different concentrations of macroalgae during the production of macaroni and

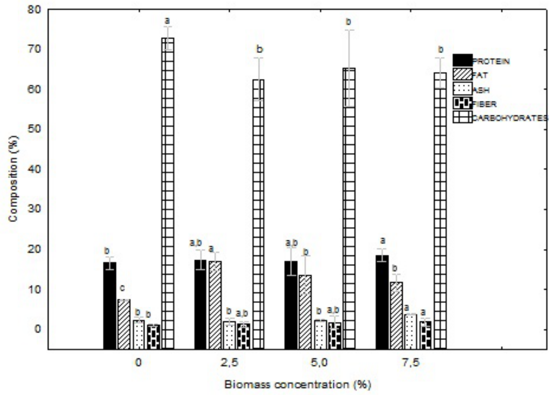

Figure 2. Proximal composition of control breads and bread enriched with macroalgal biomass
In the same determination, means with the same letter do not differ significantly at 20% probability by Tukey test.

evaluated some nutritional quality, they observed that the algae increased the protein content by 20%.

Becker (2007) concluded that algal biomass shows promising qualities as a novel source of protein. Compared to conventional plant proteins, the average quality of most of the algae examined was equal or sometimes even superior. Pádua *et al.* (2011) found a protein content of 11% (w.w-^{1}) in *Cladophora* spp. macroalgae. In the composition of sea algae, proteins stand out as able to bring benefits to food, thereby nutritionally contributing, mainly by containing amino acids that the human body needs.

The breads presented lipid concentration values ranging from 7.0 to 17.0% (w.w^{-1}), with the increase in biomass there was a decrease in the lipid content, by about 5% (w.w^{-1}). According to Figueira (2011), the lipid content of bread enriched with *Spirulina,* with samples spiked with 2, 3, 4 and 5% (w.w^{-1}), ranged from 1.24 to 2.81% (w.w^{-1}), the lipid content was also reduced with the addition of *Spirulina,* this occurred probably due to the smaller amount of this components in the algae biomass.

The ash content between all the breads did not have any significant difference, except for the bread formulation with 7.5% (w.w^{-1}), which differed significantly due to the mineral content (2.5%) present in macroalgae. Morais et al. (2006) show ash values of around 0.4% for Cookies with *Spirulina* platensis. In this study, the mineral content is more incremented due to the algal biomass, this can contribute to the supplementation of macronutrients and micronutrients required in the diet.

According to Kritchevsky *et al.* (1995), a food with fiber content of 2 to 3% (w.w^{-1}) can be considered as a good source of dietary fiber. In Brazil, decree n° 27 of the National Health Surveillance establishes the technical regulations concerning nutritional supplement information, stating that a food can be

Table 2. Technological characterization of breads (1)

Biomass of Macroalgae (%) added to bread	Specific Volume ($mL.g^{-1}$)	Hardness (fresh) (g)	Hardness (24h) (g)
0	3.75±0.01 ^{a}	252.72± 17.58 ^{b}	469.38 ±49.62 ^{a,b}
2.5	3.50 ±0.06 ^{b}	254.99±38.09 ^{b}	414.99±9.05 ^{b}
5.0	3.74 ±0.16 ^{a,b}	386.73±27.73 ^{a}	615.10±98.53 ^{a}
7.5	3.54 ±0.07 ^{a,b}	232.95±29.59 ^{b}	502.15 ±84.76 ^{a,b}

(1) In the same column, means with the same letter do not differ significantly at 5% probability by Tukey test

Table 3. Characterization of the coloration of breads (1)

Biomass of Macroalgae (%) added to Bread	L*	a*	b*	C*	h_{ab}(°)
0	68.02± 1.57 ^{a}	0.57± 0.05 ^{c}	11.30± 0.37 ^{c}	11.32±0.37 ^{c}	87.11±0.37 ^{a}
2.5	59.98± 1.13 ^{b}	0.78± 0.02 ^{b}	11.57± 0.35 ^{c}	11.60±0.03 ^{c}	86.14 ±0.03 ^{a}
5.0	52.69± 0.88 ^{c}	1.42± 0.32 ^{a}	17.36± 1.26 ^{b}	17.42±1.28 ^{b}	85.32 ±1.14 ^{a}
7.5	51.97± 0.91 ^{c}	1.66± 0.31 ^{a}	19.59± 0.56 ^{a}	19.66±0.53 ^{a}	85.15±1.04 ^{a}

