

Effect of Okara Flour Addition on the Physical and Sensory Quality of Wheat Bread

Abstract

This study was carried out to develop bread with improved nutritional quality from a soymilk residue, okara, which is considered a foodstuff low-calorie and rich in fiber. Bread was prepared by substituting wheat flour by okara at 5, 7.5 and 10%. Based on preliminary results, breads made with okara improved many nutritional aspects compare with the control (without okara). Okara increased the total dietary fiber and protein content of bread. A darkening was observed in breads with okara regarding the control sample. Control and 5-0 samples presented a structure of the crumb with a greater number of air cell than the other formulations, inducing a more uniform distribution of them and a more aerated crumb. From sensory evaluation, it could be concluded that bread with 7.5% okara presented a great acceptability. Okara represents an excellent raw material that can be utilized for dietary fiber fortification.

Keywords: Okara; Bread; Functional Food; Dietary fibre

Research Article

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Abbreviations: C: Control sample; 5-O: bread with 5% Okara; 7.5-O: Bread with 7.5% Okara; 10-O: Bread with 10% Okara; DF: Dietary fiber; SEM: Scanning electron microscopy

Introduction

Investigation on utilization of underutilized food resources or edible by-products of food industries is a promising alternative to address the aggravating world food problem [1]. Okara is the solid non-soluble fraction obtained during soymilk manufacturing. About 1.2 kg of fresh okara is produced from 1 kg of soybean processed for tofu [2]. Okara is a natural rich source of dietary fibre and protein, which can be processed to industrial useful forms such as powders, extrudates or pregelatinized powders. The main reason behind the limited use of okara as a food ingredient is its deterioration if not dehydrated or frozen rapidly [3]. It is well known that dietary fiber plays an important role in many physiological processes and in the prevention of diseases of different origin [4]. Furthermore, okara contains much protein and considerable isoflavone and mineral elements, so it possesses high nutritional value and prebiotic effects [2]. Although the presence of biologically active anti-nutritional factors is the main limitation of soya-derived foods, these factors could be eliminated satisfactorily during various pretreatment and processing conditions [1].

During drying okara, one of the most important aspects to be considered is the preservation of the protein quality, which can be affected by the drying conditions [5]. Dietary fiber (DF) is currently considered as a critical ingredient in food products such as baked goods, beverages, meat, confectionery, dairy and pasta. Most frequently, DF are incorporated into bakery products to prolong freshness due to their capacity to retain water [6]. Okara may be considered a good source of dietary fiber for the

high fiber content, low cost and wide resource. Also, there are studies which showed that okara had the functions of preventing diabetes, hyperlipidemia and obesity and present antioxidant activity [2]. Therefore, it could be useful as a functional ingredient with health-promoting attributes. Considering that wheat bread represents a staple food for the majority of the world population and contributes substantially to the intakes of certain nutrients, in the present paper we investigate the optimum level of substitution of okara for wheat flour and examine okara effects on dough texture properties, as well as on quality parameters (volume, hardness and sensory analysis) on the end-product.

Materials and Methods

Raw materials

Okara flour was prepared in the laboratory as described by [7]. Wet okara is later dried off in a Microwave oven using 40% power level (BGH, Argentina) in order to reduce the moisture content between 88-90 %. Other ingredients wheat flour, yeast, salt, water, fatty matter and sugar were purchased in local market.

Bread making procedure

Bread was prepared by substituting okara at 0%, 5%, 7.5 % and 10% levels into wheat flour. Samples were labelled as: control, 5-O, 7.5-O and 10-O respectively. The formula contained salt (1%), yeast (0.3%), water (35%), fatty matter (2.4%) and sugar (2.7%). Firstly, yeast was dissolved in water at $37 \pm 1^\circ\text{C}$. This dispersion was added to the ingredients and then was mixed with a 5-speed mixer (average mixing speed was 100 rpm) (Santini, Argentina) for 30 min. Approximately 500 g of dough was poured into aluminum rectangular moulds (90 cm²). After fermentation (at 30 °C for 75 min), baking was carried out in an air electric oven (BGH, Argentina) at 180 °C for 40 min (convection/fan). After baking,

the breads were removed from the moulds and cooled at room temperature for 30 min. Samples were packed in hermetically-sealed bags (Ziploc Brand) and stored at ambient temperature.

