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Influence of the Particle Sizeand Level of Substitution of Okara Flour in the Development of Gluten-Free Cookies

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Abstract- The research study was conducted to obtain okara flour by freeze-drying which could be used as a fiber and protein supplemented in foodformulation. The chemical composition, functional properties and the influence of particle size of okara flour were studied. Four formulations of gluten-free cookies were developed: one using manioc flour as control and three other with different proportions of okara: 15%; 30%; 50% (w/w). Physical properties as spread factor, color, pore area, shape factor, pore diameter and texture were measured. The overall results showed that okara and manioc flour have the potential to be used in the preparation of cookies with acceptable quality and improved nutrition value.Results from physical analyses evaluation indicate that the incorporation of okara at 30% without milling (30%OF) process presents a similar shape factor to the control sample, a percentage of pore area and pore diameter improved, that provides good textural properties. Therefore, the substitution of manioc flour by okara freeze-dried at 30% provides good quality to a gluten-free cookie formulation, whit better properties that the control sample.

Index Terms-okara, freeze drying, gluten-free cookies.

1. INTRODUCTION

Celiac disease is a well known type of food intolerance specifically associated with gluten and other prolamins in wheat, barley, rye, and other cereals. The only known effective treatment is a lifelong, strict glutenfree diet [1].

Gluten is the main structure-forming protein in flour, because resulting dough presents high elastic characteristics and contributes to the appearance and crumb structure of many baked products. Nevertheless, when pathologies require the removal of gluten from the diet, the replacement of gluten presents a major technological challenge [2].

An alternative source of suitable nutrients for celiac sufferers is found in soybean and its derivatives. Okarais a byproduct of the production of soybean foods such as tofu and soy milk. It is a nutrient-rich product, containing about 25% protein, 20% fat and 33% dietary fiber on a dry basis. [3]. It corresponds to the insoluble fraction that remains in the filter, when the grain of soybean has undergone a hydro-thermal treatment of crushing, grinding and leaking. It is a material of white yellowish color and soft flavor [4].Okara is usually discarded as industrial waste because it is perishable and other uses for it have not been identified, which has created social and environmental problems.

The use of by-products in the food industry has drastically increased in recent years since not only increases the economic value of raw material but also calls for alternative food source and zero-waste strategy with an increasing world population [5]. Fresh okara deteriorates rapidly due to its high moisture content, so it has to be driedas soon as possible under the appropriate conditions in order to maintain its integrity and allow its use as flour [6].

Okara could be a potential raw material for cookie making with improved bioactive value, since food fibers are recognized as important components of food diets, exerting different positive physiological effects on human health.

Cookies represent baked products containing three major ingredients: flour, sugar and fat, which are mixed together with other minor ingredients to form cookie dough [7]. The main attributes that affect cookie quality are texture, flavour and appearance [8]. Variants of gluten-free rice bread, made with rice flour instead of wheat flour, have been developed, but usually require the addition of thickeners such as xanthan gum, guar gum, or hydroxypropyl methylcellulose to trap the CO2 released by the yeast in the batter [9].

Due to the possibility of introducing okara as substitutes for manioc flour in the elaboration of cookies in order to attend groups of people with special needs (celiac), the present study aims at evaluating the effect of okara on the spread factor, instrumental color parameters, and texture properties. In addition, the comparison with a control sample formulated with manioc flour was carried out. Thus, the objective of this study is to investigate the freeze-drying technique as technology suitable to obtain a flour of okara that could be used for human supply. Additionally, we evaluated how the particle size of okara flour affects the physical properties of cookies elaborated as an alternative gluten-free product.

2. MATERIALS AND METHODS

2.1. *Raw materials*

The ingredients (manioc flour, eggs, butter, xantic gum + guar gum, sugar, vanilla essence, baking powder) were purchased in local markets. Okara (O) flour was prepared in the laboratory.