(1) In the same column, means with the same letter do not differ significantly at 5% probability by Tukey test. L^* (lightness), a^* and b^* (chromaticity coordinates), C^* (chroma) and h_{ab} (hue angle)

considered a source of fiber if it presents 3 $g.100g^{-1}$ of the ready product for solid foods. With twice the amount of this content, it is considered a food with high fiber content (Brasil, 1999). The concentration of fibers increased from 1 to 2% ($w.w^{-1}$) with increased concentrations of macroalgal biomass, there was a significant difference when a concentration of 7.5% ($w.w^{-1}$) of biomass was used. This proves that adding higher concentrations of macroalgal biomass can make the bread a good source of fiber, but bread prepared with 7.5% ($w.w^{-1}$) of macroalgal biomass would already be a good source of fiber 2% ($w.w^{-1}$) (Kritchevsky *et al*., 1995).

The carbohydrate content was 65% ($w.w^{-1}$) on average, reduced with addition of macroalgae, all breads differed significantly from the control bread. Morais *et al*. (2006) found an average of 68% ($w.w^{-1}$) of carbohydrates in cookies with *Spirulina platensis,* but the more the addition of seaweed the greater the carbohydrate content was.

The specific volume (SV) is an important measure of the ability of the flour to retain the gas with in the mass and thus making the bread rise. From all the macroalgal biomass added in the evaluated samples, the formulation with 5.0% ($w.w^{-1}$) was the one with the highest specific volume. Consequently, this sample also obtained highest total in the technological characteristics scores.

The addition of macroalgal biomass had no significant difference in the measured hardness of fresh bread and after 24 hours of baking except for the fresh bread enriched with 5% ($w.w^{-1}$) biomass, which was different from the control, presenting greater hardness, as shown in Table 2. However this may have contributed to the consistency of the bread, whereas in the preference analysis for consistency panelists preferred the bread added with 5% ($w.w^{-1}$) biomass (Figure 3). Figueira (2011) found that the hardness of the breads was unchanged with the addition of up to 4% ($w.w^{-1}$) of algae, the hardness only increased when 5% (w.w-^{1}) of algae (based on flour) was added.

Bread that has a score from 81 to 100 may be classified as good quality bread, 61 to 80 is regular, and 31 to 60 is bad (Dutcosky, 1996). In the total scores, breads made with macroalgae were classified as regular quality, with average values of 75. Table 3 shows the color parameters, L* (luminosity), a* and b* (chromaticity coordinates), chroma (C*) and hue angle (hab) values evaluated in the breads. The breads show a reduction in their luminosity values (L*) with increased macroalgal biomass concentrations, hence demonstrating that browning of the bread occurred. The biomass concentration is directly related to the color of the bread, because it interferes in the color of the obtained dough of the breads.

With the addition of 2.5% ($w.w^{-1}$) of macroalgal biomass, the color (C*) did not differ significantly from the control bread, but with the addition of 5.0 and 7.5% ($w.w^{-1}$) of macroalgal biomass, the C* value increased, indicating an increase in color intensity of the breads. The control bread is closest to the hue angle (hab) of 90°, which indicates a yellowish color. The color of the bread is directly related to the ingredients contained in the formulation and due to reactions that occur during baking. Although this difference was significant, it shows that the color of the breads with concentration of 7.5% ($w.w^{-1}$) doesn´t differ from the control bread, generating a desirable feature, facilitating the acceptance by the judges.

To determine the preferred bread formulation, the ranking test was applied to 32 potential consumers aged between 17 and 33 years, they were requested to rank the samples starting with the formulation they most preferred to the least preferred in color/odor/consistency and finally the most preferred considering the formulations of 2.5, 5.0 and 7.5% ($w.w^{-1}$) macroalgal biomass. Figure 3 shows the sensory preference evaluation of macroalgae added bread according to the ranking test.

