Research Article

SCI

Received: 19 May 2016 Revised: 23 September 2016 Accepted article published: 9 November 2016 Published online in Wiley Online Library: 14 December 2016

(wileyonlinelibrary.com) DOI 10.1002/jsfa.8131

Quality assessment of dried okara as a source of production of gluten-free flour

María V Ostermann-Porcel, Ana N Rinaldoni, Laura T Rodriguez-Furlán and Mercedes E Campderrós*

Abstract

BACKGROUND: Okara is a by-product of soymilk and of tofu elaboration that is rich in protein, fiber and vegetable oils as a source of gluten-free flour. In order to take advantage of the nutritional characteristics of okara and to be able to determine an appropriate drying methodology, microwave, rotary dryer and freeze-drying were assessed. Furthermore, flour with an enzymatic treatment was characterized as well as its functional, physicochemical, and textural properties.

RESULTS: The results showed that the physiochemical characteristics of the flour were affected by the drying process, reaching adequate water content, and high protein and fiber content. The freeze-drying process produced clearer flours with porous structure and high water absorption capacity, and with a higher protein denaturation. Okara dried by microwave and rotary dryer exhibited a denser structure with similar functional properties and improved textural characteristics such as firmness and consistency. The microwave-produced flour was darker due to the non-enzymatic browning reactions. The enzymatic treatment employed improved the consistency of the flour.

CONCLUSION: It was possible to choose the drying process to be applied according to the feasible use of the flour, intended to preserve the favorable nutritional aspects of the okara flour. Based on the results, it can be affirmed that the physicochemical properties and attributes of okara are influenced by the drying process employed. Okara dried by freeze-drying resulted in a better product because it had a low final moisture content and the highest whiteness index. The flour presented a porous structure with high solubility, which is an indicator of potential applications in foods developments.

Keywords: drying processes; enzymatic treatment; dietary fiber

INTRODUCTION

Celiac disease is caused by the uptake of gluten, which affects the mucus of the intestine and the ability to absorb nutrients. The only possible treatment is a gluten-free diet. This autoimmune condition, with an estimated worldwide prevalence of approximately 1%, also affects 2.5 million people who are undiagnosed and are at risk of long-term health complications.[1] For this reason, there is a strong need for the development of food products that increase the availability of gluten-free food in order to address the increasing demand. This constitutes a real technological challenge with wide possibilities for investigation.[2]

Flours habitually used to elaborate gluten-free products from bread-making products are based on rice, maize, and manioc. As some of these preparations have either low protein or low fiber content, biologically active components such as minerals, vitamins, oily acids, and fiber are added. One other alternative source of suitable nutrients for celiac sufferers is found in soybean and its derivatives.

Among soy derivatives, okara is a by-product of the production of soymilk and tofu. It corresponds to the insoluble fraction that remains in the filter when soybean grains have undergone a hydrothermal treatment of crushing, grinding, and leaking. It is a material of yellowish-white color and soft flavor, and which contains most of the carbohydrates, some protein and a small portion of the lipids from soybeans. Okara is a rich source of protein and dietary fiber, which plays an important role in many physiological processes and in the prevention of diseases of various origins.[3] Its proteins are of high quality, since all the essential amino acids are present, having the ability to reduce triglycerides and cholesterol (total, low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) cholesterol). The bounding amino acid is methionine, whereas the lysine content exceeds the established requirements, which would make its use feasible in the supplementation of gluten-free flour. Okara contains an essential type of omega-3 fatty acid. The following chemical composition has been reported:[4] 490 g kg^{-1} fiber; 330 g kg^{-1} protein, 198 g kg^{-1} fat and 35 g kg^{-1} ash content.

Fresh okara deteriorates rapidly due to its high moisture content. To overcome these limitations, okara must be dried as soon as possible under the appropriate conditions in order to maintain its integrity and allow its use as flour.[5] Okara was obtained

* *Correspondence to: ME Campderrós, Instituto de Investigaciones en Tecnología Química (INTEQUI-CONICET), Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Ejército de los Andes 950, 5700 San Luis, Argentina. E-mail: mcampd@gmail.com*

Instituto de Investigaciones en Tecnología Química (INTEQUI-CONICET), Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, 5700 San Luis, Argentina

by various drying processes, for instance, air jet impingement dryer, jet-spouted bed of sorbent particles, electrohydrodynamic technique and tray dryer. In order to enhance the nutritional and functional properties of the flour, several technological treatments have been applied, such as thermal treatment and chemical hydrolysis. The drying processes could produce encapsulation of the proteins inside a structure of polysaccharides, allowing the preservation or improvement of the properties of some food ingredients.[6] The sensitive ingredient remains inside the coating material, whose structure depends on the type of technology and on the conditions applied.[7] Despite the improvements, there is an increasing demand from consumers for food considered 'natural'; for that reason, the food industry has shown great interest in the use of natural enzymes as opposed to the utilization of chemical hydrolysis, which would upgrade the functional properties for the production of food ingredients.[8]

The aim of this research was to investigate drying technologies suitable to obtain an okara flour that could be used for human consumption. Three drying methods were evaluated to optimize the production of okara flour: microwave oven, rotary dryer of convective airflow, and freeze-drying technique. The purpose of this investigation was to define the usefulness of okara as an alternative gluten-free product and to test the influence of the type of drying methods according to various parameters such as physicochemical composition, functional properties, and quality attributes including color, level of oxidation, and textural parameters. The effect of an enzymatic treatment on okara before dehydration was studied using canary-seed milk, which is an enzymatic compound with a high lipase content.[9] Lipases are enzymes that are soluble in water and operate on insoluble substrata and aggregates, joined to the lipid–water interface.[10]

MATERIAL AND METHODS

Raw materials

Soybean and canary-seed (*Phalaris canariensis*) for human consumption were used as raw materials and purchased from the local market.

