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FOOD COMPOSITION AND ANALYSIS

Germination and extrusion as combined processes for reducing phytates and increasing phenolics content and antioxidant capacity of *Oryza sativa* L. whole grain flours

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Abstract

Whole rice (WR) products with low phytic acid (PA) content and enhanced bio-functional components were obtained by the combination of germination and extrusion processes. Germination conditions (24 h – 35 °C), with a previous soaking process (24 h – 20 °C), were chosen according to the remnant PA content and germination rate. Specific mechanical energy consumption, expansion, sensorial and mechanical hardness, specific volume, solubility, water absorption, free phenolic content (FPC) and antioxidant capacity were evaluated. Results indicated that 175 °C and 14 g 100 g^{-1} of moisture were the most appropriate conditions to obtain expanded products and precooked flours based on germinated WR. Selected extruded product presented less PA content (821.6 9 ± 10.3 versus 695.2 0 ± 1.6 mg 100 g^{-1}) and higher Fe bio-accessibility, FPC (45.2 9 ± 1.61 versus 66.3 5 ± 3.35 mg GAE g^{-1}) and antioxidant capacity compared with WR (34.9 5 ± 0.8 versus 54.6 3 ± 1.6 μmol trolox g^{-1}). Combining germination–extrusion processes could be a strategy to obtain expanded products or precooked flours based on WR with enhanced health benefits.

Keywords

Antioxidant capacity, extrusion, germination, phenolics content, phytic acid, whole rice

History

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Introduction

Germination is a process that involves the uptake of water by a quiescent dry seed, resulting in the elongation of the embryonic axis. For rice, the embryo is germinated after rice is soaked in water for a certain period, and process finished when the embryonic axis is ∼1 mm long (Jiamyangyuen & Ooraikul, 2008). It has been reported that germinated whole rice (WR) offers a great deal of benefits because during sprouting process, chemical composition changes dramatically as the essential compounds produce biochemical activity and energy for the formation of the plant. Hydrolytic enzymes are activated and decompose large molecular substances, such as starch, non-starch polysaccharides and proteins, to small molecular compounds. Beyond changes in nutrient levels, germination can also generate bioactive components such as ascorbic acid, tocopherols, tocotrienols and phenolic compounds, thus resulting in an increase in antioxidant activity (Moongngarm & Khomphiphatkul, 2011; Moongngarm & Saetung, 2010). Antioxidants occurring in whole grains (WG) are considered the major contributors of potential health-benefits for humans.

It is also important to note that the availability of nutrients was increased by reducing the phytate content present in WG because of the activation of endogenous phytase. Phytates are compounds that the gastrointestinal tract does not have the capacity of degraded because of it lacks the necessary enzymes (Kumar et al., 2010; Patil & Khan, 2011). The complexes formed by the phytates and minerals cannot be absorbed by proteins and lipids, and in the case of proteins they also reduce the digestibility.

There are some problems with germinated WR from the viewpoint of safety and food sanitation as it is easily contaminated by micro-organisms. To prevent micro-organism growth, extrusion cooking has been reported to be a good way to pasteurize or sterilize materials for foodstuffs. Extrusion is a widely used technology for the production of cereal-based foods, and according to the process conditions, it produces phytate hydrolysis. It is also used for producing pre-cooked cereals and pulses, suitable for making a wide variety of products, such as cream soups, baby food, snacks, meals nutritionally improved 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 very beneficial from the standpoint of the use of energy, manpower and space required for installation (González et al., 2002b).

The aim of this work is to study the conditions to obtain germinated-extruded WR products and evaluate the effects of processes on bioactive compounds, physicochemical properties and phytate content.

Materials and methods

Rice samples

Rough long rice grains (*Oryza sativa* L.) were supplied by Los Cerrillos S.A (Los Cerrillos, Santa Fe, Argentina).

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Soaking and germination laboratory study

Prior to germination, the hydration characteristics of rice samples were studied. Using the 1:2 grain-to-solution ratio, samples were soaked in distilled water at different temperatures: 20, 35, 45, 55 °C. Small samples were removed at specified times during soaking (0.5; 1; 2; 3; 5; 7; 9; 12; 24 h), and moisture content was determined in order to study water uptake.

