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RESEARCH ARTICLE

Soaking and extrusion effects on physicochemical parameters, phytic acid, nutrient content and mineral bio-accessibility of whole rice grain

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Abstract

A combination of soaking and extrusion processes of whole rice grain was studied. The effects of temperature (35–55 °C) and time (24–48 h) of soaking treatment on phytic acid (PA), protein and ashes losses using a factorial design were evaluated. Taking into account ash, protein and PA losses, whole rice was soaked 24 h at 45 °C and extruded using a Brabender single screw extruder. Effects of extrusion temperature (160–190 °C) and moisture content (14–19 g/100 g) on product characteristics were evaluated using surface response methodology. Values corresponding to the different responses were: Expansion (1.64–3.28), Specific Volume (5.68–11.06 cm^3/g), Water absorption (3.41–4.43 mL/g) and Solubility (45.44–66.20 g/100 g). The content of PA was reduced from 740.09 to 163.47 mg/100 g (77%) after both processes, resulting in a higher mineral bio-accessibility, and a 7.3% decrease of protein digestibility. Total soluble phenolics and trolox equivalent antioxidant capacity (TEAC) were affected according to the treatment. Both treatments were important to obtain a nutritionally improved whole grain product.

Keywords

Bio-accessibility extrusion, phytic acid, rough rice, soaking, whole grain.

History

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Introduction

Whole grains (WG) are defined by the ''American Association of Cereal Chemists'' (2000) as ''the caryopsis intact, ground, cracked or rolled, whose principal anatomical components (endosperm, germ and bran) are in the same relative proportions which were in the intact caryopsis'' (Marquat et al., 2003). WG are sources of dietary fiber, resistant starch and oligosaccharides, which escape gastrointestinal digestion and are fermented in the large intestine. Produced short chain fatty acids lower the pH of the colon, serve as energy source for colonocytes, and can modify the contents of blood lipids, all these changes resulting in beneficial health effects (Topping & Clifton, 2001). Furthermore, it is noteworthy that WG are rich in antioxidants, such as phenolic compounds that have been linked to the prevention of chronic diseases, such as stroke, cancer, diabetes, etc. (Fardet et al., 2008; Slavin, 2003). For that, it is important to incorporate WG in human diet.

Rice provides about half the calories for up to half of the world's population, especially in parts of Asia and South America. Worldwide, it is second in production after corn (*FAOSTAT*) but first in its contribution to human food, since corn is used for many other purposes.

Phytic acid (PA) present in WG is the main source of phosphorus storage. In foods it is normally negatively charged and has the ability to bind cations or other functional groups of positively charged molecules. The complexes formed with minerals cause that them could not be absorbed through the gastrointestinal tract and in the case of protein, the digestibility could decrease. Thus, PA content decreases nutritional quality of WG-based foods. Moreover, the intestine lacks enzymes called phytases, capable of degrading this compound (Kumar et al., 2010). To decrease phytate content and its effects on nutrients, different technological processes, such as steeping, germination, fermentation and extrusion could be used (Hotz & Gibson, 2007). Soaking of brown rice was studied in order to produce a whole grain rice product with very low PA content. However, soaking caused high losses of minerals (Albarracín et al., 2013). Thus, soaking of rough rice could be an alternative to produce a low PA content ingredient minimizing mineral losses.

Extrusion is a widely used technology for the production of cereal-based foods, suitable for making a wide variety of products, such as cream soups, baby foods, snacks and textured vegetable protein (González et al., 2002a). This process provides high versatility of operations, such as mixing, cooking, drying and partial texturing. It is also beneficial from the standpoint of the use of energy, manpower and space required for installation (González et al., 2002b). Its use reduces the cost of post-extrusion drying and guarantees an improved shelf-life of the product without the need for cooling or refrigeration and produce unique product shapes with high quality.

Currently, limited information is available on extrusion processing of previously soaked whole rice in order to obtain whole rice extruded products to be used as a basis for the development of food. For this reason, the purposes of this study

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were to investigate: (i) the effects of soaking conditions on PA and nutrient content of rough rice; (ii) the effects of extrusion conditions on the physicochemical properties, PA content and other nutrients of soaked whole grain rice.