Okara preparation

Soybeans were soaked in water for 8–10 h at ambient temperature. They were ground in a blender (SIAM LIC07) incorporating water at 100 ± 1 °C to enhance the grinding process. The ratio of water to beans is usually between 8:1 and 10:1.[3] A thermal treatment was applied for 20 min at a temperature higher than 90 ± 1 °C to reduce the activity of the trypsin inhibitor and deactivate the lipoxygenase enzyme, which provokes an unpleasant taste. Milk was separated from ground soybean slurry using a filter. About 1.1 kg fresh okara was obtained from every kilogram of soybean processed to obtain soymilk.

One fraction of the okara obtained was reserved for enzymatic treatment. A technique developed by Vergara-Olivares,[11] with some modifications, was used. Canary-seed, rich in lipases, were hydrated and disinfected in a solution of hydrogen peroxide 30 g kg^{-1} for 8–10 h. They were then rinsed three times with sterile water and ground in a blender. The milk was separated by manual pressing using a filter. The enzymatic treatment was carried out in a stove at 31 ± 1 °C for 180 min using 100 mL canary-seed milk 200 g^{-1} fresh okara.

Drying

The fresh okara obtained was dried for its conservation using one of three methods: (a) microwave oven, using 40% power level (model Quick Chef 3d, 1000 W, BGH, Argentina). The sample was placed in a cylindrical container suitable for the technique, $T_{ok} = 110 \pm 5$ °C (where T_{ok} is the temperature of the okara); (b) rotary dryer of convective airflow – the prototype elaborated in the laboratory was a cylindrical structure of metallic mesh; fresh okara was placed in a bag to avoid loss of sample and direct contact with the mesh; $T_{ok} = 70 \pm 5$ °C, $T_p = 150 \pm 20$ °C (where T_p is the temperature of the process); (c) fresh okara was placed on a stainless steel tray, frozen in a freezer at −40 °C and freeze-dried using a lyophilizer (model L-2, Rificor, Buenos Aires, Argentina) at 25 ± 2 °C and 50 µm Hg for 48 h . The temperature of the samples, in all cases, was measured using a temperature sensor, and the weight loss was monitored using an analytical balance in order to obtain information at different drying times.

The samples obtained were designated as: OM, okara dried by microwave; OF, okara freeze-dried; OR, okara dried by rotary dryer; OME, okara dried by microwave with enzymatic treatment; OFE, okara freeze-dried with enzymatic treatment; ORE, okara dried by rotary dryer with enzymatic treatment.

Physicochemical analysis

Physicochemical analyses were determined in triplicate according to standard replication AOAC (Association of Official Agricultural Chemists 1995) methods.[12] 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), fat content by Soxhlet extraction (model SZC-D, Shanghai QianJian Instrument Co. Ltd, China) (AOAC 945.39), and carbohydrate by difference. The peroxide value (PV) (AOAC 965.33) was also studied and determined.

Determination of denatured protein content

Soluble protein content was determined after isoelectric precipitation of denatured/aggregated proteins. Solutions of 10 g L^{-1} okara were adjusted to pH 4.6 using 0.1 mol L^{-1} NaOH and HCl. An aliquot of the solution was centrifuged (model 2070, Rolco centrifuge, Buenos Aires, Argentina) at 3000 rpm for 30 min. Protein concentration in the supernatants was determined by measuring absorption at 280 nm after appropriate dilution in a dissociating buffer (ethylenediaminetetraacetic acid 50 mmol L^{-1}, urea 8 mol L^{-1}) and reported as a percentage of the total protein concentration . Insoluble protein content of suspensions at pH 4.6 was defined as the difference between total protein (TP) and soluble protein (SP) content and was used to estimate the extent of denaturation in okara samples. The percentage of denatured protein (DP) content was calculated using the following equation:[13]

$$P = \left[\frac{TP - SP}{TP}\right] \times 100 \quad (1)$$

Non-enzymatic browning measurement

Duplicate samples were stored at room temperature and subsequently analyzed. The extent of browning was spectrophotometrically determined (UV–visible double-beam spectrophotometer, Shimadzu, USA) using the technique described by Delgado-Andrade *et al.*:[14] 0.5 g sample was suspended in 5 mL

deionized water; the tube was shaken vigorously for 1 min and clarified with 0.25 mL each of Carrez I (potassium ferrocyanide, 150 g L^{-1}, and Carrez II (zinc acetate, 300 g L^{-1}) solutions. The resulting mixture was centrifuged (25 min at 3000 rpm (model 2070, Rolco), the supernatant was collected in a 10 mL volumetric flask, and two further extractions were done using 2 mL deionized water. The supernatants were mixed and the volume was made up to 10 mL with deionized water. Solutions were filtered, adequately diluted if necessary, and measured at 280 nm to detect the products at an early stage of browning and low-molecular-weight Maillard compounds; and at 420 nm in order to detect the final and high-molecular-weight Maillard compounds. The constant rate of browning, k_b, was calculated from zero-order kinetics using a linear regression analysis.[15]

Functional properties

The functional properties of okara flour are important for the processing and formulation of food products. These properties include swelling capacity (SC), water holding capacity (WHC), water absorption capacity (WAC), oil binding capacity (OBC), and solubility (*S*). These functional properties were determined using the methods described by Alvarez-Restrepo *et al.*,[16] with some modifications.