2.2. *Okara preparation and freeze drying stages*

Soybean was soaked in water for 8-10 h at ambient temperature. It was ground in blender (SIAM LIC07) incorporating water at 100±1ºC to enhance the grinding. The ratio of water to beans is usually between 8:1 to 10:1 [4]. A thermal treatment was applied during 20 minutes at a temperature superior of 90±1 ºC to reduce the activity of the trypsin inhibitor and deactivate the lipoxygenase enzyme that provokes the unpleasant taste. Milk was separated from ground soybean slurry using a filter. Fresh okara was placed on stainless steel tray, frozen in a freezer at –40°C and freeze-dried using a lyophilizer (Rificor S.A., Argentina) for 48 h. The temperature of the sample was measured by a temperature sensor and the weight loss was monitored using an analytical balance in order to obtain information at different drying times.

2.3. *Physicochemical analysis*

Physicochemical analyses of okara flour, were determined according to standard replication methods[10],as follows: protein content by establishing total nitrogen by the Kjeldahl method using a Digestion Blocks and a semiautomatic Distiller (Selecta, Spain) with a conversion factor of 6.25 (AOAC 991.22); fat content by Soxhlet extraction (AOAC 945.39); moisture content by gravimetric method (AOAC 925.10), dry matter by weight difference (AOAC 925.23); ash by incineration (AOAC 945.46); and carbohydrate was determined by difference. All determinations were performed in triplicate.

2.4 Functional characterization of okara flour

Functional properties of food proteins are important in food processing and for food product formulation [11]. The functional properties such as water holding capacity (WHC), water absorption capacity (WAC) and oil binding capacity (OBC) were determined using the methods described by [12] with some modifications.

2.4.1 Water holding capacity: One gram of sample (wo) was weighed in a test tube and 30 ml of water was added. It was stirred and hydrated for 18 hours. Then, it was centrifuged at 3000 rpm during 30 minutes. The supernatant was separated and the residue was transferred to porcelain crucibles. It was weighed obtaining the value of humid residue (HR). After that, the residue was dried at 105 ± 1°C for 24 hours and was weighed with the purpose of obtaining the value of dry residue (RS). The following equation was applied:

$$WHC=(HR-RS)/RS \qquad Eq.(1)$$

2.4.2 Water absorption capacity: It expresses the maximum quantity of water that can be retained by gram of dry material in presence of a water excess under the action of a force. In a test tube, 0.5 grams of flour sample (w_o) were weighed, a water excess was added (10 ml). The slurry was shaken during 30 minutes and centrifuged at a rate of 3000 rpm for 10 minutes. The supernatant was decanted and discarded and the sediment was weighed (Ws). The results were expressed as grams of water per grams of sample.

$$WAC = (W_S - w_o) / w_o \qquad Eq.(2)$$

2.4.3Oil binding capacity: The oil binding capacity was determined using the method of [11]. A gram of the flour sample (w_0) was weighed inside a test tube and it was mixed by 10 ml of vegetable oil (V_1) using an agitator. The samples were left to rest during 30 min and then were centrifuged to 20000 rpm during 25 min. Immediately after centrifugation, the supernatant was carefully poured in a graduated cylinder of 10 ml and the volume was registered (V_2). The OBC (milliliters of oil per gram of product) was calculated as:

$$OBC = (V_1-V_2)/w_0 \qquad Eq.(3)$$

2.5 Optical Microscopy studies

The surface structure of the different samples of okara was observed with an optical microscopy (Digital Usb Microscope BW1008-500X). The samples were viewed at 60X magnification, with the purpose of determining if the different processes of drying had affected the microstructure of the okara and, consequently, its characteristics.

2.6 Cookie preparation

Four formulations were developed: one with manioc flour (without okara) as control (C) and three other with different proportions of okara and manioc flour: 50% (50-O); 30% (30-O); 15% (15-O). Cookies were baked at 180ºC for 10 min. Once baked, the cookies were cooled up to room temperature. Finally, they were packed in polypropylene bags.

2.7 Physical analysis

Different parameters that can be easily assessed at the end of the baking process include physical dimensions such as cookie length, width and height, along with measures of color. These values are essential for maintaining product quality [13].

2.7.1 *Cookie dimensions:* Diameter and thickness were measured with a vernier calliper at three different places in each cookie and the average was calculated for each. The spread ratio (SR) was calculated using the Eq.(4) [14], assigning the best quality to those samples that obtained a higher absolute value [15].