Materials and methods

Long whole rice grains (*Oryza sativa*) were supplied by Molino Los Cerrillos S.A. (Santa Fe, Argentina).

Soaking process

A surface response methodology with a 3^2 factorial experimental design (three central points) for the soaking treatment was used. Factor levels were soaking time (24, 36 and 48 h) and temperature (35, 45 and 55 °C). Approximately 50 g of rough rice was soaked with 5.5 g/L lactic acid solution (1:2 g/mL) at different experimental conditions. The moistened seeds were dried in an oven at 80 °C and dehulled using an standard rice miller (Galliver, San Salvador, Entre Ríos, Argentina), milled using a Ciclotec Sample Mill, (UD Corporation, Boulder, CO) and kept at 4 °C until the analysis.

For the soaking process at pilot plant scale, an amount of 3 kg of whole rice was previously washed in a 0.2% NaClO solution during 15 min and then soaked at the chosen condition (45 °C during 24 h in a 5.5 g/L lactic acid solution). After that, the grains were dried to almost 13 g/100 g moisture content using a tray dryer at 40 °C, dehulled and milled to grits (1190–420 µm particle size) using a roll mill with a sieve of 0.5 mm (Retsch-Muhle, Haan, Germany).

Extrusion process

Sample

The grits obtained in by pilot plant scale soaking process were used for extrusion.

Extrusion process

The extrusion process was carried out with a Brabender 20 DN single-screw extruder, using a 4:1 compression ratio screw, a 3/20 mm (diameter/length) die and a screw speed of 150 rpm. The effects of grits moisture (M) and extrusion temperature (T) were analyzed by surface response methodology using a 3^2 factorial design with triplicate of the central point, resulting 11 runs. The levels of each factor were: T: 160–175–190 °C and M: 14–16.5–19 g/100 g. Rice grits samples were conditioned by adding water to reach the moisture level corresponding to each experimental sample, 1 h before each run. All extruded samples were ground with a laboratory hammer mill (Retsch Muhle, Germany) using a 2 mm sieve and then with a Cyclotec mill (UD Corp Boulder Colorado, USA) using a 1 mm sieve and finally kept in plastic bags hermetically sealed until their evaluation.

Physicochemical properties of extrudates

Diameters were measured with a Vernier caliper on 10 pieces of sample and axial expansion (E) was determined as the ratio $E = D \times d^{-1}$, where D is the extrudate diameter (average of 10 determinations) and d is the die diameter. Extrudate specific volume (SV) was determined as $SV = \pi . (D/2)^2 . l$/weight (d.b.) (cm^3/g), corresponding to an extrudate piece of about 15 cm long (l) and diameter (D). This procedure was applied to 10 pieces and the average is reported.

Solubility (S) and water absorption (WA) were determined by the method of Anderson et al. (1969) modified by González et al. (2002a) and Torgensen & Toledo (1977); then again modified by González et al. (1995), respectively.

Proximate composition

Dry matter (DM) and ashes were determined by gravimetric measure according to AOAC 935.29 and AOAC 923.03, respectively (AOAC, 1995). Protein content was determined by the Kjeldahl method AOAC 920.53 (AOAC, 1995), using 5.95 as nitrogen-to-protein conversion factor (Juliano, 1985).

Free phosphorus, phytic acid phosphorus (WPPA and GPPA)

Free phosphorus (WFP), phytic acid phosphorus from soaking water (WPPA) and phytic acid phosphorus from whole grain rice (GPPA) were determined according to AOAC (1993) and AOAC (1995) methods.

Total soluble phenolic content

An aliquot of 0.05 g of sample was extracted with 80:20 solution of methanol/water in a vortex mixer (Decalab Eternity, Buenos Aires, Argentina) for 30 min and then centrifuged at $8000 \times g$ (Z160M Hermle, Wehingen, Germany). The supernatant was assay for polyphenol content using the Folin–Ciocalteau reagent described by Singleton et al. (1999). A standard curve with Gallic acid (ICN Biomedicals Inc., Irvine, CA) was used for calibration. Results were expressed as Gallic acid equivalent (mg GAE) per 100 g extruded product (dry basis).