Swelling capacity

The swelling capacity of a product is that which allows it to increase its volume in the presence of an excess of water. In a graduated cylinder, 2.5 g sample (w_0) was weighed, then an excess of water was added (30 mL). The mixture was manually stirred and then left to rest for 24 h at room temperature (27 ± 0.5 °C). The final volume was measured in milliliters (V_f). The swelling capacity was obtained by applying the following equation:

$$SC = V_f / w_0 \tag{2}$$

Water holding capacity

One gram of sample (w_0) was weighed into a test tube and 30 mL water was added. The mixture was stirred and hydrated for 18 h. It was then centrifuged at 3000 rpm for 30 min. The supernatant was separated and the residue was transferred to porcelain crucibles. The residue was weighed to obtain the value of humid residue (HR). After that, the residue was dried at 105 ± 1 °C for 24 h and then weighed again to obtaining the value of dry residue (RS). The following equation was applied:

$$WHC = (HR - RS) / RS \tag{3}$$

The percentage of soluble material was calculated indirectly from the WHC, using the following equation:

$$\%S = \left(w_0 - RS\right) / w_0 \tag{4}$$

Water absorption capacity

WAC expresses the maximum quantity of water that can be retained by 1 g of dry material in the presence of a water excess under the action of a force. In a test tube, 0.5 g sample (w_0) was weighed, excess of water was added (10 mL), and the mixture was shaken for 30 min. It was then centrifuged at 3000 rpm for 10 min. The supernatant was separated and the sediment was weighed (*Ws*). The results were expressed as grams of water per gram of sample:

$$WAC = \left(Ws - w_0\right) / w_0 \tag{5}$$

Oil binding capacity

OBC was determined using the method employed by Palatnik *et al.*[17] One gram of the sample (w_0) was weighed inside a test tube and mixed by 10 mL vegetable oil (V_1) using an agitator. The samples were left to rest for 30 min and then centrifuged at 20 000 rpm for 25 min. Immediately after centrifugation, the supernatant was carefully poured into a 10 mL graduated cylinder and the volume was registered (V_2). The OBC (milliliters of oil per gram of product) was calculated as follows:

$$OBC = \left(V_1 - V_2\right) / w_0 \tag{6}$$

Physical properties

Analysis of surface color

The color values of the different okara samples obtained were measured using a digital spectrophotometer (model Mini Scan EZ, HunterLab, Reston, VA, USA), which was provided with the necessary software. The equipment was calibrated with the standard white and black colors. 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.[17] Whiteness Index (WI) for each sample was calculated according to the Eqn (7):[18]

$$WI = 100 - \left[(100 - L^*)^2 + a^{*2} + b^{*2}\right]^{\frac{1}{2}} \tag{7}$$

Optical microscopy studies

The surface structure of the different samples of okara was observed with an optical microscope (model BW1008-500X digital USB microscope, Duratool, USA). The samples were viewed at 60× magnification, with the purpose of determining whether the different processes of drying had affected the microstructure of the okara and, consequently, its characteristics.

Textural profile analysis

For the textural profile analysis dough elaborated with okara–manioc starch-water was used. The substitution of 40% manioc starch had the purpose of achieving the formation of a suitable dough. All measurements were performed with a back-extrusion test using a texture analyzer (model TMS-TOUCH, Food Technology Corporation, Sterling, VA, USA). The test was carried out in a back-extrusion container (50 mm in diameter), filled 75% with the sample, using an acrylic cylinder probe (25 mm) attached to an extension bar, with a load cell of 500 N. Three replications were made at a test speed of 30 mm min^{-1} and a distance of 10 mm. Mean values were used to obtain a force–time curve, calculating the following as texture parameters: (i) firmness = maximum compression force in extrusion thrust into sample (g); (ii) consistency = area within a curve during extrusion thrust (g s); (iii) cohesiveness = maximum compression force during withdrawal of probe from sample (g); (iv) viscosity index = area within negative region of curve during probe withdrawal (g s).

Statistical analysis

Experimental data were analyzed using analysis of variance with the significance defined at $P < 0.05$. The obtained data were statistically evaluated by the Tukey–Kramer multiple comparison test in cases where two or more comparisons were considered. Otherwise, the *t*-test was used, assuming that $P < 0.05$ was statically significant.[19]

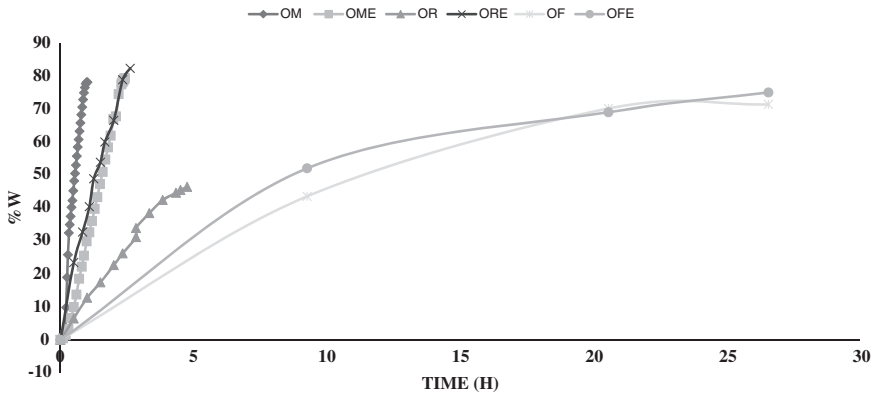

Figure 1. Curve of fresh okara dried using different methodologies. OM, okara dried by microwave; OF, okara freeze-dried; OR, okara dried by rotary dryer; OME, okara dried by microwave with enzymatic treatment; OFE, okara freeze-dried with enzymatic treatment; ORE, okara dried by rotary dryer with enzymatic treatment.