Physicochemical analysis of bread

All the methods had previously been applied to characterize the okara flour [8]. Physicochemical analyses for flour and breads samples were determined in triplicate according to standard replication AOAC methods [9]. Moisture content was determined by gravimetric method (AOAC 925.10), dry matter by weight difference (AOAC 925.23), ash by incineration (AOAC 945.46), protein content by determination of total nitrogen by the Kjeldahl method (model Pro-Nitro S, Selecta, Spain) with a conversion factor of 6.25 (AOAC 991.22), total dietary fiber content (AOAC 985.29), fat content by Soxhlet extraction (model SZC-D, Shanghai QianJian Instrument Co. Ltd, China) (AOAC 945.39), and carbohydrate by difference.

Quality evaluation of bread

To evaluate the effects of okara substitution on bread formulations, the following studies were performed:

Loaf Volume of the bread: Loaf volume was measured by small seeds displacement method [10]. A container was used to measure the volume using small grains. Flax seeds were poured into the container of known volume until the bottom was covered. The loaf was placed inside the container which was then filled to the top with more seeds. The extra flax seeds, which equal the loaf volume, were measured in a graduated cylinder. The specific volume of the loaf was calculated using the following equation:

$$SV \left(\frac{cm^3}{g} \right) = \frac{loaf\ volume\ of\ bread}{weight\ of\ bread} \quad (1)$$

Crust and crumb color of bread (CIELab system): The chromatic characteristics of the bread crust and crumb were determined using a HunterLab colorimeter, model Mini Scan EZ (HunterLab, USA) which was provided with the software. Colour values (L^* , a^* and b^*) for the control and enhanced bread formulations were recorded, each the average of four measurements at different points in the bread crumb and crust to ensure the reproducibility. L^* is the lightness variable from 100 (white) to zero (black), whilst a^* and b^* are chromaticity, +redness/greenness and +yellowness/blueness, respectively [11], [12]. The color of all breads (crust and crumb) was measured 1 h after baking. An average of four measurements for L^* , a^* and b^* values were recorded [13]. Mean values of at least 3 breads were taken for statistical purposes

Image texture analysis: At macroscopic level, two phases can be identified in bread structure: a solid (wall material) and a gaseous (air cell) one, which are partially connected. So, the nature of their connectivity, their size, uniformity and fraction area determines the structure, and consequently, the mechanical properties of the bread. Therefore, raw materials determine the structure of the bread [14]. Slices from the center of each bread were scanned with a digital camera. Analysis was performed independently for each image from a sub-image area of 4x4cm from the center on each bread slice. The images were analyzed with Image-J 1.47v

(USA). The color image was converted to 8-bits image and was analyzed in a grey scale (0 black, 255 white). The image selected was segmented by a grey-value to create a binary image by the algorithm Iso-Data [15]. From these analyses, the total number and mean size of alveolus and the area factor were obtained. Three slices from the center of each bread loaf were analyzed in each case.

Texture profile analysis (TPA): The mechanical properties of bread samples were measured using a Brookfield texture Pro CT analyzer, (USA). A double compression test was performed with a 25% compression with a 38 mm diameter cylindrical probe and a load cell of 25 N at a test-speed of 1 mm/s and 10 mm of distance. Breads were cut into 2 cm thick slices. Tests were conducted after 1 day in the central part of the slice. The parameters obtained from the curves were hardness (N), consistency (N.s) and cohesiveness [16].