Trolox equivalent antioxidant capacity

The antioxidant capacity was estimated according to Cian et al.'s (2014) ABTS•+ radical cation decolorization assay. To estimate the trolox equivalent antioxidant capacity (TEAC), a concentration-response curve for the absorbance at 734 nm for ABTS•+ as a function of concentration (0–2.5 mmol L^{-1}) of standard Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) in 0.01 mmol L^{-1} PBS (pH 7.4) was performed. The absorbance reading was taken at 6 min after initial mixing. Results were expressed as µmol trolox/g extruded product (d.b).

Losses produced by soaking

Percentage losses of dry matter, ashes, proteins and PA were estimated as the difference between initial and final content related to the initial value.

Protein digestibility

Protein digestibility (PD%) was determined as described by Rudloff & Lönnerdal (1992) with modifications. Approximately 2.3 g of sample was dispersed in 10 mL of distilled water, adjusted to pH 2 with 6 mol/L HCl and pepsin was added in order to have 1/16 enzyme/protein ratio. The samples were kept in dark, in a shaking water bath at 37 °C for 30 min. Then, the pH was gradually increased to 7.0 with 1 mol L^{-1} $NaHCO_3$ and 2.5 mL of pancreatin solution (0.4 g/100 mL in 0.1 mol L^{-1} $NaHCO_3$) was added and incubated for 1 h at 37 °C. Digested samples were immediately placed in boiling water for 10 min to inactivate the enzymes. On 5 mL aliquot of the digested samples, 5 mL of 200 g/L TCA was added, stayed 30 min at 4 °C and centrifuged 30 min at $5000 \times g$. PD% was defined as non-protein nitrogen (NPN) after digestion, in relation to the total nitrogen content (TN).

$$PD\% = 100 \times NPN/TN.$$

Mineral bio-accessibility

A modification of the widespread *in vitro* Miller et al. (1981) method, according to Drago et al. (2005) was followed.

The samples were ground previously and then prepared to 10 g solid/100 g dispersion using deionized water. Aliquots (25 g) of homogenized samples were adjusted to pH 2.0 with 4 mol/L HCl and after addition of 0.8 mL pepsin digestion mixture (160 g/L pepsin (Sigma P-7000) solution in 0.1 mol/L HCl), were incubated at 37 °C for 2 h in a shaking water bath. At the end of pepsin digestion, dialysis bags containing 20 mL of 0.15 mol/L PIPES (piperazine-N,N0-bis [2-ethane-sulfonic acid] disodium salt) buffer (Sigma P-3768) were placed in each flask and were incubated for 50 min in a shaking water bath at 37 °C. Pancreatin-bile mixture (6.25 mL of 25 g/L bile (Sigma B-8631), 4 g/L pancreatin (Sigma P-1750) solution in 0.1 mol/L $NaHCO_3$) was then added to each flask and the incubation continued for another 2 h. Then, bag contents were weighted and analyzed for its mineral content by flame atomic absorption spectroscopy. Mineral dialyzability was calculated from the amount of each dialyzed mineral expressed as a percentage of the total amount present in each sample.

$$\text{Dialyzable Mineral (DM\%)} = [D/(W \times A)] \times 100,$$

where, D is the total amount of dialyzed mineral (mg); W is the weight of sample (g) and A is the concentration of each mineral in the sample (mg/g).

Statistical analysis

The results were expressed in dry basis. Each experiment was performed at least in duplicate and each assay was performed by triplicate. Response surface and analysis of variance was carried out using the software Statgraphics Plus 5.1 (Warrentong, VA). The statistical differences among samples were determined using the least significant difference (LSD) test with a level of signification $\alpha = 0.05$.