SR= (Diameter of cookies)/(height of cookies) Eq.(4)

2.7.2 Analysis of surface color: The color values of the cookies were measured in three different zones of the crust using a digital spectrophotometer Mini Scan EZ (Hunterlab, USA) which was provided with the software. A chronometer was calibrated with the standard black and white color. The results reported are averages of three measurements in each sample using CIELAB L*, a*, b* values. L* value is the lightness variable from 100 for perfect white to zero for black, while a* and b* values are the chromaticity values, +redness/ -greenness and +yellowness/-blueness, respectively [11].

2.7.3Texture measurements: The mechanical properties of cookies with added freeze-dried okara samples were measured using a TMS-TOUCH texture analyzer (Food Technology Corporation, USA) with a probe attached to an extension bar and a 500 N load cell and a platform. A double compression test was performed with a 25% compression with a 38 mm diameter cylindrical probe and a load cell of 500N at a pre-speed of 30 mm/min a test-speed of 70 mm/min and a post-test of 100 mm/min. The parameters obtained from the curves were hardness (N), consistency (N.s) and cohesiveness.

*2.7.4Microstructure analysis:*A digital image analysis (DIA) system was used to analyze the cookies. For alveolus diameter measurements, the muffins were cut on a horizontal plane and images of cut surface were acquired with a digital camera. Eight replicates of each formulation tested were analyzed. The images were analyzed with Image-Pro Plus 6.0 (Media Cybernetics Inc, Bethesda, USA) and the statistical analysis performed with GraphPadInStat. From these analyses, the percentage of aeration area (mm), pore diametric (µm), mean shape factor were obtained.

The pore diameter measurements were performed manually with the software Image-Pro Plus 6.0 in 8 replicas and were made between 80-100 measurements per image. The pore was considered whit an oval shape, which has two diameters larger one than the other. Para cada poro se realizaron dos mediciones: diametro mayor y diametro menor de la circunferencia. Besides, for the analysis the images were color based segmented in two zones: (i) the zone with alveolus or bubbles (white) and (ii) the zone without alveolus (black). The bright or white objects were measured and calculated de pore area, pore diameter for each pore and the total area. For area percentage of aeration the results were expressed as the percentage of area total of alveolus with respect to the total area. From the experimental data of pore diameter for each pore obtained with the software the shape factor was calculated as:

Shape factor=(Lower pore diameter)/(Bigger pore diameter) Eq. (5)

A perfect circle has shape factor of 1.0 and a line has a shape factor of 0.0.

2.8Statistical 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 GraphPadInStat Software Inc.[16]

3. RESULTS AND DISCUSSION

3.1 Physicochemical characterization of okara flour

The quality of okara was evaluated before being incorporated in the cookies. The protein content was 34.61±1.49%; fiber 20.37±1.20%; fat 19.10±4.67%; ash 3.44±0.41%; moisture 1.38±0.14%; total carbohydrates 21.11±1.58%. Similar results were reported[17]; [18]. However, it is common knowledge that the chemical composition of okara depends on the procedure followed to obtain it.

3.2 Microstructure and functional properties of okara flour

The study of optical microscopy revealed that okara flour (OF) without milling process presents a porous structure with formation of thin capillaries (Fig. 1).

The structure of OF provides better functional properties compared to the grounded okara flour (GOF), (**Error! Reference source not**

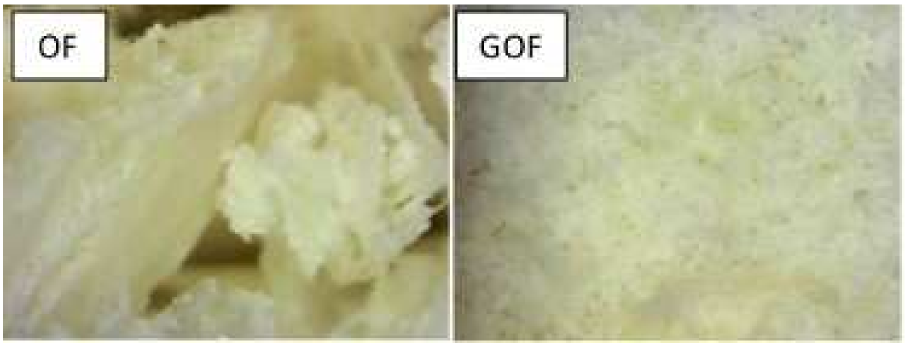

Fig.1: Photographs taken with an optical digital microscope of OF and GOF samples.

