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Bread containing *Brassica oleracea var. acephala* as a source of copper and manganese

Pão de forma contendo *Brassica oleraceae* var. *acephala* como fonte de cobre e manganês

Érica Menezes Salvino[1*]; Janeeyre Ferreira Maciel[2]; Roberto Kelwin Lopes da Costa e Lopes[3]; Larissa Raphaela Gonçalves de Farias Feitosa[4]

Abstract

The aim of this study was to add dehydrated kale (*Brassica oleracea* var. *acephala*) to the formulation of a loaf of bread at concentrations of 2.5% and 5%, and submit the products obtained to physico-chemical, sensory and chemical composition analysis. We also designed a control loaf of bread for comparison purposes. The addition of dehydrated kale increased the pH and acidity of the bread; however, it did not affect the specific volume, which ranged from 4.57 to 4.08 cm^3/ g, and the water activity, which was 0.95, making it possible to obtain products with satisfactory technological characteristics. In the sensory test, the kale bread showed good acceptance, with mean scores ranging from 6.5 to 8.4, and outstanding softness. The color of the bread with 5% kale was the only attribute that obtained a mean score below the one obtained for the control bread. The addition of dehydrated kale promoted greater increase in the fiber (133-281%), calcium (176-297%), phosphorus (201-232%), potassium (208-318%) and magnesium (181-300%) content of the bread; however, only for copper (140-160%) and manganese (76-118%), were increments sufficient to make the products good sources of these minerals. As regards the level of oxalic acid, the concentrations obtained were far below the level considered as risky to health. Therefore, the addition of dehydrated kale to the formulation of bread resulted in products with good sensory acceptance, increased their nutritional value, and offered consumers a new choice of bread.

Key words: Sensory acceptability, kale, bakery products

Resumo

Nesse estudo o objetivo foi adicionar couve (*Brassica oleraceae* var. *acephala*) desidratada à formulação de pão de forma, nas concentrações de 2,5% e 5%, e submeter os produtos obtidos as análises físico-químicas, sensoriais e de composição química. Ainda, foi elaborado um pão controle, para fins de comparação. A adição de couve desidratada promoveu elevação no pH e acidez dos pães, entretanto, não interferiu no volume específico, que variou de 4,57 a 4,08 cm^3/g, e na atividade de água, que foi de 0,95, sendo possível obter produtos com características tecnológicas satisfatórias. No teste sensorial, os pães adicionados de couve apresentaram boa aceitação, com escores médios variando de 6,5 a 8,4, destacando-se quanto à maciez. A cor do pão com 5% de couve foi o único atributo que obteve escore médio abaixo do obtido para o pão controle. A adição de couve desidratada promoveu maior

[1] Nutricionista, Drª em Ciência e Tecnologia de Alimentos, Universidade Federal da Paraíba, UFPB, João Pessoa, PB, Brasil. E-mail: ericamsal@hotmail.com
[2] Engª de Alimentos, Profª Drª, Universidade Federal da Paraíba, UFPB, João Pessoa, PB, Brasil. E-mail: janeeyre@ct.ufpb.br
[3] Discente em Engenharia de Alimentos, UFPB, João Pessoa, PB, Brasil. E-mail: kelwinl@hotmail.com
[4] Engª de Alimentos, M.e em Ciência e Tecnologia de Alimentos, UFPB, João Pessoa, PB, Brasil. E-mail: larissargfarias@gmail.com
[*] Author for correspondence

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elevação no teor de fibras (133-281%), cálcio (176-297%), fósforo (201-232%), potássio (208-318%) e magnésio (181-300%) dos pães, entretanto, somente para cobre (140-160%) e manganês (76-118%) os incrementos foram suficientes para tornar os produtos fontes desses minerais. Quanto aos teores de ácido oxálico, as concentrações obtidas foram bem abaixo do considerado de risco a saúde. Portanto, a adição de couve desidratada à formulação de pão de forma resultou em produtos com boa aceitação sensorial, com incrementos no valor nutricional, além de oferecer ao consumidor uma nova opção de consumo desse tipo de pão.
Palavras-chave: Aceitação sensorial, couve, produtos de panificação

Introduction

Kale (*Brassica oleracea* var. *acephala*) is a source of calcium, magnesium, potassium and fiber (LISIEWSKA et al., 2009; SIKORA; BODZIARCZYK, 2012).It also contains high concentrations of ascorbic acid antioxidants (CAMPOS et al., 2009), carotenoids and chlorophyll (KORUS, 2013), substances which reduce the concentration of free radicals in the body and have been proved to prevent certain chronic degenerative diseases (LIGOR et al., 2013).