Results and discussion

Soaking treatment

Table 1 shows water free phosphorous (WFP) and water phosphorus from phytic acid (WPPA) in the soaking media; percentage losses of grain phosphorus phytic acid (GPPA), dry matter (DM), protein and ashes. Table 2 shows p values of the regression model coefficients corresponding to each response. It can be observed that lack-of-fit is not significant ($p > 0.05$) in all cases, which means that regression models are adequate to explain the effect of factors (T and t) on each response.

The effects of time and temperature on WFP and WPPA were significant in the linear terms of time (t) and temperature (T), in T^2 for WFP, in T^2 and $t \times T$ for WPPA.

Table 1. Effect of soaking conditions on WFP, WPPA and percentage losses of GPPA, DM protein and ash.

Soaking (Temperature–time)	WFP (mg/100 g)	WPPA (mg/100 g)	GPPA losses %	DM losses %	Ash losses %	Protein losses %
35 °C–24 h	8.5	2.2	23.73	1.15	5.35	1.07
35 °C–36 h	9.86	2.38	26.10	1.27	6.04	3.14
35 °C–48 h	13.16	3.23	27.08	1.77	6.09	5.00
45 °C–24 h	13.64	4.71	36.68	1.56	8.41	5.78
45 °C–36 h	28.97	6.87	39.72	1.75	9.19	6.59
45 °C–36 h	28.45	7.67	42.87	1.70	9.96	6.05
45 °C–36 h	29.75	5.81	38.28	1.78	11.11	6.44
45 °C–48 h	31.55	11.46	46.99	2.18	14.26	6.73
55 °C–24 h	41.03	19.73	51.08	2.55	20.16	9.90
55 °C–36 h	48.87	23.75	54.64	2.71	28.69	11.89
55 °C–48 h	53.67	31.85	54.12	2.94	31.94	11.96

Figure 1(a) shows the main effects of time and temperature on WFP. Soaking at 55 °C during 48 h released the highest amount of WFP (53.67 mg P/100 g d.b). Phosphorus occurs in the grain as phytic acid (PA) or attached to other components. P release into the soaking medium can be due to different processes. Firstly, via enzymatic hydrolysis of higher inositol phosphates (IP6-IP5) to lower inositol phosphates (IP4, IP3, IP2, IP) and inorganic phosphate, induced by the enhanced activity of endogenous cereal phytases during soaking; and secondly, by diffusion in the medium. In this case, PA hydrolysis is the main mechanism involved in P release and increase with temperature because of phytase activation. Figure 1(b) shows that the maximum value of WPPA is reached at 55 °C during 48 h (31.85 mg P/100 g d.b) meaning temperature favored phytate diffusion in acidic media.

Figure 2 shows surface response for the GPPA losses. The values of percentage losses range 23.7–54.64. The highest losses were obtained at 55 °C which is in agreement with the higher values of WFP and WPPA corresponding to the release of P from PA mediated by hydrolysis and PA diffusion in the water. PA hydrolysis could be attributed mainly to the action of phytases whose maximum activity would be at temperature around 55 °C.

Table 2. p Values of the polynomial regression model coefficients corresponding to WFP, WPPA and percentage losses of GPPA, DM, P and ash for the soaking process.

	Response					
Source of variation	WFP	WPPA	GPPA losses	DM losses	Protein losses	Ash losses
Time (t)	**0.0063**	**0.0129**	0.1009	**0.0037**	**0.0095**	**0.0162**
Temperature (T)	**0.0006**	**0.0011**	**0.0048**	**0.0006**	**0.0008**	**0.0014**
t^2	0.0510	0.1685	0.9942	**0.0425**	0.2002	0.7843
$t \times T$	0.0731	**0.0272**	0.9534	0.1045	**0.0337**	**0.0294**
T^2	**0.0354**	**0.0088**	0.4284	**0.0111**	0.0785	**0.0107**
Lack-of-fit	0.0703	0.7824	0.4461	0.2933	0.1196	0.3140
R^2	0.9772	0.9972	0.9725	0.9953	0.9832	0.9901

Bold values indicates significant differences ($p < 0.05$).