found.). The values obtained of water holding capacity (WHC) for the sample OF were similar to the functional characterization performed by [19]. OF presented a higher water absorption than OFG.Previous studies demonstrated that milling process generated damage to starch granules, affecting the water absorption properties of the flour [20]. Water absorption index is an important processing parameter and has implications for viscosity. It is also essential in bulking and consistency of products, as well as in baking application. Oil absorption index was important since oil acts as flavor retainer and increases the mouth feel of foods, improvement of palatability and extension of shelf life particularly in bakery or meat products where fat absorptions are desired [21].

Table 1: Functional properties of flour samples

Property	OF	GOF
WAC	7.55±0.40[a]	5.69±0.44[b]
WHC	7.34±0.17[a]	5.57±0.16[b]
OBC	4.76±0.67[a]	6.02±0.20[b]

3.3 Moisture content of cookies

Data on the moisture content of cookies are presented in **Error! Reference source not found.**. A higher moisture content was observed in those samples in which the okara was not grounded as compared to samples with the same percentage of okara. This could be associated with the porous structure previously mentioned. All samples presented a moisture content under the maximum limit of 12% established by [22]. Cookies without grounded flour presented a higher moisture content due to its better water-holding capacity of okara flour. All samples with the incorporation of grounded okara flour presented less moisture content withrespect to those samples with the same substitution of okara.

Table 2: Moisture content of gluten-free cookies.

Sample	Moisture content (%)
C	8.71±0.71[a]
15% OF	8.28±2.30[a]
15% GOF	7.33±1.88[a]
30% OF	7.61±0.28[a,b]
30% GOF	6.67±0.49[a,c]
50% OF	7.96±0.18[a,b]
50% GOF	7.01±0.11[a,c]

The moisture content was found to be the highest in control cookie. The results showed that the moisture content gradually decreased with the increase in okara flour. This may be explained for the amount of total dry solid in the okara flour with high emulsifying properties compared to manioc flour. Similar results were found for soy flour [23].

3.4 Physical properties of cookies

3.4.1 Spread ratio and analysis of surface color

Results of the physical characteristics (spread factor and color) of cookies are given in Table 3.

The spread ratio (SR) is an important characteristic for determining the quality of cookies. Cookies with higher spread ratios are considered to be the most desirable. An increase in okara content provoked an increase in the spread ratio of the cookies, which is directly related with the height of the cookies, whereas the diameter was generally not affected. The use of grounded okara flour decreased the SR of cookies (Table 3). The flour whit a milling treatment contain a higher level of damage starch than coarse flour. Higher levels of damaged starch generated an increase dough viscosity, which lead to smaller cookies[20]. Previous studies reported that the increasing levels of wheat bran and rice flour decreased the spread factor, however the use of whole flour increase the spread factor [24]. Recent studies found that the milling process significantly affect the flour capacity for make cookies [25]. The differences in the spread factor might be due to altered water absorption capacity of each particular flour (Table 1), [20].

Table 3: Spread factor and color of cookies formulated with okara flour with and without milling process.

The surface color of a baked products is, together with texture and taste, a very important element for the initial acceptability of baked goods by consumers [20]. The increase in color values may be attributed to the interaction of protein and sugar at baking temperatures, resulting in a higher degree of Maillard reaction [26]. The color surface of cookies was evaluated across the space CIELAB, and darkening was verified due to the incorporation of okara. If L* values are compared, statistically significant differences were found among all samples, samples formulated with grounded okara flour present a major lightness compared with those samples with the same okara substitution. When comparing a* and b*, with the replacement of okara, an increase in the values was observed when grounded okara flour was used (*P* <0.05). This result indicates a browning of the crust with the incorporation of okara, demonstrating a marked reddish-dun color and an increase in yellowness. The protein content was negatively correlated with lightness of a cookie, indicating that the Maillard reaction played a major role in color formation.[27].