Scanning electron microscopy

The microstructure of breads was analyzed by scanning electron microscopy (SEM, LEO1450VP, Zeiss, Germany). The samples were mounted on double-sided adhesive carbon on aluminum sample holders. The micrographics were determined under VP mode (variable pressure), using 200X magnifications. The low vacuum mode of SEM is a special type, where the chamber can be maintained at low vacuum at 70 Pa, while that the column remains under vacuum. In this way, it is possible to observe biologically sensitive samples without dehydrating or metalizing with gold [17].

Sensory analyses of breads

Sensory evaluation tests were performed on bread samples by 80 untrained panel members. The sensory analyses consisted of a comparison test (preference) between the samples which were coded with three-digit numbers and were presented in a randomized order. Apples were provided between samples to cleanse the palate. Then, a statistical analysis was performed using the Turkey-Krumer test, which determines whether the sums of the total orders for each sample differ significantly [18].

Statistical analysis

Results are expressed as means with standard deviations of analysis performed in triplicate. One-way analysis of variance and Tukey's test were used to establish the significance of differences among mean values at $p \leq 0.05$. The statistical analyses were performed using GraphPad InStat Software Inc.

Results and Discussion

Drying of okara

From the drying curves it was possible to estimate the time needed to obtain flours with the characteristics established by CAA [19]. Figure 1 shows the moisture content of okara during the drying process using a microwave oven. Initially the surface of the okara was very humid, proved by the existence of a continue water film. The film was constituted by free water without mass transfer

resistance. The fact that fresh okara has a high moisture level ($\cong 80\%$) facilitated the penetration of electromagnetic radiation through the polar molecules, allowing an almost instantaneous warming in the totality of the sample [8]. Starting at time zero, the free water content corresponds to point A. At the beginning of the drying process, the solid is at a lower temperature than it will be at the end, and the evaporation rate is increasing. At point B, the temperature reaches an average value of $91\text{ }^{\circ}\text{C}$ (near the boiling point) and evaporation begins to be maximum. It is observed in the Figure 1 (a) that the segment BC is a straight line with maximum slope. The drying rate becomes constant; it is represented in the section BC. At point C, in both figures, the drying speed begins to decrease until reaching the point D. The CD segment represents the period of decreasing speed. In this stage, the water was removed. The solid material increased the temperature and the drying rate decreased rapidly tending to become zero, as the final moisture was approached [20].

Physicochemical analysis

The okara quality was evaluated before being incorporated into the bread. The proximate composition of okara flour was determined in the laboratory. The protein content was $34.61 \pm 1.49\%$; fiber $20.37 \pm 1.20\%$; fat $19.10 \pm 4.67\%$; ash $3.44 \pm 0.41\%$; moisture $1.38 \pm 0.14\%$; and total carbohydrates $21.11 \pm 1.58\%$. Our results were in agreement with [21] and [22]. However, it is a common knowledge that the chemical composition of okara will depend on the procedure followed to obtain it. The results of chemical analyses performed on breads are summarized in Table 1. As expected, the protein and fiber content increased with the level of substitution of okara flour. The replacement with okara flour produced a decrease in carbohydrates and an increase in ash content. The moisture content was not significantly affected, however, since protein and fiber has hygroscopic nature, the samples retained more moisture during the baking cycle [23], [24].