Laboratory germination was evaluated at 90–95 g 100 g^{-1} relative humidity and 35 °C using an oven (Bioelec, Santa Fe, Argentina). In brief, around 500 g of rough rice were washed with a NaClO solution (120 mgL^{-1} available chlorine), soaked in distilled water during 24 h at 20 °C, and germinated in an oven during three different times (24, 36 and 48 h). Rice grains were considered as germinated seeds when the young radicle, white root emerging from the lower end of the rice seed, was visible. The germination rate was determined by sampling around 100–200 grains from the germination basket and counting the germinated seeds (Cáceres et al., 2014). The results of three replicates were calculated as the percentage of germinated kernels to total kernels sampled.

Germinated grains were dried in an oven (Bioelec) at 40 °C until 13 g 100 g^{-1} of moisture content. Then, they were dehulled and milled (Ciclotec Sample Mill, UD Corp., Boulder, CO) to obtain germinated WR flour. Phytic acid (PA) was determined according to AOAC Anion-Exchange Method (AOAC, 1995) and AOAC Phosphorus Method (AOAC, 1993).

Soaking and germination at pilot plant scale

Around 6 kg of grains were washed in a NaClO solution (120 mgL^{-1} available chlorine) for 15 min, soaked in distilled water for 24 h at room temperature (20 °C), and finally germinated at 35 °C and 90–95 g 100 g^{-1} relative humidity for 24 h in an oven (Bioelec). Germinated grains were dried at 40 °C until 13 g 100 g^{-1} of moisture content. Finally, the grains were dehulled and milled to obtain grits with a particle size between 1.190 and 0.420 mm using a roll mill (Retsch-Muhle, Germany).

Extrusion process

To evaluate the effects of extrusion on physicochemical properties of extrudates based on germinated WR flour, a 3^2-factorial design was used. The independent variables were the temperature of extrusion (160, 175, and 190 °C) and moisture content (14, 16.5 and 19 g 100 g^{-1} sample) with the central point (175 °C – 16.5 g 100 g^{-1} sample) processed by triplicate. Samples were conditioned by adding water to reach the moisture level corresponding to each experimental sample, 1 h before each run.

Extrusion process was carried out with a Brabender (Duisburg, Germany) 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. While the extruder feeding section was maintained cold atmosphere by circulating water through the jacketed device, the metering and die sections were both kept at the temperature corresponding to each run by using the heat control device of the extruder. The feeding rate of the extruder was at full capacity. Experimental samples were taken after stationary state was established, then torque (Brabender Units – BU) and mass output (g min^{-1}) were measured.

All extruded samples were dried in an oven at 40 °C (Bioelec) until a moisture content of 6 g 100 g^{-1} was reached, this moisture level was considered adequate for texture evaluation. Each dried sample was divided into several portions and kept in plastic bags hermetically sealed until their evaluation.

Extrusion response evaluation

Specific mechanical energy consumption (SMEC) was measured according to González et al. (2002a) using the following formula: SMEC (J g^{-1}) = $k \times T \times N \times Qa^{-1}$, where k is: 61.6×10^{-3}, T is torque in BU, N is screw speed (rpm) and Qa (g min^{-1}) is the mass output, referring to feeding moisture level.

Product response evaluation

Diameters were measured with a caliper (Vernier, 0–150 mm, Stronger Argentina) on 10 pieces of sample, and expansion (E) was determined as the ratio $E = D\ d^{-1}$, where D is the extrudate diameter (average of 10 determinations) and d is the die diameter. Extrudate specific volume (SV) was obtained by calculating the volume/weight (dry basis) ratio ($cm^3\ g^{-1}$), corresponding to extrudates pieces of about 10 cm long. Solubility (S) and water absorption (WA) were determined according to González et al. (2002a). Sensorial hardness (SH) was evaluated by a trained panel (three judges) with a quality descriptive analysis according to Murray et al. (2001) using a hardness nine-point scale, the highest score (9) corresponding to the hardest sample. The score given to each sample was obtained by consensus among the judges.

Mechanical hardness (MH) was measured using a Model 4411 Instron Texturometer (Shakopee, MN) by determining the compressive strength of a piece of 5 cm length, with a load of 1000 N and compression rate of 10 mms^{-1} (similar to chewing speed), according to Park et al. (1993). Each procedure was performed at least five times.

Composition of samples

Proteins, moisture, ash and extractable lipids were determined by AOAC methods (1995). Assessment of minerals (Fe and Zn) was made by Flame Atomic Absorption Spectroscopy using an Atomic Absorption spectrophotometer Analyst 300 (Perkin Elmer, Waltham, MA). Dietary fiber was determined by AOAC 985.29 (2000) with a commercial Megazyme kit (Wicklow, Ireland).

PA was determined according to AOAC anion-exchange method and AOAC phosphorus method (AOAC, 1993, 1995).