This high-water-content vegetable cannot be maintained at room temperature for more than a few days, but its life may be extended for months or even years through the application of dehydration (MWITHIGA; OLWAL, 2005), a process which promotes the concentration of nutrients and other compounds present in the kale. In this case, in addition to providing significant amounts of fiber and minerals, dehydrated kale also happens to be considered a good source of protein.

Among the dehydrated vegetables widely used by the food industry, the most common are spinach, beetroot and carrot (HAMERSKI et al., 2013), but the consumption of fresh kale still dominates at the domestic level, and it may be added in the preparation of juices (FEIBER; CAETANO, 2012), sautéed (LUCIA et al., 2011) or cooked, when used as an ingredient in elemental diets or in dishes like soups and rice (CALHEIROS; CANNIATTI-BRAZACA, 2011; AYAZ et al., 2006).

Therefore, the use of dehydrated kale in the formulation of processed food constitutes an excellent alternative for increasing the consumption of this vegetable, especially by people who live in urban areas and eat out more frequently, replacing main meals with snacks (IBGE, 2011) like sandwiches (TEIXEIRA et al., 2012).

Bread has long been used for enrichment, being widely consumed by individuals from different age groups and social classes worldwide (HOBBS et al., 2014; LÓPEZ-NICOLÁS et al., 2014) and for making up for deficiencies in nutrients which are essential to health like minerals and vitamins (SABANIS et al., 2009).

In this study, the goal was to enrich loaves of bread with dried kale in concentrations which promoted improvement of their nutritional value and might result in products with good sensory acceptance.

Materials and Methods

Materials

The ingredients Dona Benta™ wheat flour, instant dry yeast, salt, sugar, Adipan dough improver (ingredients: corn starch or cassava, sugar, polysorbate 80, calciumstearoyl-2-lactyltate, ascorbic acid and alpha-amylase enzyme) and hydrogenated vegetable fat, all used in the production of loaves, were acquired from a store in the city of João Pessoa, Brazil.

Obtaining dehydrated kale

The process of obtaining dehydrated kale consisted of the stages of selection, cleaning, bleaching and drying of kale leaves.

Initially, the stalks were discarded and the leaves were visually evaluated; only the ones without injuries and which were uniform in color and texture were kept. Subsequently, the selected leaves were washed with tap water and immersed for 15 minutes in chlorinated water (150 ppm). Then, they were rinsed with distilled water and the excess was removed with paper towels.

The cleaned leaves were cut and subjected to bleaching, which was done by exposing the leaves to water vapor (100°C) for three minutes with subsequent cooling under refrigeration at a temperature of approximately 5°C.

The blanched leaves were kept in a drying cabin for a period of about five hours under conditions of average air velocity of 1.00 $m.s^{-1}$ measured by an anemometer (Veloci Check, model 8330-M, São Paulo, Brazil), air temperature 55°C ± 2° C and relative humidity of approximately 13%, both measured with dry bulb and wet bulb thermometers (LAMBRECHT, model 761, Göttinger, Germany) attached to the top of the entrance to the drying camera. Once dry, the leaves were ground in a food processor (Magic Bullet, Model MB-1001, USA) at full speed and sifted through a mesh 60 screen to obtain a homogeneous powder. The powder obtained (moisture: 5.56%, pH: 6.02; acidity: 4.29 mL of NaOH0.1N/100 mL) was vacuum packed (Selovac, 120-B, São Paulo, Brazil) in polyethylene plastic bags of low density and kept at room temperature.

Preparation of breads

Formulation

Three formulations of loaves were prepared, two with the addition of dehydrated kale, at concentrations of 2.5% (F1) and 5.0% (F2), based on 100g of flour, and one a control loaf without kale. The selection of the concentrations of kale used in this research was based on preliminary tests, and formulations at concentrations above 5% were excluded because they showed low specific volume. The basic ingredients used in the formulation of the loaves were: special wheat flour (100g), water (52g), instant dry yeast (1.5g), salt (1.8g), crystal sugar (6.0g), dough improver (1.0g) and hydrogenated vegetable fat (3.0g).