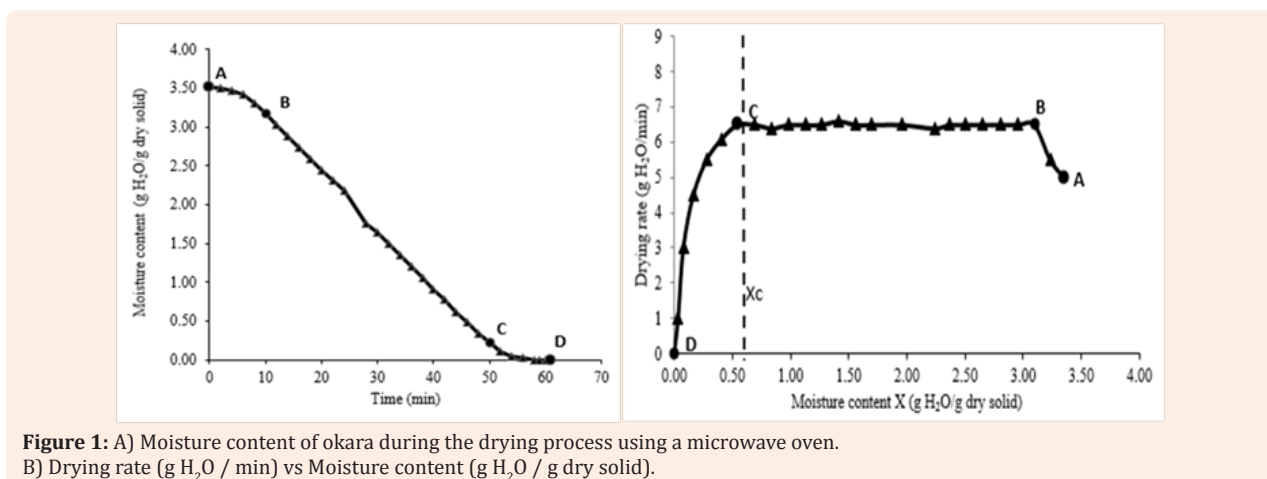


Figure 1: A) Moisture content of okara during the drying process using a microwave oven. B) Drying rate (g H₂O / min) vs Moisture content (g H₂O / g dry solid).

Table 1: Proximate composition of breads with different concentration of okara (O).

Sample	Protein (g/100g)	Fat (g/100g)	Ash (g/100g)	Moisture (g/100g)	Carbohydrates (g/100g)	Fiber (g/100g)
Control	7.45 ± 0.25 ^a	3.95 ± 0.30 ^a	1.35 ± 0.10 ^a	49.35 ± 3.80 ^a	37.89 ± 3.70 ^a	0.012 ± 0.006 ^a
5-O	8.85 ± 0.37 ^b	5.28 ± 0.20 ^b	1.67 ± 0.08 ^a	51.14 ± 5.50 ^a	32.3 ± 5.70 ^{ab}	0.74 ± 0.100 ^b
7.5-O	9.80 ± 0.37 ^c	6.76 ± 0.50 ^c	1.63 ± 0.10 ^a	53.59 ± 3.50 ^a	26.85 ± 4.20 ^{ab}	1.31 ± 0.170 ^c
10-O	9.98 ± 0.25 ^c	6.84 ± 0.60 ^c	1.74 ± 0.09 ^a	54.92 ± 3.10 ^a	24.58 ± 3.80 ^b	1.87 ± 0.200 ^d

Means followed by different letters in the same row are significantly different ($P < 0.05$)

Quality evaluation of bread

Specific volume of breads: The specific volume for the different experimental bread types are shown in Table 2. The specific volume, which is the ratio between the volume and weight, has been adopted in the literature as the most reliable measure of bread [25]. The result shown that, the higher the replacement of okara, the lower the specific volume. This behavior could be due to the dilution of gluten, or to the interaction between gluten

and fiber. This phenomenon could be also a result of the fiber weakening or crippling dough structure and reducing CO₂ gas retention [6]. Moreover, the absence of gluten in okara flours had a great influence on the bread' rheological properties.

Crust and crumb color of breads: The values relating to the color analysis of the crumb and the crust of the experimental bread samples are listed in Table 3. Such analyses have shown considerable differences regarding L*, a* and b* between samples

with and without okara. The results showed that when the color crust and crumb of breads were evaluated across the space CIELAB, darkening was verified due to the incorporation of okara. The increase in color values may be attributed to interaction of protein and sugar at baking temperatures resulting in a higher degree of Maillard reaction. This result indicates a browning of the crust and crumb with the incorporation of okara, demonstrating a marked reddish-dun color and an increased yellowness.