Free phenolic content (FPC): The samples were dispersed at 10 g 100 mL^{-1} in a 80:20 methanol/water solution and extracted for 30 min using a vortex mixer (Decalab Eternity, Argentina), then centrifuged at $10\,000 \times g$ for 10 min (Z160M Hermle, Germany). The supernatant obtained was determined for polyphenol content applying the technique described by Singleton et al. (1999) using the Folin–Ciocalteau reagent. A standard curve with gallic acid (GAE) (ICN Biomedicals Inc., USA) solutions was used for calibration. Results were expressed as GAE equivalent (mg GAE) per 100 g extruded product (dry basis).

Bound Phenolic Content (BPC): For BPC, the previous residue from methanol extraction (in FPC) was alkaline hydrolyzed with a 2 $molL^{-1}$ NaOH solution by Qiu et al. (2010). Then, three consecutive hexane extractions were performed to remove lipids. Polyphenols released were extracted with ethyl acetate, evaporated to dryness in a Rotavapor (Büchi, Switzerland), and redissolved in a 50-mL methanol per 50 mL water solution. Finally, BPC were determined by Singleton et al. (1999).

To estimate the total equivalent antioxidant capacity (TEAC), a concentration–response curve for the absorbance at 734 nm for ABTS+ as a function of concentration of standard Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) solution (0–2.5 $mmolL^{-1}$) in 0.01 $mmolL^{-1}$ (PBS, pH 7.4) was performed according to Cian et al. (2014).

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 molL^{-1} 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 molL^{-1} $NaHCO_3$ and 2.5 mL of pancreatin solution (0.4 g 100 mL^{-1} in $NaHCO_3$ 0.1 molL^{-1}) 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. For 5 mL aliquot of the digested samples, 5 mL of a 200 g L^{-1} trichloroacetic acid (TCA) solution was added, left for 30 min at 4 °C and centrifuged for 30 min at 5000 × *g*. PD % was calculated as non-protein nitrogen (NPN) after digestion, in relation to the total nitrogen content (TN).

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

Non-protein nitrogen

Donovan & Lönnerdal (1989) stated that NPN was determined by the semi-micro Kjeldahl in the soluble fraction of a 0.23 g 100 mL^{-1} sample dispersion, after protein precipitation with equal volume of a 20 g 100 mL^{-1} TCA solution and centrifugation at 10 000 × *g* for 20 min.

Mineral bioaccesibility

The method of Drago et al. (2005) was followed in this work. Mineral dialyzability was calculated from the amount of each dialyzed mineral expressed as a percentage of the total amount presented in each sample.

Statistical analysis

Each assay was performed in triplicate. Response surface and analysis of variance was carried out using Statgraphics Plus 5.1 software (Statistical Graphics Corporation, Warrentong, VA). The statistical differences among samples were determined using least significant difference test. The accepted level of significance was $p < 0.05$. Figures were drawn using OriginPro 8 (OriginLab Corporation, Northampton, MA).

Results and discussion

Soaking and germination process

Figure 1 shows water uptake of rough rice at different temperatures (20, 35, 45, 55 °C) for 24 h. Rice moisture content prior to soaking was 12.5 g 100 g^{-1} and was gradually increased to 30–33 g 100 g^{-1} at different times and temperatures. Since all samples reached appropriated moisture content at 24 h, soaking at 20 °C (room temperature) for 24 h prior to germination step was performed.

Results of laboratory rice germination at 35 °C showed that around 75 ± 5% of the grains were germinated after 24 h and that longer times did not allow obtaining higher rates of germination.

In regards of PA content, soaking–germination laboratory study involved ~30% losse at soaking step (560.7 mg PA 100 g^{-1} of sample), taking into account the initial content 821.69 mg PA 100 g^{-1} of sample. Then, germination process reduced the PA content ~30–40% depending on the germination time (24, 36 and 48 h), and 36 h of germination represented the best condition with the lowest level of PA. These results indicate that 24 h soaking time was sufficient to activate endogenous phytases of grains and both processes account for PA content reduction of 40% with respect to WR.