Elaboration process

Initially, the dry ingredients along with the fat were homogenized in a blender with spirals (Steel, ST-005, São Paulo, Brazil), at slow speed for five minutes and at high speed for 10 minutes (until it reached the point of veil). During the process, chilled water at approximately 10 ° C was gradually added. Then, the 24°C fresh dough was kneaded, left to rest for 10 minutes and divided into 650 g pieces. After manual modeling, individual portions were placed in pans (22 cm x 11 cm x 7 cm) previously coated with hydrogenated vegetable fat and transported to the fermentation chamber (Nova Ética, series 400D, São Paulo, Brazil), and kept there for about an hour and 40 minutes at 35 ± 1 °C. After this period, the loaves were baked in a gas oven (Turbo Progás, Caxias do Sul, RS, Brazil) for 20 minutes at 200°C. They were then cooled for three hours, sliced, packed in polyethylene bags and stored at room temperature until the time of analysis.

Physico-chemical evaluation of the loaves

Total titratable acidity (TTA) and pH

The pH was determined in a previously calibrated potentiometer (Quimis, 0400, São Paulo, Brazil), and the acidity was titrated with NaOH 0.1 mol/L to final pH of 8.5 expressed in mL of NaOH 0.1 mol/L (ROBERT et al., 2006).

Specific volume

The volume of the loaves was determined 24 hours after processing by the seed displacement method according to the procedure described by AACC (2000), and the rapeseed was replaced by millet seed (SHITTU et al., 2008). The specific volume (cm^3/g) was based on the ratio of the volume (cm^3) and the dough mass (g).

Water activity (A_w)

The water activity in the bread crumb was determined in Aqualab equipment (series 3, Decagon, Pullman, USA) according to the procedure described by AOAC (2000).

Texture

The texture profile of the loaves was assessed with the aid of a texturometer model TA-XT plus (Stable Micro Systems, Surrey, UK) fitted with a cylindrical probe of compression with a 35 mm diameter. The texture parameters determined were firmness, chewiness, springiness and cohesiveness. The instrumental analysis of the texture was conducted under the following conditions: pre-test, test and post-test speed of 2.0 mm/s, 5.0 mm/s and 5.0 mm/s, respectively; with a distance of 20 mm, 20g type of trigger and time between the two compressions of 5 sec. For analyzing these parameters, the ends of the loaf were removed, resulting in a cylinder approximately 6.0 cm long (CARR; TADINI, 2003).

Sensory evaluation

Formulations of bread containing 2.5% and 5% of kale powder were subjected to an acceptance test by 77 tasters (56% female and 44% male) recruited among students and staff members of the Federal University of Paraíba (UFPB). This test was performed at the Laboratory for Sensory Analysis at UFPB in individual cabins at a temperature of 22°C under white light, and the samples comprised a quarter of a slice of bread including crumbs and crust, obtained 24 h after production. The samples were served in monadic order, in disposable white plates numbered with three randomly coded digits, accompanied by mineral water.

During analysis, the tasters evaluated the color attributes of the slice of bread, aroma, crumb softness, flavor, appearance and overall acceptability using a nine-point hedonic scale (9 = really liked and 1 = really disliked), according to IAL (2005). The breads were considered accepted when they obtained averages ≥ 6 (liked slightly) (ROCHA; CARDOSO SANTIAGO, 2009). In the purchase intent test, a five-point scale (1 = definitely would not buy and 5 = definitely would buy) was used.

Acceptance testing was conducted after approval by the Research Ethics Committee of the Center for Health Sciences at the Federal University of Paraiba (protocol nº 0278/2013).

Chemical composition of breads

Samples of bread were evaluated for their chemical composition according to the following methodology:

- moisture, ash, proteins, according to the analytical procedures of AOAC (2000);
- total dietary fiber, according to the analytical procedures of AOAC (2005);
- lipids by the method described by Bligh and Dyer (1959);
- total and reducing sugars as described by Somogyi (1945);
- minerals by the use of atomic absorption spectrophotometry in a Varian spectrophotometer, model Spectr AA-200 VARIAN for Ca (calcium), Mg (magnesium), Cu (copper), Fe (iron), Mn(manganese) and Zn (zinc); by the method of flame photometry for K (potassium) (AOAC, 2005) and by the UV/*vis* Espectrofotometro (model Spectr AA-200 VARIAN, Mulgrave, Australia) e (QUIMIS, Q798U, São Paulo, Brazil);
- oxalic acid, by the method described by Moir (1953);
- phytic acid was determined by the colorimetric method described by Latta and Eskin (1980);

- tannins were determined by the colorimetric method based on the reduction of the Folin-Dennis method, according to AOAC (2000).