RESULTS AND DISCUSSION

From the drying curves it was possible to estimate the time needed to obtain flours with the characteristics established by the Código Alimentario Argentino (CAA)[20] and optimization of the technologies employed. Figure 1 shows the percentage of weight loss of the sample of okara during the drying process using a microwave and a rotating dryer. It was observed that at the beginning of the process there was an increase in the percentage of weight loss which was linear with the time of drying (positive slope); the speed of drying remained constant in all the devices. This was the consequence of the fact that 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.[21] In the samples dried by microwave, a greater slope was observed, which represents a major loss of water in less time. The fact that fresh okara has a high moisture level (≅800 g kg^{-1}) facilitated the penetration of electromagnetic radiation through the polar molecules, allowing an almost instantaneous warming in the totality of the sample.[22]

The lower drying speed obtained with the rotary dryer would be because at the temperature of the process (65–75 °C) physical and chemical changes took place in the surface of the sample. Those changes led to the formation of a superficial, hard and semi-impermeable film. This phenomenon could reduce the speed of dehydration and allow the okara to dry in the surface while remaining humid in its interior.[23]

The freeze-drying samples showed low moisture content, which could be favorable for conservation and could improve the expected lifetime of the flour. This technology has been associated with a relatively high energetic cost; consequently, its utility would depend on the development of special applications of the flour.

The results of the physicochemical characterization and peroxide value of the okara are shown in Table 1. It is observed that only flours obtained by microwave and freeze-drying with and without enzymatic treatment presented moisture below 150 g kg^{-1}, which is the established CAA limit for commercial flours. The surface of the matter was not particulate with the rotary dryer although it was adhesive, which resulted in the formation of agglomerations that were difficult to dry.[23] Similar results were reported by Ma *et al.*[4] and Vishwanathan *et al.*[24] No significant difference in fat content was found among samples. The enzymatic treatment decreased the protein content but increased the fiber content in the samples. However, it is common knowledge that the chemical composition of okara will depend on the procedure followed to obtain it.

PV value is crucial when selecting a flour due to the fact that this parameter could affect its organoleptic characteristics, its nutritional quality and, therefore, its lifetime. Enzymatic treated samples presented a higher PV value. The lipases could accelerate the oxidation of the lipids provided that the free fatty acids are more capable of oxidation than the triglycerides.[25] Degradation of the triglyceride was reflected in the PV obtained. Nevertheless, the peroxide index of the samples presented statistically significant differences, demonstrating that the different processes implemented to obtaining flour of okara affect the degree of oxidation.

Micrographs were taken in order to establish a relation between the microstructure of okara flour and the denatured protein content according to the drying process carried out. Figure 2 shows both the denatured protein content according to each drying process and the images of the flour obtained. The temperature is an important parameter to control since it has an impact on the agglomeration and coating formation of the particles, influencing the efficiency of the encapsulation.[7] Optical microscopy revealed that the freeze-dried sample had a porous structure with a thin capillary formation. The samples dried by microwave and rotary dryer had smooth and rounded structures in a dense matrix. The images are also shown in the Fig. 2. Similar structures were found by Vishwanathan *et al.*[24]

The highest percentage of denatured protein was obtained by freeze-drying. During freeze-drying the protein is subjected to freezing and drying stress, by which its activity can be lost.[13] Furthermore, okara proteins are highly insoluble, with a high content of hydrophobic amino acids,[25] suggesting low protein–sugar–water interactions, which would act as cryoprotectors of proteins. This behavior could produce the crystallization of the carbohydrates present in the sample, increasing the denatured protein content. Therefore, if the saccharides crystallized, the protein would not be protected from conformational changes or denaturation.

As previously mentioned, the use of microwave and rotary dryer produced a better isolation of the proteins inside a dense matrix structure (Fig. 2), without surface cracks. During the process of freeze-drying, a barrier of porous structure was formed between the active agent and the surroundings. This porous structure provided little protection[7] for this reason: a major

Table 1. Proximate composition and peroxide value (PV) of dried okara

Sample	Moisture (g kg^{-1})	Ash (g kg^{-1})	Fiber (g kg^{-1})	Protein (g kg^{-1})	Fat (g kg^{-1})	Total carbohydrate (g kg^{-1})	PV (meqO_2 kg^{-1} fat)
OM	13.8 ± 0.57a	34.4 ± 1.67a	203.7 ± 5.02a	346.1 ± 6.09[a]	191.0 ± 19.08a	211.1 ± 11.25a	0.6 ± 0.0300a
OF	36.2 ± 0.49b	22.4 ± 2.03b	231.4 ± 9.6ab	333.9 ± 3.20ab	189.8 ± 12.30a	187.4 ± 13.5a	0.47 ± 0.023b
OR	176.8 ± 2.85c	18.0 ± 2.74b	224.5 ± 1.64a	307.85 ± 22.00ab	147.4 ± 40.41a	163.7 ± 9.40b	0.53 ± 0.026b
OME	149.0 ± 0.28d	26.8 ± 2.94 ac	214.7 ± 2.56a	296.0 ± 1.30b	155.5 ± 36.08a	158.0 ± 19.5b	0.72 ± 0.036a
OFE	33.8 ± 0.03b	24.4 ± 1.58b	271.9 ± 14.3b	314.4 ± 10.50ab	215.0 ± 26.85a	140.5 ± 15.2b	0.68 ± 0.034a
ORE	374.5 ± 1.32e	15.1 ± 0.43bc	232.6 ± 15.8ab	244.5 ± 3.14c	172.7 ± 15.73a	207.0 ± 10.1a	0.86 ± 0.043c