Texture profile analysis: Breads enriched with okara presented greater firmness, cohesion and chewability than control sample. There are different factors that (Table 4) increase the viscosity of the dough such as: dilution of the gluten's matrix, the formation of defects in gluten due to soybean's fiber, the exchange of disulfide bonds between soy's proteins and gluten's proteins [26].

Table 3: Crust and crumb color of breads.

Sample	Crust			Crumb		
	L*	a*	b*	L*	a*	b*
Control	63.08 ± 0.5 ^a	9.53 ± 0.2 ^a	35.96 ± 0.01 ^a	70.09 ± 0.4 ^a	0.81 ± 0.2 ^a	13.95 ± 0.1 ^a
5-0	52.82 ± 1.9 ^b	15.53 ± 0.2 ^{b,d}	27.72 ± 0.06 ^b	67.88 ± 0.5 ^{a,b}	2.07 ± 0.1 ^b	18.8 ± 2.4 ^b
7.5-0	47.65 ± 4.8 ^b	13.39 ± 1.2 ^{c,d}	27.68 ± 0.08 ^b	68.03 ± 1.5 ^a	2.95 ± 0.09 ^c	18.94 ± 1.1 ^b
10-0	46.47 ± 3.5 ^b	14.17 ± 0.6 ^d	27.43 ± 0.03 ^b	64.82 ± 1.8 ^b	3.8 ± 0.2 ^d	20.23 ± 1.0 ^b

Means followed by different letters in the same row are significantly different ($P < 0.05$).

Table 4: Texture profile analysis of breads.

Sample	Firmness (g)	Adherence (mj)	Chewability (mj)	Cohesion (mj)
Control	579 ± 166 ^a	0.02 ± 0.03 ^a	43.8 ± 14 ^a	0.28 ± 0.01 ^a
5-0	686.6 ± 49 ^a	0 ± 0 ^a	49.7 ± 3.5 ^a	0.32 ± 0.01 ^{a,b}
7.5-0	688.7	0 ± 0 ^a	49.8 ± 2.8 ^a	0.36 ± 0.04 ^b
10-0	691.8 ± 64 ^a	0.02 ± 0.03 ^a	50.3 ± 4.5 ^a	0.37 ± 0.03 ^b

Means followed by different letters in the same row are significantly different ($P < 0.05$).

Scanning electron microscopy

At macroscopic level, two phases can be identified in bread structure: a solid (wall material) and a gaseous (air cell) one, which are partially connected. So, the nature of their connectivity, their size, uniformity and fraction area determines the structure, and consequently, the mechanical properties of the bread. Therefore, raw materials determine the structure of the bread [14]. Electron micrographs were made in order to establish the relationships between the microstructure of breads and their composition. In the Figure 2 images obtained for the breads with different concentrations of okara are shown. The micrographs of breads elaborated with okara show a different structure compare with the control sample. This is possibly due to the fibers and proteins present in the okara flour, which might interfere in the structure of the matrix, diminishing the gas retention capacity in the dough. Excess amounts of insoluble dietary fiber had an adverse effect on the formation of gluten network and reduced the quality of bread due to gluten dilution effect or gluten -fiber interaction [6].

Regarding to adherence, samples did not showed statistically significant difference. In general, the incorporation of saccharides and proteins in bread formulations increased the hardness of the samples [12]. Results were similar to values reported by [27].

Table 2: Specific Volume (SV) of breads.

Sample	SV (cm ³ /g)
Control	2.95 ± 0.98 ^a
5-0	2.78 ± 0.77 ^a
7.5-0	2.52 ± 0.27 ^a
10-0	2.06 ± 0.68 ^a

Means followed by different letters in the same row are significantly different ($P < 0.05$).