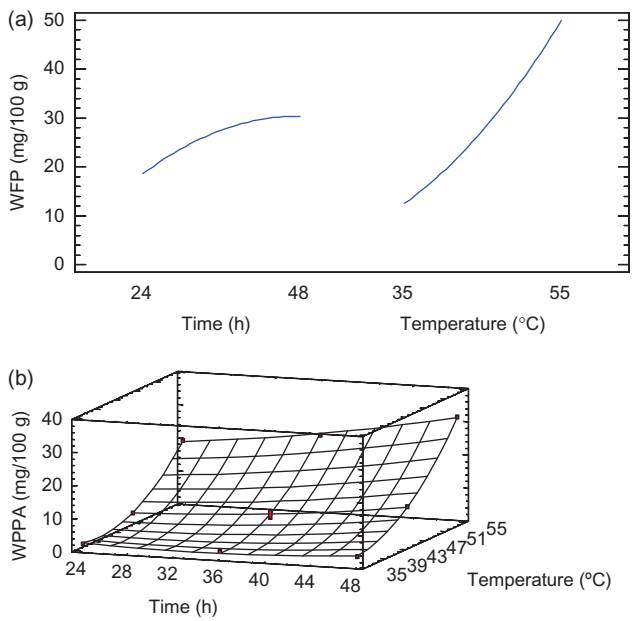

Figure 1. (a) Main effects of temperature and time on water free phosphorous (WFP); (b) surface response for water phosphorous from phytic acid (WPPA) release in soaking media.

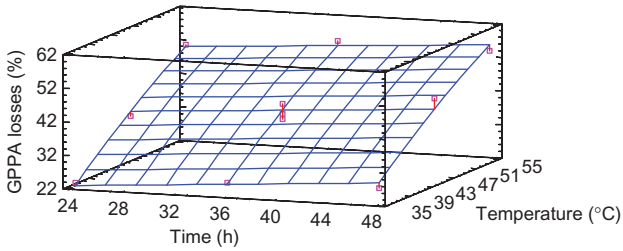

Figure 2. Surface response for grain phosphorus phytic acid (GPPA) losses corresponding to soaking experimental design.

Table 3. Effect of extrusion processes on Expansion (E), Specific Volume (SV), Water Absorption (WA) and Solubility (S), Total Soluble Phenolic Compounds (TSPC) and Trolox Equivalent Antioxidant Capacity (TEAC) of extruded soaked whole rice flour.

Extrusion (Temperature and moisture)	E	SV (cm^3/g)	WA (mL/g)	S (g/100 g)	TSPC (mg GAE/g)	TEAC (μmol trolox/g)
160 °C 14%	3.28	8.42	3.75	59.31	23.41	34.42
160 °C 16.5%	2.92	6.72	4.05	55.48	17.73	29.21
160 °C 19%	2.67	5.68	4.35	45.44	16.61	27.68
175 °C 14%	3.04	9.86	3.66	65.31	29.60	35.20
175 °C 16.5%	2.69	8.45	4.04	64.49	25.27	36.05
175 °C 16.5%	2.65	8.83	3.77	66.20	23.77	34.48
175 °C 16.5%	2.63	8.91	3.51	58.88	22.95	32.81
175 °C 19%	2.33	7.60	3.69	56.20	21.32	32.35
190 °C 14%	2.07	10.99	3.41	61.28	36.77	39.95
190 °C 16.5%	1.82	11.06	3.58	65.14	36.27	39.57
190 °C 19%	1.64	8.20	4.43	61.14	32.07	38.22

Several authors studied optimum conditions to achieve the maximum enzymatic activities in several cereals. Yoshida et al. (1975) reported that acidic conditions in the soaking medium (lactic acid) favor hydrolysis because endogenous phytases have an optimum of action around pH 4–5. In the case of whole grain rye, optimum pH and temperature of phytase activity are at 5.5 and 48 °C, respectively (Tijskens et al., 1997). Peers (1953) reported pH 5.15 and temperature slightly higher than 55 °C for whole grain wheat and Egli et al. (2003) observed optimum conditions for buckwheat phytases at pH 5.0 and 55 °C.