Means followed by different letters in the same row are significantly different ($P < 0.05$).
OM, okara dried by microwave; OF, okara freeze-dried; OR, okara dried by rotary dryer; OME, okara dried by microwave with enzymatic treatment; OFE, okara freeze-dried with enzymatic treatment; ORE, okara dried by rotary dryer with enzymatic treatment.

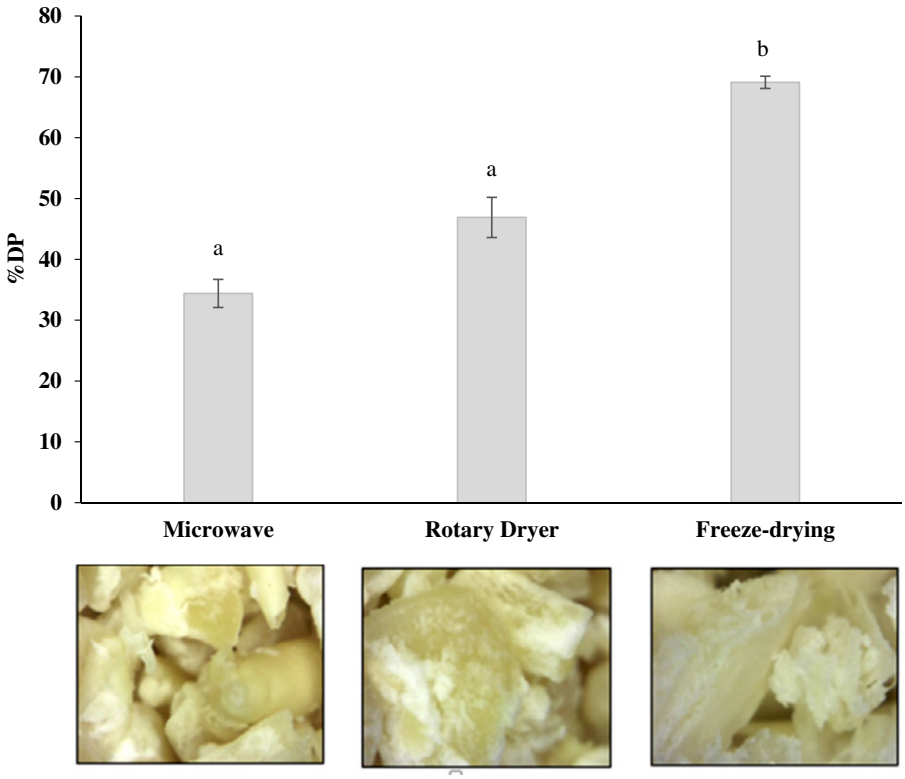

Figure 2. Percentage of denatured protein contain (%DP) and photographs of each sample taken with an optical digital microscope.

protein denaturalization could take place due to a major contact between the exterior and the interior of the coating. A test of solubility was further carried out. The samples were dissolved in distilled water, while spectroscopy determined the protein concentration in the solution. The results showed that the freeze-drying samples (OF, OFE) presented greater protein solubility (20.95 ± 1.56; 19.81 ± 2.96), substantiating a better interaction of the proteins with the environment due to its porous structure.

Non-enzymatic browning appears during the technological processes or the storage of diverse products. It hastens for the heat during the operations of boiling, pasteurization, and dehydration.[25] Non-enzymatic browning is a consequence of Maillard reactions between reducing sugar samples and amino acids, culminating in the formation of a dark pigment called melanoidin. The temperature exercises an important influence on the speed of the Maillard reaction, especially when it is higher than 120 °C.[23] Those samples dried in the microwave presented higher non-enzymatic browning values compared with the other drying processes employed (OM = 0.104 ± 0.001; OME = 0.086 ± 0.008; OR = 0.076 ± 0.005; ORE = 0.074 ± 0.006; OF = 0.072 ± 0.002; OFE = 0.06 ± 0.001, $P < 0.05$) due to the temperatures applied in the drying process. Also, the condensation between the functional carbonyl and amino acid groups generated during the Maillard reaction implies minor solubility and digestion of the proteins,[25] which is in accord with the result of solubility analysis.

Table 2 shows the results of the functional properties of the okara. It provides information about interactions between okara and water, predicting the processing and mass formation as well as its possible behavior in other food matrices. The values obtained for SC and WAC represent the high fiber content present in the

Table 2. Results of functional properties of okara flour obtained by the different drying methods assayed

Property	OM	OF	OR	OME	OFE	ORE
SC (mL water g^{-1} sample)	7.39 ± 0.37a	7.99 ± 0.4a	7.58 ± 0.38a	5.80 ± 0.29b	9.19 ± 0.46a	–
WAC (g water g^{-1} sample)	5.6 ± 0.120a	7.55 ± 0.44b	4.27 ± 0.16a	4.9 ± 0.20a	7.29 ± 0.028b	–
WHC (g water g^{-1} sample)	5.22 ± 0.035a	7.34 ± 0.17b	5.89 ± 0.18a	5.47 ± 0.15a	7.56 ± 0.200b	–
OBC (g oil g^{-1} sample)	2.61 ± 0.440a	4.76 ± 0.67b	2.31 ± 0.00a	1.88 ± 0.21a	5.88 ± 0.820b	–
S (g soluble portion g^{-1} sample)	0.05	0.13	0.29	0.21	0.11	–