Moongngarm & Saetung (2010) studied brown and rough rice germination for 48 h at 28–30 °C, with a 48-h presoaking treatment in distilled water (room temperature), and accomplished

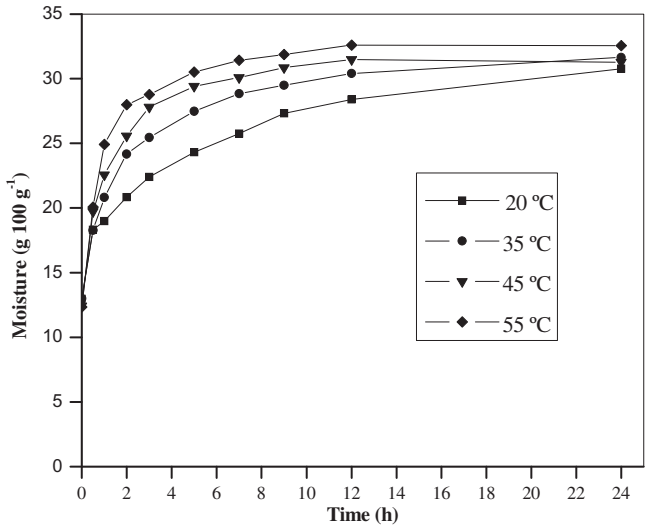

Figure 1. Water uptake of rough rice at 20, 35, 45 and 55 °C for 24 h.

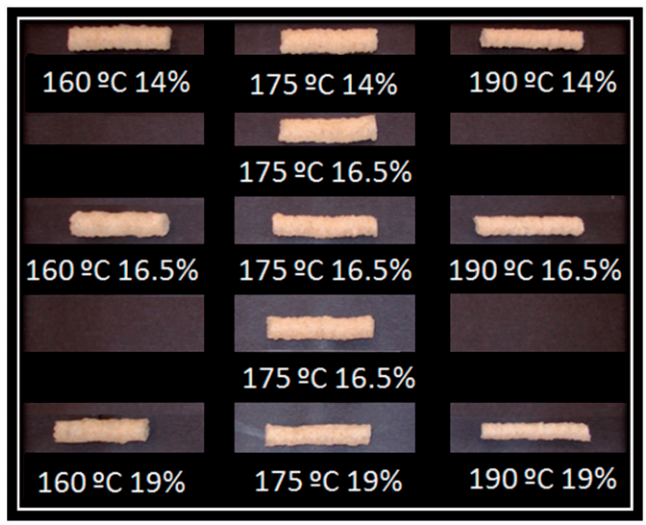

Figure 2. Extrudates products based on germinated WR obtained in the conditions of temperature and moisture content of the experimental design.

the same PA removal (~30%) as obtained in this study with the 24 h of germination and 24 h presoaking.

Moongngarm & Khomphiphatkul (2011) showed ~13% loss of PA for rough rice after 48-h soaking and 24-h germination treatment, but greater loss (60%) after 4 days germination.

Extrusion process

Figure 2 shows that the extrudates were obtained with germinated WR flour as per the experimental procedure described earlier. Table 1 shows the results of extrusion process and the properties of extruded products obtained by different extrusion conditions, and Table 2 lists the corresponding ANOVA for each response. It is observed that for all responses the lack of fit was not significant ($p > 0.05$), meaning that regression models are adequate to explain the effect of factors (*T* and *M*) on each response.

The highest values of SMEC and *E* corresponded to the sample obtained at 160 °C and 14 g 100 g^{-1} of moisture. Both responses presented an inverse relation with *T* and *M* (Figure 3a and b). As *T* and *M* increased, friction level in the extruder decreased, and

Table 1. SMEC, Expansion (E), SV, Solubility (S), WA, SH, FPC and TEAC.

Sample	SMEC	E	SH	SV	S	WA	FPC	TEAC
160 °C 14 g 100 g^{-1} M	502.07	3.26	7	6.05	42.31	5.43	43.49	25.79
160 °C 16.5 g 100 g^{-1} M	496.92	3.21	9	5.75	39.83	5.05	39.35	21.32
160 °C 19 g 100 g^{-1} M	457.01	2.83	8	4.90	33.11	5.48	26.23	17.37
175 °C 14 g 100 g^{-1} M	479.75	3.16	6	6.64	38.93	5.92	47.73	25.66
175 °C 16.5 g 100 g^{-1} M	384.35	2.60	5	7.43	39.21	5.40	60.05	23.81
175 °C 16.5 g 100 g^{-1} M	385.95	2.73	4	7.50	40.53	5.55	62.71	23.90
175 °C 16.5 g 100 g^{-1} M	382.75	2.75	4	7.76	41.00	5.55	64.58	23.44
175 °C 19 g 100 g^{-1} M	361.43	2.62	5	7.44	35.48	6.18	63.30	21.32
190 °C 14 g 100 g^{-1} M	345.99	2.64	2	10.28	41.36	5.43	72.06	26.65
190 °C 16.5 g 100 g^{-1} M	343.99	2.43	3	9.41	40.93	6.05	74.24	30.34
190 °C 19 g 100 g^{-1} M	325.04	2.06	4	7.70	33.27	6.65	72.51	27.13

SMEC: J g^{-1}; SV: cm^3 g^{-1}; S: g 100 g^{-1}; WA: g water g^{-1}; FPC: mg GAE 100 g^{-1}; TEAC: μmol Trolox g^{-1}.