Image texture analysis : Image analysis was performed on each scanned bread slice to provide a more detailed view of the bread texture. It can be observed in Table 5 that the number of air cell in the range of sizes of 0.5-4 mm² (average size 1.4 mm²) decreases when increase the concentration of okara. Control and 5-0 samples presented a structure of the crumb with a greater number of air cell than the other formulations, inducing a more uniform distribution of them and a more aerated crumb. In the range of 4-100 mm² (average size 13 mm²) the number of air cell increases with the content of okara and the shape factor value approaches to 0, it indicate an increasingly elongated form, which represent a collapse in the structure of the crumb with the consequent compaction of it.

Sensory characteristics of breads enriched with okara

The effects of okara addition on the sensory characteristics of breads are presented in Figure 3. The sensory analysis was carried out with the 3 formulations of bread enriched with okara,

in order to determine sensory attributes such as: color, manual texture, taste and overall preference. It was concluded from the multiple comparisons test that the color of the 5-0 and the manual texture of 7.5-0 were statistically preferred. While flavor

and overall preference attributes did not present significant difference in sample ordering. It is important to highlight that all the formulations were accepted by the panelists, and the bean flavor was not perceived by them.

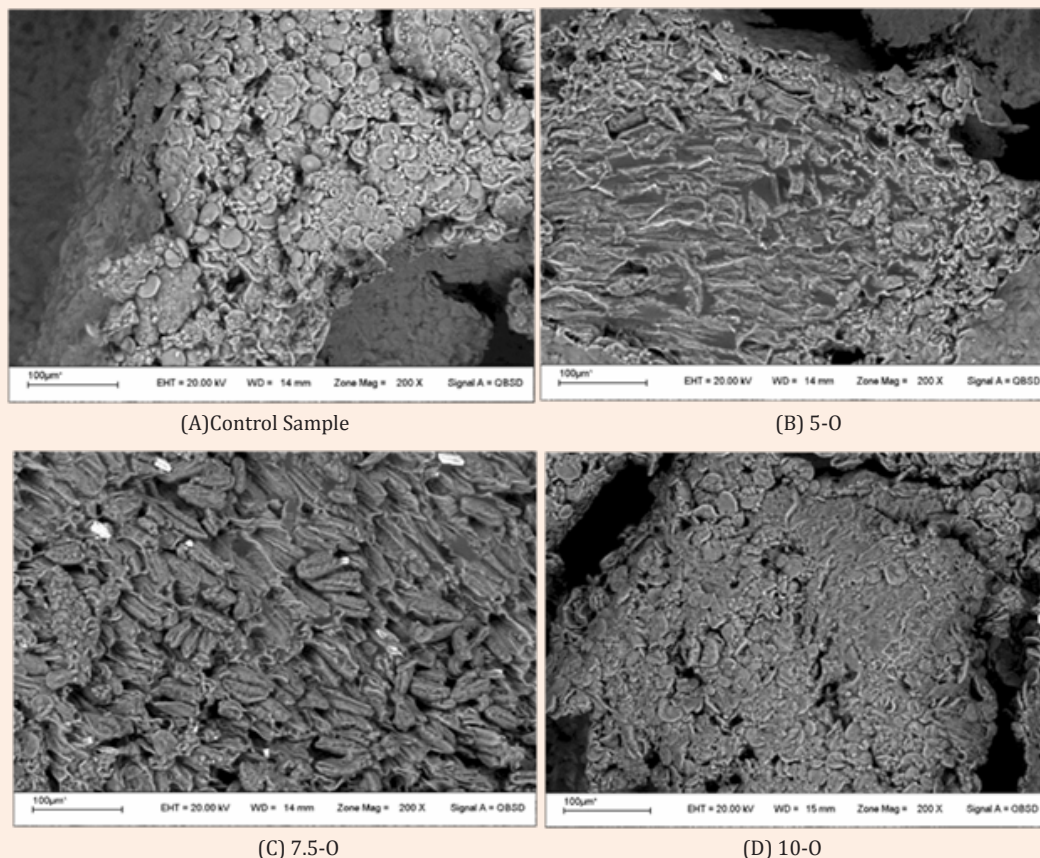


Figure 2: Scanning electron microscopy (SEM) of bread matrix. Magnification: 200x.