Means followed by different letters in the same row are significantly different ($P < 0.05$).
OM, okara dried by microwave; OF, okara freeze-dried; OR, okara dried by rotary dryer; OME, okara dried by microwave with enzymatic treatment; OFE, okara freeze-dried with enzymatic treatment; ORE, okara dried by rotary dryer with enzymatic treatment; SC, swelling capacity; WAC, water absorption capacity; WHC, water holding capacity; OBC, oil binding capacity; S, solubility.

samples. The work carried out by Hoyos Sánchez and Palacios Peña[26] provided evidence that the flours with high fiber content and non-gluten-forming proteins can increase water absorption, since these components compete for water molecules. The freeze-drying samples (OF, OFE) presented a major capacity of water absorption due, probably, to the porous structure previously described, which results in a higher availability of proteins and higher solubility.

WHC plays an important role in the texture quality of various foods, e.g., soups, ground meat, dressing, sauces, and baked products. Water adsorption without protein dissolution leads to swelling, which provides properties such as consistency, thickening, viscosity, and adherence.[17,27] It was observed anew that OF and OFE present a better capacity of water retention due to the fact that their capillary structure increases their affinity for water.

OBC did not exhibit a statistical difference between the okara dried by microwave and that dried in a rotary drier. However, freeze-dried samples presented the highest values due to their structure.

Similar results were found in samples of okara analyzed by Hernández *et al.*[28] Also, okara flour featured a high fiber and protein content compared to soy flour (30 g kg^{-1}; maximum limit established by the CAA) and to other flours derived from legumes such as chickpea flour and carob.[29,30]

The results of color measurements on the different samples are shown in Table 3. All samples present an L^* value higher than 65, which reflects that those are light-colored flours. The a^* value shows a slight deviation towards the positive values. The b^* positive value indicates the degree of yellowness. The samples of OF and OME, when compared to b^* value, showed statistically significant differences with regard to the other samples studied. All the samples displayed a WI superior to 56. However, the lyophilized samples were those which exhibited a major clarity, whereas the lowest values of WI corresponded to the samples dried by microwave, which could be due the reactions of non-enzymatic browning generated in the process. In addition, the flours elaborated using an enzymatic treatment presented lower values compared to the flour produced without enzymatic treatment.

The results obtained in the profile analysis of texture are shown in Fig. 3. The samples dried by microwave and rotary dryer (OM, OR) presented higher values of firmness (Fig. 3A) and consistency (Fig. 3B), while, when the enzymatic treatment was applied, an increase in consistency was observed. In the dough elaborated with enzymatic treatment, some enzymes act in the network of proteins forming covalent bonds between them. The structural network of the dough is strengthened and therefore

Table 3. Color measurements on okara flour obtained by the different drying methods assayed.

Sample	L^*	a^*	b^*	WI
OM	69.50 ± 0.17a	4.01 ± 0.16a	23.92 ± 0.12a	60.17
OF	74.85 ± 0.13a	2.29 ± 0.19b	19.50 ± 0.09b	68.09
OR	69.53 ± 0.36a	4.19 ± 0.49a	25.17 ± 0.23a	60.26
OME	66.94 ± 0.23a	5.12 ± 0.30a	27.79 ± 0.50b	56.51
OFE	67.01 ± 0.19a	2.76 ± 0.08b	20.07 ± 0.32a	65.50
ORE	66.05 ± 0.10a	4.95 ± 0.30a	23.78 ± 0.60a	58.26

Means followed by different letters in the same row are significantly different ($P < 0.05$).
OM, okara dried by microwave; OF, okara freeze-dried; OR, okara dried by rotary dryer; OME, okara dried by microwave with enzymatic treatment; OFE, okara freeze-dried with enzymatic treatment; ORE, okara dried by rotary dryer with enzymatic treatment; L^*, lightness variable; a^*, +redness/−greenness variable; b^*, +yellowness/−blueness variable; WI, whiteness index.

the firmness and elasticity are improved.[11] The cohesiveness of the samples (Fig. 3C) did not present statistically significant differences from that of the flours without enzymatic treatment (OM, OL, OR), indicating that the drying process did not influence this parameter. The enzymatic treatment also increased the cohesiveness of the masses ($P > 0.05$). This could occur since the enzymatic treatment could modify interparticle interactions through structural changes. Thus some enzymes featured in the canary-seed milk form intramolecular and intermolecular links between the proteins of the flour, originating a multifaceted network. Despite the temperatures used, the okara proteins retained their functionality, which probably indicates a good interaction between these and other components of the dough. The behavior of the proteins precipitated by heat could provoke a greater adhesiveness of the material when their content was increased. The viscosity index (Fig. 3D) was influenced not only by the type of drying technique employed but also by the presence or absence of enzymatic treatment. The enzymatic treatment and the temperature used in the process could produce structural changes in the samples.