Sample	Spread ratio	Color		
		L*	***a****	***b****
C	3.1±0.1^{a}	74.75±0.84^{a}	8.99±0.12^{a}	20.52±0.54^{a}
15% OF	4.8±0.3^{b}	85.49±0.34^{c}	1.73±0.42^{b}	13.75±1.09^{b}
15% GOF	5.2±0.1^{b}	79.69±0.44^{b}	6.27±0.30^{a}	22.27±0.09^{a}
30% OF	6.4±0.1^{c}	81.59±1.89^{b}	2.27±1.20^{b}	13.72±1.40^{b}
30% GOF	5.9±0.1^{d}	80.72±1.60^{b}	4.62±0.09^{b}	16.77±0.06^{c}
50% OF	7.0±0.2^{e}	81.01±0.29^{b}	2.59±0.80^{b}	18.95±0.09^{c}
50% GOF	6.9±0.5^{c,e}	79.30±0.42^{b}	3.03±0.62^{b}	23.18±1.02^{a}

Table 4. Effect of freeze-dried okara with and without milling process on texture parameters (hardness, consistency and cohesiveness) in cookies gluten-free. CS: control sample; OF: freeze-dried okara; OFG: grounded freeze-dried okara.

Sample	Time (days)	Hardness (N)	Consistency (N.s)	Cohesiveness
C	0	22.55 ± 1.98	10.00 ± 0.48	1.35 ± 0.17
	7	29.75 ± 6.32	14.57 ± 3.56	1.17 ± 0.20
	14	41.66 ± 10.72	18.48 ± 3.94	1.08 ± 0.10
	20	131.37 ± 24.26	50.01 ± 17.26	1.11 ± 0.20
15%OF	0	93.03 ± 22.08	17.63 ± 3.20	1.35 ± 0.18
	7	107.30 ± 12.22	43.60 ± 2.03	1.28 ± 0.23
	14	82.00 ± 4.90	36.15 ± 2.43	1.26 ± 0.06
	20	204.68 ± 32.00	83.11 ± 10.00	1.06 ± 0.08
15%GOF	0	60.66 ± 17.69	18.79 ± 0.43	1.57 ± 0.15
	7	74.38 ± 5.71	30.04 ± 3.32	1.64 ± 0.14
	14	93.95 ± 16.38	38.10 ± 4.18	1.36 ± 0.09
	20	165.28 ± 39.72	74.10 ± 9.50	1.16 ± 0.14
30%OF	0	38.21 ± 7.04	16.39 ± 1.82	2.07 ± 0.46
	7	81.23 ± 15.36	30.38 ± 3.42	1.92 ± 0.31
	14	86.29 ± 39.54	38.92 ± 16.06	1.67 ± 0.10
	20	179.82 ± 28.92	69.67 ± 20.44	2.21 ± 0.36
30%GOF	0	219.53 ± 45.07	94.58 ± 22.45	2.60 ± 0.77
	7	196.27 ± 27.47	84.72 ± 9.61	1.58 ± 0.16
	14	83.36 ± 16.67	30.10 ± 7.31	1.53 ± 0.11
	20	121.14 ± 22.58	42.22 ± 11.2	1.78 ± 0.37
50%OF	0	108.74 ± 13.89	41.73 ± 3.96	2.03 ± 0.12
	7	192.92 ± 53.42	92.08 ± 6.97	2.03 ± 0.10
	14	129.66 ± 5.81	49.00 ± 0.90	2.06 ± 0.11
	20	86.90 ± 31.50	32.30 ± 13.17	1.90 ± 0.70
50%GOF	0	225.00 ± 35.02	102.00 ± 13.52	1.94 ± 0.27
	7	192.06 ± 47.82	70.25 ± 20.56	1.64 ± 0.05
	14	90.44 ± 38.26	34.26 ± 7.69	1.59 ± 0.30
	20	45.11 ± 2.91	21.34 ± 1.06	1.60 ± 0.46

3.5.2Texture measurements

The hardness and consistency increased with the increase of okara content. Freeze-dried okara had a high fiber and protein content (Table 4), hence these component contributed in a sticky dough and thus reducing extensibility of dough, obtaining a harder cookie [28]; [29]. Similar results were found for cookies formulated with fresh okara and soy flour, in which the addition of soy flour caused an increase in firmness [29]. Recent studies reported that addition of fiber at 30% (w/w) to biscuit resulted in harder texture[30]. Also, has been reported that increasing the amount of rolled oats raised the hardness of cookies [31]. The increased of substitution grade of Buriti endocarp flour in gluten-free cookies as source of dietary fiber generated a harder and smaller sample than the control cookie [32].