Table 2. Degrees of significance (p values) of the polynomial regression model coefficients, corresponding to SMEC, Expansion (E), SV, Solubility (S), WA, SH, FPC and TEAC.

	Response							
Source of variation	SMEC	*E*	SH	*SV*	*S*	WA	FPC	TEAC
Temperature (T)	**0.0050**	**0.0083**	**0.0088**	**0.0026**	0.9040	**0.0094**	**0.0026**	**0.0492**
Moisture (M)	**0.0239**	**0.0121**	0.5528	**0.0333**	**0.0118**	**0.0187**	0.8443	0.1193
T^2	0.0861	0.5095	0.1264	0.4959	0.7368	0.1304	0.1227	0.9936
$T \times M$	0.8734	0.4095	0.4778	0.0863	0.6105	**0.0212**	0.0602	0.1853
M^2	0.3589	0.6196	0.8479	0.0937	**0.0369**	**0.0216**	0.0736	0.9653
Lack of fit	*0.1530*	*0.2413*	*0.1441*	*0.0606*	*0.2163*	*0.1116*	*0.0721*	*0.8029*
R^2	*0.9303*	*0.9383*	*0.8725*	*0.8966*	*0.8921*	*0.9038*	*0.9124*	*0.9076*

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

consequently SMEC decreased. Since only linear terms of T and M were significant, a linear tendency expansion was followed. SMEC showed similar tendency, and the slight surface curvature of response (Figure 3a) could be attributed to the effect of the T^2 term, which though not significant, it was not negligible (p: 0.0861).

In regards of SH, Figure 3(c) shows the behavior of SH related to the T and M extrusion conditions, indicating a decrease with the increase in T. The response surface for the SH had linear tendency because the unique significant variable was the temperature of extrusion term. The highest value of SH corresponded to the sample obtained at 160 °C – 16.5 g 100 g^{-1} M (SH = 9) and the lowest ones at 190 °C – 14 g 100 g^{-1} M (SH = 2).

Sample extruded in the condition of 175 °C and 14 g 100 g^{-1} of moisture was the most enjoyable from the sensory point of view (SH = 6). It presented good expansion and intermediate hardness, with higher SV than the condition of 160°C and 14 g 100 g^{-1} of moisture, indicating higher Cooking Degree (CD).

Regarding MH, force values were between 26.2 and 34.6 N, was lower for the samples obtained at 190 °C and higher at 160 °C. A linear relationship between MH and SH was observed ($y = 1.0132x + 25.125$) and there was a good correlation between both responses ($R^2 = 0.8273$). It was known that the SH and MH were related to the CD and decreased when T increased. Furthermore, it was found that the sample extruded at 160 °C – 19 g 100 g^{-1} moisture had one of the highest values of SH (SH = 8) and the lowest SV (4.9 cm^3 g^{-1}). Thus, based on these parameters, it would have the lowest CD.

Regarding SV (Figure 3d), only linear T and M terms were significant and the lowest value was obtained at 160 °C – 19 g 100 g^{-1} M. An inverse correlation between SH and SV (SH = 14.936 − 1.327 SV; $R^2 = 0.9227$) was found. SV can be considered as a good indicator of CD. Also, an inverse relationship between SV and other responses was observed, as T increases CD increases, affecting negatively to E, SMEC and SH. These results were according to those found by Pastor-Cavada et al. (2013) for extrusion of whole grain rice flours.

In regards to hydration properties of extruded flours, ANOVA results show that for S, only the linear term of M and the corresponding quadratic term M^2 were significant. S was inversely related with M, meaning that certain dextrinization reached at low M conditions would be due mainly to mechanical effects (friction level), but at high M content, thermal effects would predominate.

In case of WA, the ANOVA results show that all terms, except T^2, were significant. The highest value of WA corresponds to the higher T and M (6.65 g water g^{-1} sample). Once cooking is completed, WA should be increased with M and decreased with T (González et al., 2002a), thus the results would indicate an incomplete destruction of native granular structure.