Also, pH was determined by using a digital pH meter (QUIMIS, 0400, São Paulo) according to AOAC standards (2000) and acidity of the samples according to the method described by Robert et al. (2006). All analyses were performed in triplicate, with five distinct repetitions, 15 determinations of each analysis in total.

Statistical analysis

The results of physico-chemical, sensory and chemical composition analyses of sliced bread were subjected to analysis of variance (ANOVA) and Tukey's test at a significance level of 5%. The software used was Assistat Version 7.7 Beta.

Results and Discussion

Physico-chemical evaluation of breads

Tables 1 and 2 show the results for the physicochemical analyses for the loaves of bread.

Breads with kale showed elevated pH and acidity compared with the control bread (Table 1), ranging from 6.22 to 6.39 and from 2.55 to 2.89 mL of NaOH0.1N/10 g of bread, respectively, which was expected, especially in relation to the acidity as kale is a relatively acidic vegetable (acidity; 4.29 mL of NaOH0.1 N/100 mL).

For the pH, the values observed were greater than those recommended (5.4 to 6.1) in breads (OURA et al., 1982), and for acidity they were below 3.0 ml of NaOH 0,1N/10 g of bread, limit commonly observed in breads added only of yeast (QUÍLEZ et al., 2006; BELZ et al., 2012).

Table 1. Values of the physico-chemical analysis of loaves.

Parameters	Formulations		
	CL	F1	F2
pH	$6.22^{c}\pm0.01$	$6.28^{b}\pm0.03$	$6.39^{a}\pm0.01$
Acidity (mL de NaOH 0,1N/10g de pão)	$2.55^{b}\pm0.16$	$2.83^{a}\pm0.75$	$2.89^{a}\pm0.27$
Specific volume (cm^3/g)	$4.57^{a}\pm0.36$	$4.51^{a}\pm0.31$	$4.08^{a}\pm0.23$
Water activity	$0.95^{a}\pm0.01$	$0.95^{a}\pm0.00$	$0.95^{a}\pm0.00$

CL- control loaf; F1 – loaf with 2.5% kale powder; F2 – loaf with 5% kale powder.
Mean ± standard deviation of triplicate analyses, with five repetitions. Means followed by the same letter do not statistically differ from each other. Tukey's test at 5% probability level was applied.

Table 2. Texture evaluation values of loaves of bread.

Texture Profile	Formulations		
	CL	F1	F2
Firmness (N)	$2.93^{c}\pm0.29$	$3.49^{b}\pm0.25$	$4.92^{a}\pm0.07$
Elasticity	$0.97^{a}\pm0.01$	$0.97^{a}\pm0.00$	$0.96^{a}\pm0.01$
Cohesiveness	$0.71^{a}\pm0.04$	$0.69^{ab}\pm0.01$	$0.66^{b}\pm0.01$
Chewiness(J)	$2.01^{b}\pm0.21$	$1.96^{b}\pm0.22$	$3.11^{a}\pm0.12$

CL- control loaf; F1 – loaf with 2.5% kale powder; F2 – loaf with 5% kale powder.
Mean ± standard deviation of triplicate analyses, with five repetitions. Means followed by the same letter do not statistically differ from each other. Tukey's test at 5% probability level was applied.

With respect to the specific volume, values greater than 4.0 were observed and are considered satisfactory results when compared with those (4.45 to 5.41) in other studies involving loaves of bread (OLIVEIRA et al., 2011; SILVA et al., 2009). The observed increase in the pH of bread with kale did not affect this characteristic, despite the growth of yeasts during fermentation being favored at pH around 5.0 (QUÍLEZ et al., 2006).

For water activity, values observed in the three formulations were close to those reported in the literature for a loaf of bread (LAZARIDOU; BILIADERIS, 2007).

The texture profile results are shown in Table 2. The addition of the dehydrated kale to the formulation of loaf led to an increase in firmness and chewiness, whereas cohesiveness was reduced as of 5% concentration. Regarding elasticity, there was no significant difference between the samples ($p>0.05$). Similar results were observed in loaves enriched with inulin and Okara flour (SILVA et al., 2009; PERESSINI; SENSIDONI, 2009). Possibly, these products promoted increase in fiber content of breads, a condition that contributed to the increase in firmness. This same effect may have been responsible for the results observed in bread with kale. According to Gandra et al. (2008) and Oliveira et al. (2007) the increase in fiber content leads to an increase in the firmness of breads.