The sample 30%OF presented a particular behavior. The initial hardness of the sample 30%OF did not present a significant difference with the initial values of C (P>0.05) and was much lower than 30%GOF (P<0.001), (Table 4). These results are in agreement with their improved microstructure that presented a higher percentage of aeration area, pore diameter and shape factor (Table 4). The higher hardness of 30%GOF may be due to the lower moisture content (≅ 12%, w/w). The sample 30%OF presented an initial higher staling rate due to a higher hardness at seven days of storage. Nevertheless, during the rest of the storage this sample did not present a significant difference in hardness with the C (P>0.05), (Table 4).

No statistically significant difference was observed among 30%OF, 15%OF and 15%GOF during the storage period (P>0.05). The sample 30%GOF presented a high initial hardness and then diminished during the storage period, reducing the integrity and resistance of the sample to a force of compression. This phenomenon could be related to a higher staling due to the lower moisture content, since higher water content delays the staling processes of baked products [33]. Water works embedding themselves between the chains of polymers, reducing the force of attraction between them and, thus, being the final product softer, influencing in the shelf-file of the product. Staling is a phenomenon that describes the deterioration of product quality, and is associated with the increases of the hardness during storage. The staling or retrogradation including gelation and recrystallization of starch granules[33].

No statistically significant difference was observed for the parameters hardness and consistency between 15% OF and 15% GOF during most of the storage period. Sample 15% OF presented higher values of harness and consistency in day seven, demonstrating a higher initial staling rate. However, the consistency between day fourteen and twenty of analysis was similar between in both samples (P>0.05).

The initial consistency of the sample 30%OF did not present a significant difference with the initial values of 15%OF and 15%GOF and was similar to C (P>0.05). However, the sample 30%GOF presented a higher initial consistency than 30%OF, 15%OF, 15%GOF and CS (P<0.001). From day 7 of storage, the consistency of 30%GOF diminished during storage time due to the higher fracturability and lower integrity of the sample matrix. This may be due to their lower moisture content that increase the initial values of consistency and accelerates the staling processes of the product. Sample 30%OF did not present different in the consistency whit the control sample during de storage (between 14 and 20 days), (P>0.05). Previous studies performed related the lower consistency with the higher symmetry of the air cells [34]. This is in agreement with our previous results, where the sample 30%OF presented lower values of consistency and higher values of shape factor similar to the C.

The initial hardness and consistency of the sample 50%OF was lower than 50%GOF, this may result from the lower moisture content, (P<0.001), (Table 4). Similar results were found in cookies formulated from fresh okara, which the increase in hardness was linearly related to decreasing moisture content [29]. The hardness and consistency of 50%OF increased with time until seven days of storage, and started to diminish, the product became easily breakable, and the final texture of the cookies turned scratchy and brittle. The increase of rigidity and susceptibility to fracture related to the staleness of the products[33].

The cohesiveness of the samples raised with the increase of okara content between 15 and 30% and with the process of okara grinding (P<0.05), which may increase the interparticle contact surface, rising their interactions, obtaining a more cohesive matrix. Additionally, the cookies formulated with unground okara were not so homogeneous and the final texture of the cookies became less cohesive because of the differences between the particle size of flour and freeze-dried okara[35]. However, when okara content is higher than 30% (w,w), (50%OF and 50%GOF), cohesiveness decreases. This reduction can be due to the impeding of the intermolecular interaction among ingredients [36]. Additionally, all samples show a decreased of cohesiveness during studied storage time. Accordingly, the increased hardness can be explained by the increase of cohesiveness, as well as an increase in dough consistency due to decreases of free water [29].

3.5.3Microstructure of cookies

During baking the air bubbles are entrapped within the batter expand due to the temperature increase, due to the starch gelatinization and protein denaturation that conferring the cake structure [37].