Moreover, it was observed that even though the regression models could describe the effects of extrusion conditions on S and WA, the range of values corresponding to S (33.11–42.3 g 100 g^{-1} sample) and WA (6.65–5.05 g water g^{-1} sample) was smaller than those observed for corn or rice grits obtained from pure endosperm. González et al. (2002a) reported that values for extruded grits coming from degermed and dehulled grains much higher than those obtained in this study for germinated whole grain rice. Since S is a direct indicator of CD, a lower CD would be obtained when grits from WG are used in comparison with the refined ones (González et al., 2013). As a consequence of the presence of bran and germ particles, shorter range of S and WA values are obtained and also an incomplete cooking process is attained by starch granules. The perturbation of the particles transport inside the extruder not only would retard the cooking process of starchy particles caused by friction and thermal effects, but also could broaden the residence time distribution of particles

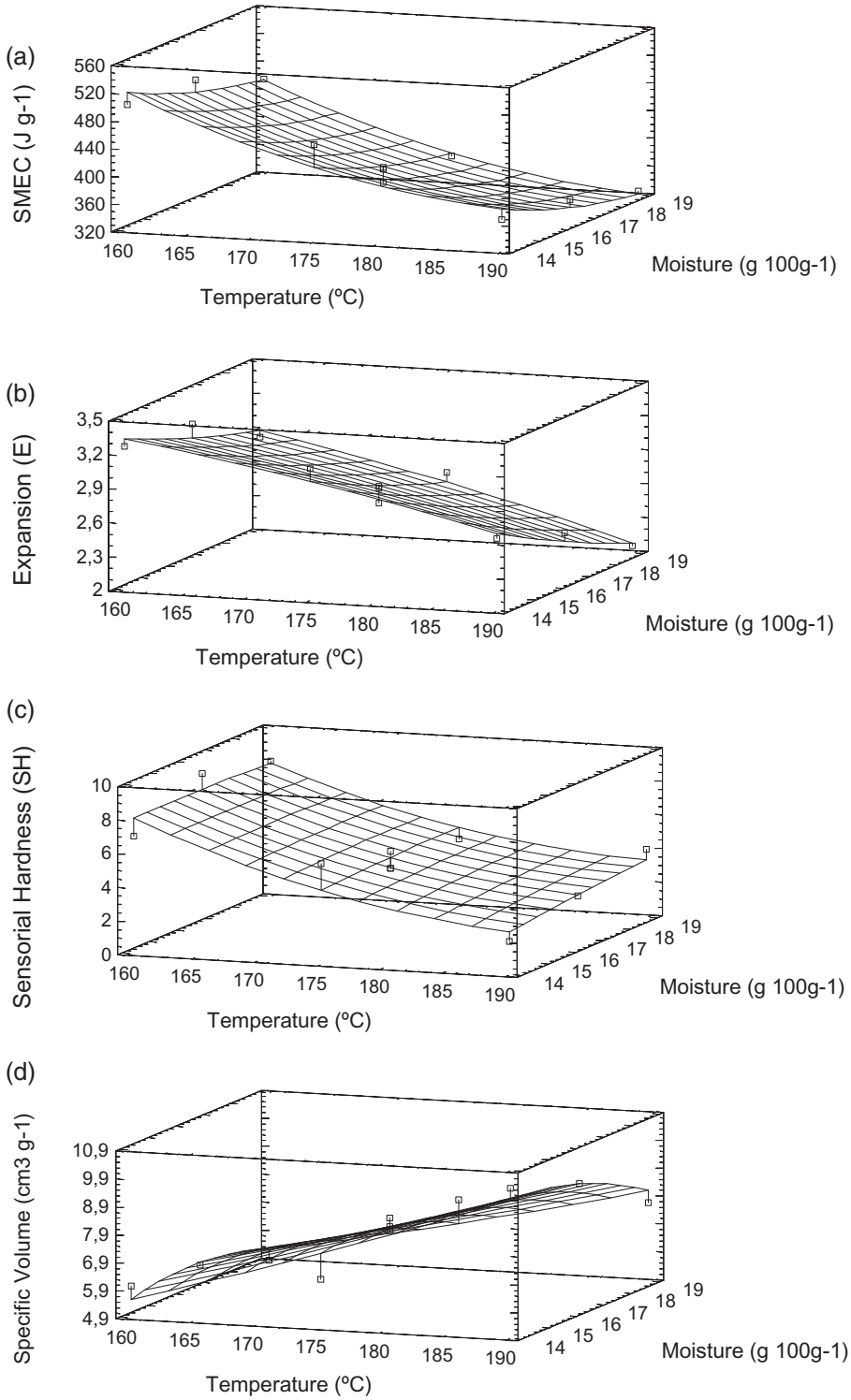

Figure 3. Surface responses corresponding to the effects of extrusion temperature and moisture content on (a) SMEC, (b) Expansion, (c) SH, (d) SV.