In relation to the attributes color, odor and consistency, there was no significant difference at 95% level of significance between the bread formulations containing 2.5, 5.0 and 7.5% ($w.w^{-1}$) of

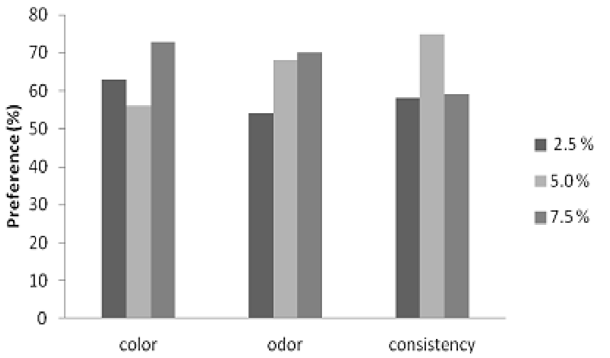

Figure 3. Sensory evaluation of preference of macroalgae added bread by ranking test with biomass concentrations of 2.5, 5.0 and 7.5% (w.w^{-1}).

macroalgal biomass. According to Figueira (2011), who evaluated breads containing 3% and 5% (w.w-1) of *Spirulina platensis*, there was no significant difference between the breads of the different concentrations of microalgal biomass. Kritchevsky *et al*. (1995) in sensory evaluation by 36 judges, 22 judges preferred bread enriched with 3% (w.w^{-1}) of macroalgal biomass.

In this study the preference by the judges was the formulation with 7.5% (w.w^{-1}) biomass, except in consistency and therefore it is possible to choose the formulation with the greatest concentration of macroalgal biomass (7.5% w.w^{-1}), since this increased the protein (18.6% w.w^{-1}) and fiber (2% w.w^{-1}) content of the bread, and, moreover, lipid content (12% w.w^{-1}) was lower, satisfying the necessecity of desired nutritious food, with low fat content.

Adding to the vast research on bakery products enriched with new ingredients or flour, the algae have emerged as one of these new ingredients. The use of microalgae has already been tested by Morais *et al.* (2006), who developed chocolate cookies enriched with dry *Spirulina platensis* biomass. These authors analyzed the composition, sensory characteristics and digestibility of cookies and observed an increase in carbohydrate content by 68.6% (w.w^{-1}), protein (7.7% w.w^{-1}), digestibility (16.2% w.w$^{-1)}$ and acceptance by the judges. Figueira (2011) also suggests the use of microalgae for the enrichment of bread, because they present an increase in protein content from 15.59% to 39.04% (w.w^{-1}) in the bread.

In Brazil, ANVISA classifies the algae biomass as one of the new foods, however there are still no new foods enriched with macroalgal biomass in markets in the country (Kritchevsky *et al.*, 1995). Whereas in countries from other different continents, such as Thailand (Khuantrairong and Traichaiyaporn, 2011), France (Rava *et al.*, 1993) and Hawaii (Traichaiyaporn *et al.*, 2004), the use of algae as a food or ingredient is widespread. The reality in Brazil is different, even though there is occurrence of macroalgae on the coast due to suitable climate and territory available for its cultivation. To apply for registration of products based on algae with no history of consumption in the country, the product must be specified through a document including the identification of the algal species, the location of cultivation of the algae and levels of inorganic contaminants (arsenic, cadmium, lead and mercury). In the case of a mixture of algae, a specification of all of them must be supplied (Brasil, 1998).

Conclusion

Bread was formulated with different concentrations of macroalgal biomass that resulted in increase in the content of proteins and fibers and a reduction of lipid content in breads with increasing macroalgal biomass concentration. Sensory and technological characteristics in bread with macroalgal biomass were slightly modified with respect to the control bread.

In this study the addition of macroalgal biomass rich in nutrients from *Ulva* spp. and *Cladophora* spp. indicates a new alternative in the enrichment of breads and consumer acceptance. The use of macroalgae is a viable alternative, and higher concentrations of biomass can be tested in future research, since the higher tested concentration (7.5% w.w^{-1}) did not impair the technological and sensory characteristics of the breads. The macroalgal biomass can be produced in a controlled environment, or collected in natural environments. Whichever way, it must meet the legal requirements of each country for use in human food.

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