Other factors contribute to the changes in the texture of the loaves, such as: water quantity in the dough, differences in moisture retention capacity of wheat flour, the storage time of the product, reduction in the protein content of wheat (SILVA et al., 2009; ESTELLER; LANNES, 2008) and acidity. According to Hassan et al. (2013), the decrease in pH, associated with the increase in acidity, results in lower firmness of loaves, because of the greater activity of protease and amylase enzymes present in wheat flour. As regards chewiness and cohesiveness, no reports on the influence of acidity in these characteristics were found.

Sensory evaluation

The results of the acceptance test of both formulations of loaves with dehydrated kale and of the control bread are shown in Table 3. All samples evaluated in the sensory test were suitable for consumption according to results obtained in the microbiological analysis previously performed (BRASIL, 2001).

Table 3. Mean values and standard deviations of scores on the sensory acceptance test of loaves of bread formulations with 0%, 2.5% and 5% dehydrated kale.

Attributes	Formulations		
	CL	F1	F2
Color	$7.5^{a} \pm 1{,}35$	$7.5^{a} \pm 1.21$	$6.5^{b} \pm 1.74$
Aroma	$7.1^{b} \pm 1{,}26$	$7.6^{a} \pm 1.21$	$7.0^{b} \pm 1.49$
Flavor	$7.3^{ab} \pm 1{,}19$	$7.8^{a} \pm 1.09$	$7.1^{b} \pm 1.59$
Softness	$7.2^{b} \pm 1{,}15$	$8.4^{a} \pm 0.88$	$8.3^{a} \pm 1.03$
Global acceptance	$7.4^{ab} \pm 1{,}05$	$7.8^{a} \pm 0.89$	$7.1^{b} \pm 1.26$

CL- control loaf; F1 – loaf with 2.5% kale powder; F2 – loaf with 5% kale powder.
Mean ± standard deviation of triplicate analyses, with five repetitions. Means followed by the same letter do not statistically differ from each other. Tukey's test at 5% probability level was applied.

The three formulations tested were acceptable in terms of all sensory attributes, with mean scores ranging from 6.5 to 8.4, values between the hedonic terms "slightly liked" and "really liked."

The loaf with dehydrated kale added in the concentration of 2.5% had a better aroma and smoothness than the control bread, not differing from the latter, however, in color and taste attributes.

With the increase in the concentration of this ingredient to 5%, there was significant reduction in the acceptance of color, and flavor and aroma showed similar acceptance to the control bread.

Loaves with kale showed a better acceptance for softness, which was not expected, given the increase in firmness and chewiness thanks to the addition of this ingredient. Further, it was found that the increase in the concentration of kale from 2.5% to 5% resulted in a decrease in acceptance for the aroma, taste and color attributes.

Chemical composition of loaves

The average moisture content of breads ranged from 34.37% to 35.57% (Table 4), values close to those found by Borges et al. (2012) and Oliveira et al. (2011) in traditional loaves (35.43% and 34.85%, respectively). As regards the maximum and minimum limits of desirable moisture in loaves, no reports were found in the researched literature. In Brazil, the maximum allowed was 38%; however, the resolution that established this pattern (BRASIL, 2000) was withdrawn in 2005 (BRASIL, 2005).

The addition of dehydrated kale to the loaves promoted significant increases in fiber content, potassium, phosphorus, calcium and magnesium, 133-280%, 208-318%, 201-232%, 176-297% and 181-300%, respectively (Table 4). Despite significant increases ($p < 0.05$), the breads with cabbage could not be classified as sources of these nutrients, as they did not contain the minimum 15% of RDI established by Brazilian law for solid foods (BRASIL, 2012). In relation to calcium, the minimum amount required corresponds to 150 mg $100g^{-1}$, and this study obtained 130 mg $100g^{-1}$in the loaf with 5% dried kale, very close to the recommended value. Therefore, the amount reached significantly contributes to the recommended daily intake of this mineral.

Table 4. Average levels of the chemical components of loaves of bread.