inside the extruder (some particles would reach the die very fast, without suffering much change and remaining in native state). The presence of native starch granules observed in almost all the samples confirmed this statement. As a consequence, an increase in severity in extrusion conditions, which normally would give higher *S* values, leads to a reduction in the proportion of native granules and a lower increase in *S*, which explains both the lower *S* values and the narrow value range for WA and *S*, at the set of extrusion conditions used in this study.

On the other hand, González et al. (2013) evaluated the effects of extrusion on the physicochemical properties of brown rice flour without pretreatment (soaking or germination), using the same equipment and similar conditions. Compared with extruded products based on germinated WR, these expanded samples presented lower *SV* values (8.64–4.53 versus 10.28–4.9 $cm^3\,g^{-1}$ sample) and *S* (33–21 versus 42.31–33.11 g $100\,g^{-1}$ sample) and higher WA values (7.7–5.1 versus 6.65–5.05 mL water g^{-1} sample). This indicates that CD of the extruded germinated WR is higher than that achieved in the extrusion of brown rice flour, thus germination would produce changes in the components and/or the structure of the grain that facilitates cooking in the extruder.

Figures 4(a) and (b) show the effects of extrusion conditions on FPC and TEAC. ANOVA results indicate that the linear term of *T* was significant in both responses (*p:* 0.0026 and 0.0492,

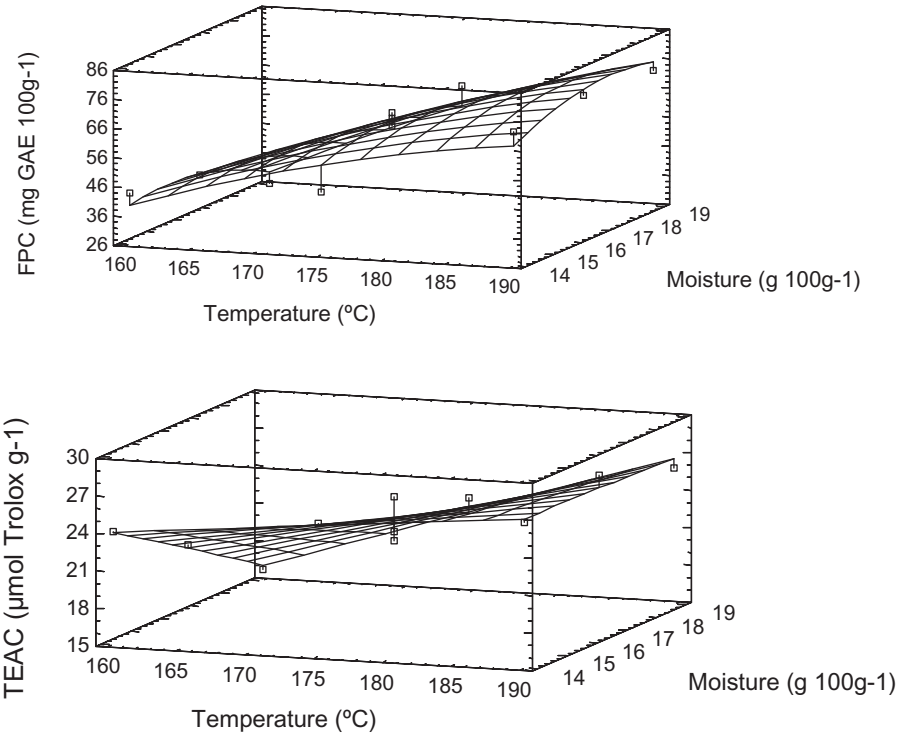

Figure 4. Surface response corresponding to the effects of extrusion temperature and moisture content on (a) FPC and (b) TEAC.

respectively). The values of FPC of brown rice flour and germinated rice flour were 37.04 mg and 49.38 mg GAE 100 g^{-1} sample, indicating that after germination FPC increases ~33%. Moongngarm & Saetung (2010) showed 40% increase in total phenolic content after germination of rough rice. During germination, the breakdown of the cell wall carried on by enzymatic hydrolysis could increase the amount of free phenolic forms (Moongngarm & Khomphiphatkul, 2011). Extruded samples had FPC ranged 26.23–74.24 mg GAE 100 g^{-1} sample. Thermal process during extrusion could decrease the content of phenolics due to decarboxylation of phenolic acids (Repo-Carrasco-Valencia et al., 2009) or may release bound phenolic acids from the breakdown of cellular constituents of the matrix (Brennan et al., 2011; Dewanto et al., 2002) which explain the maximum value obtained (74.24 mg GAE 100 g^{-1}), since around 71% of polyphenols are bound polyphenols. These compounds survive digestion in the stomach and intestine and reach the colon, where they may produce a favorable antioxidant environment (Fardet et al., 2008).