DM losses for whole rice were between 1 and 3%, which can be considered negligible taking into account that, a 30% of DM losses were observed when brown rice was soaked (Albarracín et al., 2013). Ash losses ranged 5.35–31.94% and protein losses, 1.07–11.96%. Dry matter lost during soaking was constituted by 33% protein, 6% ashes and 37% PA.

From the results discussed above, the soaking of rough rice at 45 °C and 24 h was selected for obtaining soaked flour for the extrusion experiments since it combine low nutrient losses with important PA losses.

Extrusion

Table 3 shows the results of the evaluation of extruded products and Table 4 the corresponding ANOVA. It is observed that the lack of fit was not significant ($p > 0.05$) in all cases, which means that regression models were adequate to explain the effect of factors (*T* and *M*) on each response.

Figure 3 shows extruded products obtained at different conditions. It is possible to observe the different expansion reached by the extrudates. *E* values ranged between 1.64 and 3.28, the highest values corresponding to 160 °C and 14% M condition,

Table 4. *p* Values of the polynomial regression model coefficients corresponding to the effect of extrusion variables on Expansion (E), Specific Volume (SV), Water Absorption (WA), Solubility (S), Total Soluble Phenolic Compounds (TSPC) and Trolox Equivalent Antioxidant Capacity (TEAC) of extruded soaked whole rice flour.

Source of variation	Response					
	E	SV	WA	S	TSPC	TEAC
Temperature (*T*)	**0.0006**	**0.0040**	0.3776	0.1003	**0.0037**	**0.0217**
Moisture (*M*)	**0.0021**	**0.0058**	0.1262	0.1326	**0.0203**	0.1037
T^2	**0.0053**	0.4606	0.3866	0.2877	0.0878	0.5875
$T \times M$	0.1170	0.9241	0.5112	0.2149	0.4665	0.2612
M^2	0.2026	0.1868	0.8208	0.3381	0.3788	0.9711
Lack-of-fit	0.2188	0.1181	0.4500	0.9786	0.3279	0.7407
R^2	99.46	94.49	61.90	91.29	0.9749	0.9428

Bold values indicates significant differences ($p < 0.05$).

as it was expected according to previous works using whole rice grits (González et al., 2013a). The effects of *T* and *M* were significant in both linear terms and the quadratic term of *T*. *E* is an important characteristic of snack type products. It is directly related to melt viscoelasticity and describes the degree of puffing undergone by the sample as it exits the die of the extruder (González et al., 2006).

SV has been proposed as a good indicator of degree of cooking (DC) for cereal products extruded, which is directly related to granule structure destruction (González et al., 2002a). The ANOVA results show that only linear terms of *T* and *M* were significant. SV values ranged 11.06 and 5.68 (cm^3/g) that would correspond to the highest and lowest DC. The inverse effect of *M* on SV is attributed to the fact that as M increases friction level inside the extruder decreases and consequently starch granules destruction is reduced.

Water absorption (WA) and Solubility (S) values ranged 3.41–4.43 (mL/g of dry matter) and 45.44–66.20 (g/100 g), respectively. For both responses, none of the model terms were significant, so that other type of function would be necessary if a mathematical model would be desired. However, the lowest value of WA was obtained at 190 °C and 14%, which suggests that at this extrusion condition starch granule destruction is maximized and consequently swelling would be minimized.

In the case of *S* the effects of *T* and *M* are clearer. *S* was inversely affected by *M* at 160 and 175 °C and directly by *T* at 16.5 and 19% *M*. A direct relation was observed between *S* and *SV* ($S = 2.89SV + 35$; with a $R^2 = 0.612$); confirming that *S* increases as DC increases. These results are in agreement with those published elsewhere for maize and whole grain maize extrusion (González et al., 2002a; Pastor-Cavada et al., 2011).