CONCLUSION

The drying of okara was undertaken to enhance the stability of the dry powder. Physicochemical analyses revealed that the principal nutritional advantage of okara is its high fiber (200–270 g kg^{-1}) and protein (230–340 g kg^{-1}) content. The enzymatic treatment

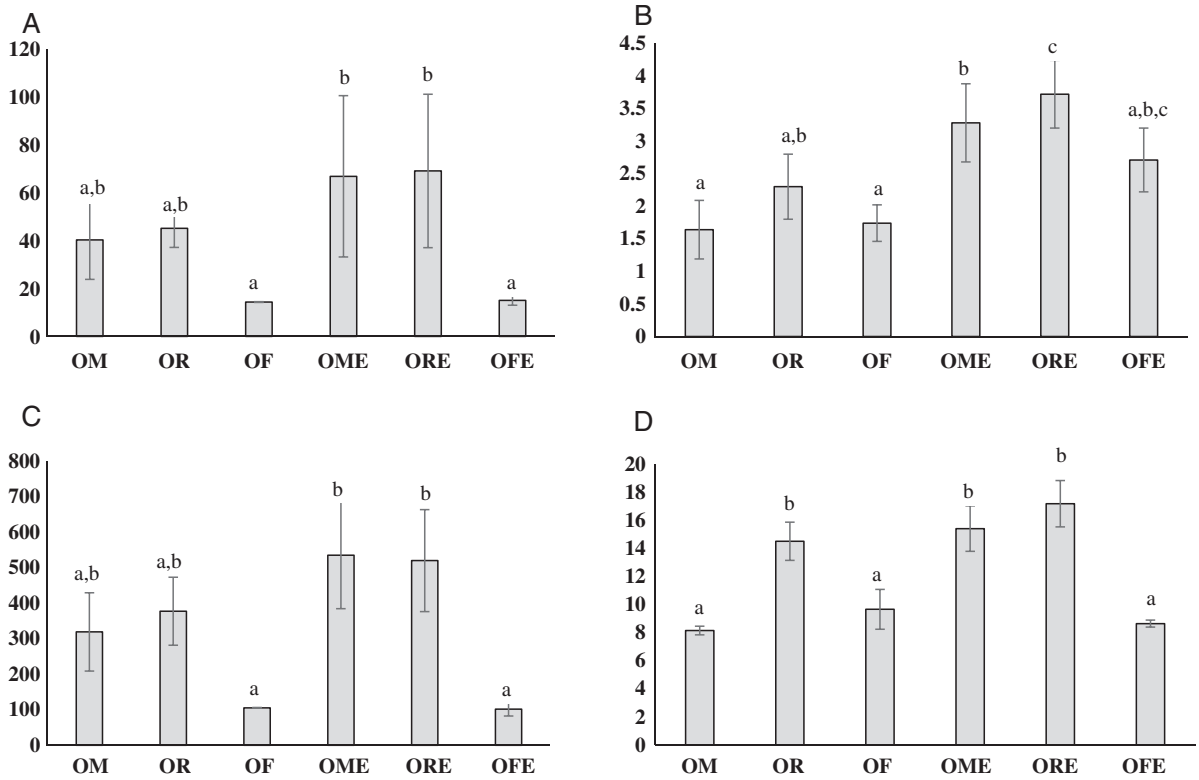

Figure 3. Textural profile analysis of dough elaborated with okara–manioc flour. **(**A) Firmness (N); (B) cohesiveness (N); (C) consistency (N s); (D) viscosity index (N s). All values are means with standard deviation ($n = 3$). Different letters within the same column differ significantly from each other ($P < 0.05$). OM, okara dried by microwave; OF, okara freeze-dried; OR, okara dried by rotary dryer; OME, okara dried by microwave with enzymatic treatment; OFE, okara freeze-dried with enzymatic treatment; ORE, okara dried by rotary dryer with enzymatic treatment.

affected the composition of okara and produced structural changes in the samples, evidenced by textural analysis. The different processes implemented in obtaining the flour affected the degree of oxidation. The experimental results showed that the use of microwave produced a greater loss of water in a shorter time, and smooth and rounded structures in a dense matrix, like the flour obtained by rotary dryer. The lower drying speed resulting from the rotary dryer was the consequence of the formation of a superficial, hard and semi-impermeable film; also, the flour obtained presented the lowest whiteness index, basically by reactions of non-enzymatic browning. The freeze-drying process produced okara flour with major protein denaturation but presented a porous structure with a low final moisture content and a high solubility, which is an indicator of potential application in food development.

ACKNOWLEDGEMENTS

Financial support was provided by the SCyT, UNSL (Project 2–3114), and PICT 2012–0155 (ANPCyT). We would also like to acknowledge fellowships of Eng. Ostermann-Porcel (ANPCyT).