Composition	Formulations		
	CL	F1	F2
Moisture (%)	$34.65^{b}\pm0.49$	$34.86^{ab}\pm1.33$	$35.57^{a}\pm1.12$
Proteins (g $100g^{-1}$)	$8.33^{b}\pm0.05$	$8.61^{a}\pm0.08$	$8.66^{a}\pm0.03$
Lipids (g $100g^{-1}$)	$3.72^{a}\pm0.21$	$3.30^{b}\pm0.09$	$3.22^{b}\pm0.05$
Ashes (g $100g^{-1}$)	$1.64^{c}\pm0.05$	$1.77^{b}\pm0.03$	$1.93^{a}\pm0.04$
Starch (g $100g^{-1}$)	$39.75^{a}\pm0.41$	$37.68^{b}\pm0.52$	$36.75^{b}\pm0.62$
Dietary fiber (g $100g^{-1}$)	$0.52^{c}\pm0.05$	$1.21^{b}\pm0.04$	$1.98^{a}\pm0.04$
Calcium (mg $100g^{-1}$)	$32.81^{c}\pm0.43$	$90.61^{b}\pm0.06$	$130.34^{a}\pm0.05$
Phosphorus (mg $100g^{-1}$)	$27.57^{c}\pm0.41$	$83.00^{b}\pm0.15$	$91.63^{a}\pm0.21$
Potassium (mg $100g^{-1}$)	$35.43^{c}\pm0.27$	$108.98^{b}\pm0.09$	$148.11^{a}\pm0.19$
Magnesium (mg $100g^{-1}$)	$8.43^{c}\pm0.11$	$23.74^{b}\pm0.06$	$33.75^{a}\pm0.08$
Iron (mg $100g^{-1}$)	$2.23^{c}\pm0.01$	$2.95^{b}\pm0.01$	$3.48^{a}\pm0.01$
Zinc (mg $100g^{-1}$)	$0.56^{c}\pm0.01$	$0.72^{b}\pm0.01$	$0.86^{a}\pm0.01$
Manganese (mg $100g^{-1}$)	$0.17^{c}\pm0.02$	$0.30^{b}\pm0.01$	$0.37^{a}\pm0.00$
Copper (mg $100g^{-1}$)	$0.05^{c}\pm0.01$	$0.12^{b}\pm0.01$	$0.13^{a}\pm0.00$
Total oxalic acid (mg $100g^{-1}$)	$0.05^{c}\pm0.13$	$0.38^{b}\pm0.12$	$0,51^{a}\pm0.21$
Phytic acid (mg $100g^{-1}$)	$0.04^{c}\pm0.02$	$0.12^{b}\pm0.04$	$0.34^{a}\pm0.01$
Tannins (mg $100g^{-1}$)	$0.09^{c}\pm0.00$	$0.41^{b}\pm0.03$	$0.63^{a}\pm0.03$

CL- control loaf; F1 – loaf with 2.5% kale powder; F2 – loaf with 5% kale powder.
Mean ± standard deviation of triplicate analyses, with five repetitions. Means followed by the same letter do not statistically differ from each other. Tukey's test at 5% probability level was applied.

For copper and manganese, the observed increases were sufficient to classify the loaf containing 2.5% dried kale as a source of manganese and the loaf with 5% as a source of manganese and copper. Copper is an important cofactor of enzymes involved in iron metabolism and may prevent hematologic abnormalities including anemia (GRIFFITH et al., 2009), amino acid metabolism, cholesterol and carbohydrates (MERDIVAN et al., 2004).

As regards iron and proteins, the quantities present in the control bread already met the standards required by legislation to classify the bread as a source of these nutrients. Considering that adding kale promoted increases in these nutrients, the two formulations which received this ingredient can also be classified as sources of iron and protein (BRASIL, 2012).

The addition of the dehydrated kale to loaves of bread also increased the concentration of factors that affect the bioavailability of nutrients. For oxalic acid, the amount obtained (Table 4) was far below the threshold considered a risk to health (2g kg^{-1} body weight) (SAVAGE, 2000), and for phytic acid and tannins no reports were found in the literature.

Conclusion

The addition of dehydrated kale in the formulation of loaves of bread contributed to the improvement of their nutritional value, promoting a significant increase in levels of fiber and minerals; they were notable for being a source of copper and manganese. The loaves constitute an excellent alternative for stimulating the consumption of this vegetable, especially by people in the habit of replacing main meals with snacks.

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