The results obtained by González et al (2013b) for whole grain of the same rice variety extruded at the same extrusion conditions were: Expansion: 2.46–3.6, Specific Volume: 3.47–8.64 cm^3/g, Water Absorption: 5.1–7.7 ml/g and Solubility 21–33 g/100 ml. The comparison of these values with those obtained with extruded soaked whole rice indicates that DC reached by soaked whole grain is higher than that of un-soaked one. It would suggest that soaking, besides promoting losses of soluble proteins, would produce structural changes in grit particles causing a reduction of mass output (data not shown) increasing residence time and consequently higher DC.

TSPC ranged 16.61–36.77 mg GAE/100 g (d.b.) and TEAC, 27.68–39.95 μmol trolox/g (d.b.). Figure 4(a and b) show surface response for TSPC and TEAC, respectively. Taking into account initial content of TSPC of whole rice grains (34.63 ± 2.12 mg GA/100 g) soaking-extrusion processes reduced TSPC from 0 to 52%, depending of extrusion conditions. Phytochemicals may exist in

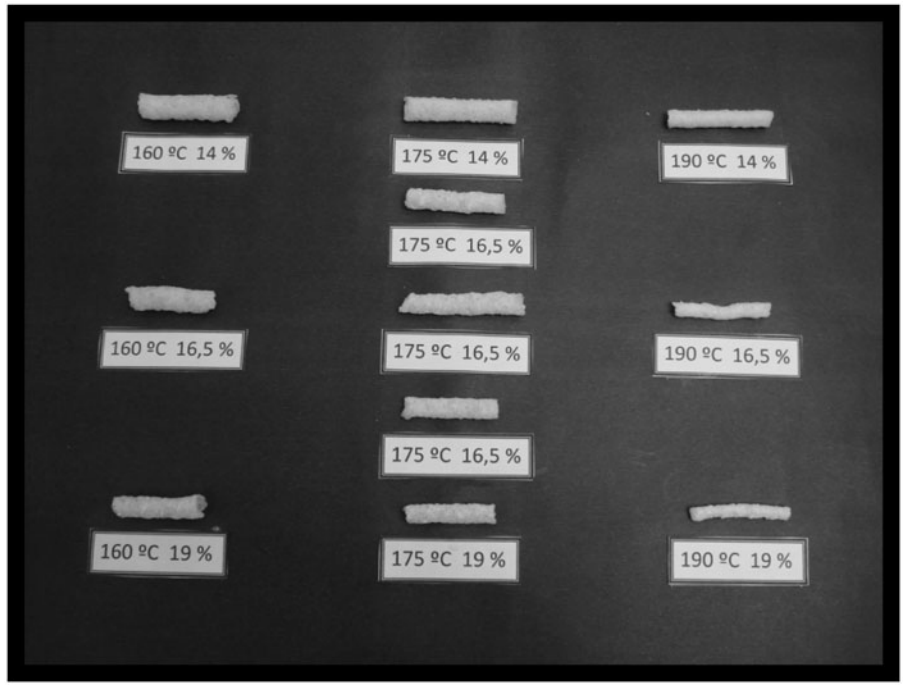

Figure 3. Extruded products of soaked whole rice grains at different temperature and moisture content.

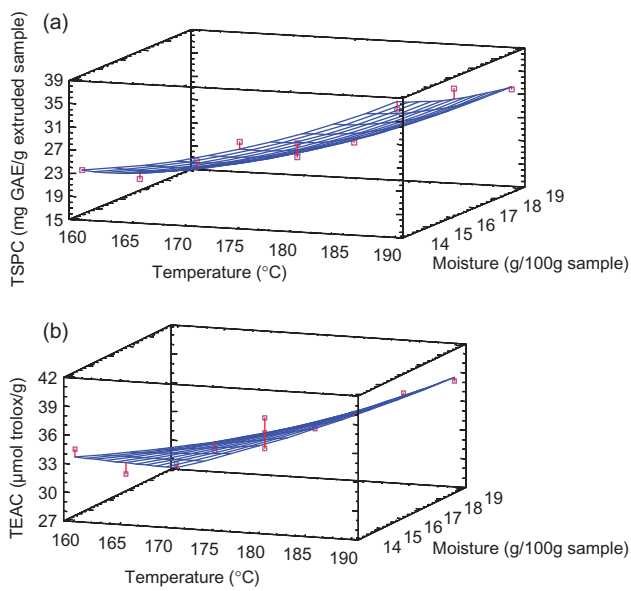

Figure 4. Surface response for (a) total soluble phenolic content (TSPC) and (b) trolox equivalent antioxidant capacity (TEAC) corresponding to extrusion experimental design.