REFERENCES

1 Saccone G, Berghella V, Sarno L, Maruotti GM, Cetin I, Greco L *et al.*, Celiac disease and obstetric complications: a systematic review and metaanalysis. *Am J Obstet Gynecol* **214**:225–234 (2016).
2 Sansone FP, Mencherini T, Russo P, Gasparri F, Giannini V, Lauro M *et al.*, Enhanced technological and permeation properties of a microencapsulated soy isoflavones extract. *J Food Eng* **115**:298–305 (2013).
3 Redondo-Cuenca A, Villanueva-Suárez MJ and Mateos-Aparicio I, Soybean seeds and its by-product okara as sources of dietary fibre: measurement by AOAC and English methods. *Food Chem* **108**:1099–1105 (2008).
4 Ma CY, Liu WS, Kwok KC and Kwok F, Isolation and characterization of proteins from soymilk residue (okara). *Food Res Int* **29**:799–805 (1997).
5 Wachiraphansakul S and Devahastin S, Drying kinetics and quality of okara dried in a jet spouted bed of sorbent particles. *LWT – Food Sci Technol* **40**:207–219 (2007).
6 Buera P, Schebor C and Elizalde B, Effects of carbohydrate crystallization on stability of dehydrated foods and ingredient formulations. *J Food Eng* **67**:157–165 (2005).
7 Ray S, Raychaudhuri U and Chakraborty R, An overview of encapsulation of active compounds used in food products by drying technology. *Food Biosci* **13**:76–83 (2016).
8 Vidal-Guevara ML, Periago MJ and Ros G, Influencia del tratamiento enzimático sobre la calidad de la proteína del guisante. *Anales Vet (Murcia)* **19**:43–60 (2003).
9 Estrada-Salas PA, Montero Morán GM, Martínez-Cuevas P, González C and Barba de la Rosa AP, Characterization of antidiabetic and antihypertensive properties of canary seed (*Phalaris canariensis* L.) peptides. *J Agric Food Chem* **62**:427–433 (2014).
10 González-Bacerio J, Rodriguez-Hernandez J and Del Monte-Martinez A, Las lipasas: enzimas con potencial para el desarrollo de biocatalizadores inmovilizados por adsorción interfacial. *Rev Colomb Biotecnol* **12**:124–140 (2010).
11 Vergara-Olivares P, Efecto de adición de enzima transglutaminasa en el desarrollo de pan a base de harina de quínoa (*Chenopodium quinoa* Willd) (2011). [Online]. Available: http://www.repositorio.uchile.cl/handle/2250/116490 [18 November 2016].
12 AOAC, *Official Methods of Analysis* (16th edn). Association of Official Analytical Chemists, Arlington, VA (1995).
13 Rodriguez-Furlán LT, Pérez-Padilla A and Campderrós ME, Inulin like lyoprotectant of bovine plasma proteins concentrated by ultrafiltration. *Food Res Int* **43**:788–796 (2010).

14 Delgado-Andrade C, Rufián-Henares JA and Morales FJ, *Assessing the Generation and Bioactivity of Neo-formed Compounds in Thermally Treated Foods*. Atrio, Granada (2009).
15 Miao S and Roos YH, Isothermal study of nonenzymatic browning kinetics in spray-dried and freeze-dried systems at different relative vapor pressure environments. *Innov Food Sci Emerg Technol* **7**:182–194 (2006).
16 Alvarez-Restrepo C, Gallardo-Cabrera C, Lopera-Cardona SM and Umaña-Gallego JH, Caracterización de harinas alternativas de origen vegetal con potencial aplicación en la formulación de alimentos libres de gluten. *Rev Aliment Hoy* **22**:33–46 (2013).
17 Palatnik DR, Ostermann-Porcel MV, González U, Zaritzky N and Campderrós ME, Recovery of caprine whey protein and its application in a food protein formulation. *LWT – Food Sci Technol* **63**:331–338 (2015).
18 Zhu K, Kanu PJ, Pierre-Claver I, Zhu K, Qian H and Zhou H, A method for evaluating Hunter whiteness of mixed powders. *Adv Powder Technol* **20**:123–126 (2009).
19 SAS, User Guide: Statistic. SAS Institute, Cary, NC, USA (1989).
20 CAA, *Código Alimentario Argentino.* [Online]. Administración Nacional de Alimentos, Medicamentos y Tecnología Médica, Capítulo IX. Available: http://www.anmat.gov.ar/alimentos/codigoa/capitulo_ix.pdf.
21 Geankoplis CJ, *Transport Processes and Unit Operations* (3rd edn). Prentice Hall, Upper Saddle River, NJ, pp. 579–634 (1993).
22 Giraldo-Giraldo GA, Conservación de frutas tropicales por métodos combinados (2012). [Online]. http://repositorio.uniquindio.edu.co/bitstream/123456789/258/1/Informe%20408%20Definitivo.pdf.
23 Perussello CA, Estudo dos parametros de processo e modelagem numerica da secagem du residuo solido da prodicao do extrato hidrossoluvel de soja (okara). Working paper, Pontificca Universidade Catolica do Parana (2008).
24 Vishwanathan KH, Singh V and Subramanian R, Influence of particle size on protein extractability from soybean and okara. *J Food Eng* **102**:240–246 (2011).
25 Cheftel JC and Cheftel H, *Introducción a la bioquímica y tecnología de los alimentos, Vol. I*. Acribia, Zaragoza, Spain (2000).
26 Hoyos Sánchez D and Palacio Peña G, Utilización de harinas compuestas de maíz y garbanzo adicionadas con fibra de cáscara de piña para sustitución de harina de trigo en productos de panificación. Working paper, Universidad del Valle, Colombia (2015).
27 Yu J, Ahmedna M and Goektepe I, Peanut protein concentrate: production and functional properties as affected by processing. *Food Chem* **103**:121–129 (2007).
28 Hernández M, Pinzón-Bedoya ML and Carvajal DC, Use of okara flour as a partial substitute for wheat flour in regional typical bread. *@limentech Ciencias y Tecnología alimentaria* **11**:43–50 (2013).
29 Aguilar N, Albanell E, Miñarro B and Capellas M, Chickpea and tiger nut flours s alternatives to emulsifier and shortening in gluten-free bread. *LWT – Food Sci Technol* **62**:225–232 (2015).
30 Tsatsaragkou K, Gounaropoulos G and Mandala I, Development of gluten free bread containing carob flour and resistant starch. *LWT – Food Sci Technol* **58**:124–129 (2014).