Fig.2 show the microstructure images of the samples with added freeze-dried okara with and without a process of grounded. Two phases can be a solid (wall material) and a gaseous (air cell) one, which their size, and fraction area determines the structure, and consequently, the mechanical properties of the samples[33]

The incorporation of freeze-dried okara and the process of grounded affects the the microstructure of cookie as was observed in Fig. 2. The incorporation of okara did not affect the percentage of aeration area (P>0.05), except for the sample with okara at 30% without grounded (30%OF), which generated a particular behavior, because increases the percentage of aeration area from 21.63 ± 3.49% (C) to 56.52 ± 3.62% (P<0.001). The incorporation okara that contains hydrocolloids like fiber and proteins stabilizing air cells in the dough, and preventing cell coalescence during cooking, obtained higher size gas cells and aeration area[38].

Table 5 shows that the pore diameter decreased with the incorporation of okara in the samples 15%OF, 30%GOF, 50%OF and 50%GOF, and did not was found differences between C and 15%GOF (P>0.05).

However, the formulation 30%OF raised the pore diameter from 166.7 ± 14.5 µm (C) to 336.9± 36.2 µm. This effect could be related to batter viscosity, since the batter viscosity must be adequate in order to retain the air incorporated during mixing and the air produced during baking [39]. Previous results show that a small increase of fibre percentages increase the batter viscosity of cakes, which could help to retain gases. However, a high increase of fiber percentage generated a high batter viscosity, which could impede expansion and diminish the air retention and final cake volume [34].

Table 5. Area percentage of the air cells, pore diameter, shape factor of gluten-free cookies for the control and cookies with freeze-dried okara with and without milling process at different concentrations. C:

control sample; OF: freeze-dried okara; GOF: grounded freeze-dried okara.

Sample	Area of the air cells (%)	Pore diameter (um)	Shape factor
C	21.63 ± 3.49[a]	166.7 ± 14.5[a]	0.83 ± 0.01[a]
15%OF	28.54 ± 7.64[a]	125.0 ± 13.9[b]	0.71 ± 0.04[b]
15%GOF	27.52 ± 4.61[a]	164.6 ± 13.7[a]	0.73 ± 0.01[b]
30%OF	56.52 ± 3.62[b]	336.9 ± 36.2[c]	0.80 ± 0.02[a]
30%GOF	26.90 ± 4.63[a]	107.8 ± 16.6[b]	0.69 ± 0.05[b]
50%OF	26.15 ± 2.26[a]	120.6 ± 9.3[b]	0.70 ± 0.02[b]
50%GOF	25.79 ± 3.00[a]	109.1 ± 8.5[b]	0.54 ± 0.02[c]