free, soluble conjugate and insoluble bound forms (Adom & Liu, 2002). Phenolic compounds may undergo decarboxylation due to high barrel temperature and high-feed moisture (Brennan et al., 2011) and alteration of molecular structure of phenolic compounds (Altan et al., 2009). Moreover, different *T–M* conditions could produce loss of phenolics or reduced extraction. In this sense, higher moisture content may promote polymerization of phenols, leading to reduced extractability (Repo-Carrasco-Valencia et al., 2009). However, extrusion process could contribute to free some bound phenolic compounds in the matrix, TSPC being a balance of the losses and the release of phenolics.

TEAC of whole rice is mainly due to the presence of bioactive compounds, including polyphenols, γ-oryzanol and tocopherols, found mostly in the outer layers (Butsat & Siriamornpun, 2010). Soaking-extrusion increased in almost all conditions TEAC activity compared to initial value of the brown rice (13.39 ± 0.16 µmol trolox/g). Moreover, TEAC was correlated with TSPC ($R^2 = 0.8996$). Other studies have reported significant correlation for TEAC of rice samples and phenolic content (Choi et al., 2007; Shen et al., 2009) and for other cereals like wheat, oats, barley and rye (Fardet et al., 2008).

In order to obtain a final product with high expansion ratio and an intermediate degree of cooking, extrusion condition at 16.5% *M* and 160 °C was selected. Table 5 shows PA content, protein digestibility and mineral bio-accessibility of the different products obtained at pilot plant scale at selected conditions. The results show that PA content was reduced by 52% by soaking and 77% by soaking-extrusion. Soaking process slightly decreased PD% probably due to the loss of soluble proteins or the change in protein structure by acid soaking. Extrusion process did not further change PD% of soaked rice. Fe and Zn bio-accessibility were enhanced markedly by soaking rough rice, probably due to the degradation of PA. Then, a slightly decreased in both mineral bio-accessibility is caused by extrusion process. However, soaked-extruded products have higher bio-accessibility than brown rice.

Conclusions

Both, soaking and extrusion processes of whole grain rice ensure lower levels of PA by increasing activity of naturally present phytase and lixiviation or degradation. Extrusion-cooking produce extrudates with different physicochemical characteristics enhanced antioxidant activity and allow obtaining a product with good Fe and Zn bio-accessibility. Thus, both treatments were important to obtain a nutritionally improved whole grain product.

The products obtained could be consumed directly as expanded product (snacks) or as pre-cooked whole flour useful to be incorporated in formulated products. It is important to point out that these products are made with whole grains ''free of gluten'' and they have very low amounts of phytases which are considered anti-nutrient components.

Table 5. Phytic acid content, protein digestibility and Zn and Fe bio-accessibility of brown rice, soaked and soaked-extruded products.

Sample	Phytic acid content (mg/100 g)	Protein digestibility (%)	Mineral bio-accessibility (%) Zn	Mineral bio-accessibility (%) Fe
Whole rice	740.09 ± 10.32^{a}	82.85 ± 2.56^{a}	11.46 ± 0.52^{c}	11.21 ± 1.15^{c}
Soaked whole rice (45 °C–24 h)	352.19 ± 2.14^{b}	76.74 ± 3.12^{b}	16.24 ± 0.82^{a}	17.13 ± 0.82^{a}
Soaked and extruded whole rice (160 °C- 16.5%H)	163.47 ± 7.86^{c}	76.81 ± 0.81^{b}	14.60 ± 0.73^{b}	14.50 ± 0.38^{b}
p Value	<0.0001	0.0163	<0.0001	<0.0001

Mean ± standard deviation; different letters mean significant differences between samples ($p < 0.05$).

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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