- A) Control.
- B) 5-0.
- C) 7.5-0.
- D) 10-0.

Table 5: Number of air cell in different ranges and shape factor.

	N° air cell (N)		Mean size of air cell		Shape factor	
	0,5 - 4 mm ²	4 - 100 mm ²	0,5 - 4 mm ²	4 - 100 mm ²	0,5 - 4 mm ²	4 - 100 mm ²
Control	44	11	1.436	12.825	0.202	0.093
5-0	41	12	1.315	13.655	0.197	0.083
7.5-0	39	13	1.311	12.489	0.212	0.08
10-0	34	16	1.529	13.052	0.208	0.064

Means followed by different letters in the same row are significantly different (P < 0.05).

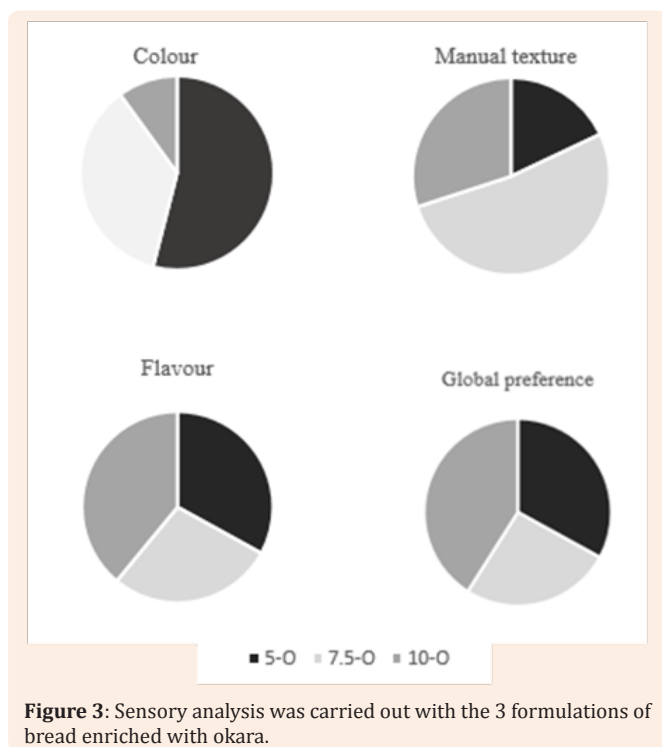


Figure 3: Sensory analysis was carried out with the 3 formulations of bread enriched with okara.

Conclusion

Okara is a rich source of nutrients that could be utilized in bakery industry. The results obtained in this study indicated that the combination of wheat flour and okara flour allowed the development of bread with enhanced functional properties. Fresh okara was dried by microwave. It was possible to obtain a dehydrated product with 0.5% moisture content in 61 minutes. The physicochemical analyses revealed that the principal nutritional advantage of bread samples elaborated with okara flour and wheat flour is its high fiber and protein content. According to the physical properties, the incorporation of okara in the formula reduced the size of the product, giving rise to harder and darker breads. Also, an increase in okara content decreased the specific volume of bread, this phenomenon could be a result of the fiber weakening or crippling dough structure and reducing CO₂ gas retention. Regarding to the structure of the crumb: the quantity of alveoli increases when okara content is higher and the elongated forms predominate, as consequence, a more compact crumb was obtained, as was corroborated by electronic microscopy. It was observed that bread enriched with okara presented higher firmness, cohesion and chewability compare with control sample. However, the samples exhibited great acceptable sensory characteristics among consumer panel members. Regarding to flavor and global preference attributes samples did not present significant difference. It is important to highlight that all the formulations were accepted by the panelists, and bean flavor was not perceived. Okara could be utilized to improve the nutritional quality of breads. The okara substitution in bakery industry would be beneficial in reducing the usage of wheat flour and also

overcome the waste disposal problem prevailing in the soymilk industries.

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Conflicts of Interest

None of the conflicts.

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