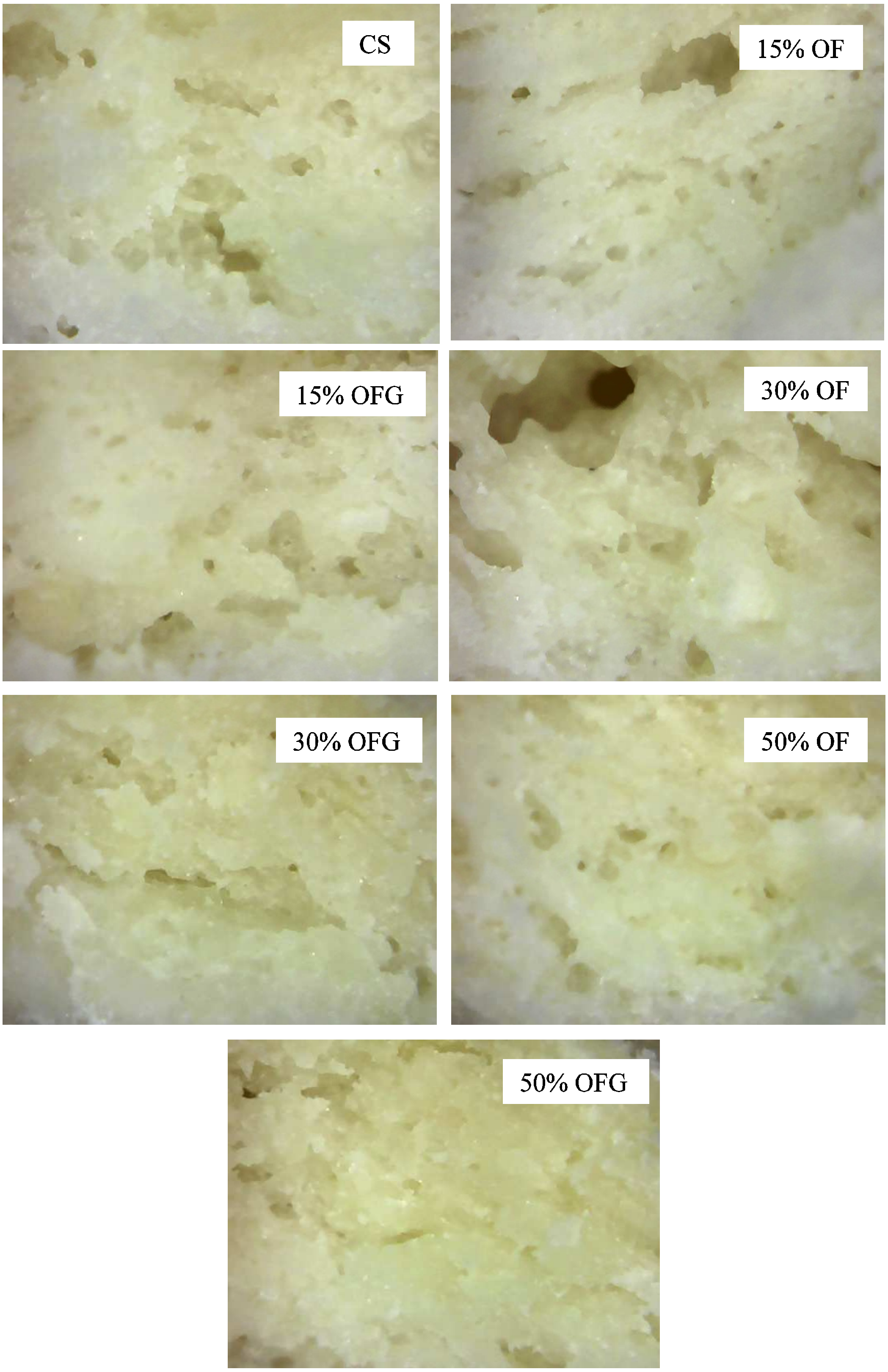

Fig. 2: Microscopy of gluten-free cookies with with freeze-dried okara with and without milling process at different concentrations. C: control sample; OF: freeze-dried okara; GOF: grounded freeze-dried okara. Magnifications 50x.

The shape factor indicates the symmetry of the air cells or pores, that's affect the texture of the cookies [34]. The cookies with freeze-dried okara generate a decreased of shape factor, except for the sample 30%OF, that statistically increased the value. Besides, this sample did not showed statistically significant differences with the control sample (P>0.05).

The incorporation of 30%OF helps to the properly expansion of air bubbles during baking and improves microstructure, porosity and volume increase. Resulted structure was more aerated and voluminous. It aids in retention of moisture, prolongs freshness and promotes good crust color. The improved microstructure can be due to the fiber at this concentration confers some strength to the network during thermal treatment that counteracted the collapse [36].

4. CONCLUSIONS

Okara flour is a rich source of fiber dietary and protein content. It can be used in food formulation in order to increase their nutritional value. OF and GOF samples presented moisture below 15%. The principal nutritional advantage of okara is its high fiber and protein content.

The samples presented similar functional characteristics. Nevertheless, OF sample presented a major capacity of water absorption due, probably, to its porous structure which leaves a higher availability of proteins. It was also observed that OF presented a better capacity of water retention due to the fact that its structure in capillary shape increases its affinity for water.

According to the physical properties of cookies, the incorporation of protein in the formula reduced the size of the product (thickness and width). Samples formulated with grounded okara flour present a major lightness compared with those samples with the same okara substitution. A higher moisture content was observed in those cookies samples in which the okara was not grounded. This could be associated with the porous structure previously mentioned for OF sample.

The results indicated that the incorporation of freeze-dried okara at 30% without a milling process allows to obtain cookies with very similar physical characteristics to the control sample with a higher aeration area and symmetry and a similar factor shape and textural parameters.

This study have demonstrated the potential of okara flour in the gluten-free cookie production.

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