Regarding antioxidant capacity (TEAC), WR presented 13.39 µmol Trolox g^{-1} sample, which increases by germination (15.85 µmol Trolox g^{-1} sample) and extrusion (17.4–30.3 µmol Trolox g^{-1}). Antioxidant capacity and anti-radical activity of extruded products are dependent not only on the level of bioactive compounds, but also on the composition of bioactive compounds (Brennan et al., 2011). TEAC was not significantly correlated with phenolic content. This may be due to some phenolic compounds occurring in the system, other than phenolics with ABTS scavenging activity (Altan et al., 2009), or due to antioxidant capacity of other compounds, such as products formed through Maillard reaction during heat treatments (Yilmaz & Toledo, 2005).

Taking into account all the characteristics analyzed above, the extrusion conditions selected for obtaining an extruded product based on germinated whole grain rice flour produced at pilot plant scale were 175 °C and 14 g water 100 g^{-1}. The composition of germinated (G) and extruded–germinated (GE) products from WR obtained at pilot plant scale is shown in Table 3.

Protein content of the products decreased after germination process and was not affected by extrusion. In regards of NPN, the germination increased the content around 36% respect to the sample without treatments and the extrusion of germinated flours further increase NPN, indicating a certain protein hydrolysis due to extrusion. Veluppillai et al. (2009) found a decrease in protein content as germination days increased, with increase in amino acid content and attributed this to the increased level of protease activity as germination proceeded.

There was an increase in PD of germinated WR (p: 0.0021), attributed to the degradation of storage proteins, which may be more easily available to pepsin attack (Bhise et al., 1988). Then, extrusion process further decreases PD% of germinated WR, leading to a similar PD% as in WR without treatment.

It is also well established that extrusion thermomechanically denatures and reorients proteins in starchy foods leading to changes in digestibility and levels of amino acids (Bjork & Asp, 1983; Della Valle et al., 1994; Perez-Navarrete et al., 2007). However, the magnitude of protein transformations due to extrusion is a function of pre and postprocessing operations, and their interactions (Onyango et al., 2004). Most of the available bibliographies consider only the individual effects of the processes. However, in this study we evaluate the combined influence of germination and extrusion on PD.

Regarding PA content, results showed ~15% losses of PA caused by germination at pilot plant scale. This value was lower than that obtained at laboratory scale and quite lower than the 29% observed by Wei et al. (2012) at similar germination conditions (24 h – 30 °C). However, the combination of processes (germination–extrusion) induced a significant reduction of the initial PA content (38%).

Extractable lipid content decreased around 27% during germination, probably due to the increase in the activity of the lipolytic enzymes, which hydrolyzes lipids into free fatty acids and glycerol, and mobilizes them to provide energy for embryo growth (Chanlat et al., 2011). Extruded samples had the lowest content of extractable lipids. Several researches indicated that lipid degradation produced by the high temperatures reached in

Table 3. Composition in dry basis of WR, G and GE.

Components	WR	G (35 °C – 24 h)	GE (175 °C – 14 g 100 g^{-1} M)
Protein (g 100 g^{-1})	7.38 ± 0.10^{a}	6.65 ± 0.10^{b}	6.68 ± 0.40^{b}
NPN (mg 100 g^{-1})	4.7 ± 0.10^{b}	6.5 ± 0.40^{a}	6.4 ± 0.20^{a}
PD (g 100 g^{-1})	82.8 ± 1.60^{b}	100.9 ± 1.00^{a}	83.6 ± 2.40^{b}
PA content (mg 100 g^{-1})	821.69 ± 10.30^{a}	695.20 ± 1.60^{b}	506.84 ± 7.19^{c}
Extractable lipids (g 100 g^{-1})	2.26 ± 0.19^{a}	1.63 ± 0.05^{b}	0.47 ± 0.03^{c}
Dietary fiber (g 100 g^{-1})	5.47 ± 0.61^{a}	5.68 ± 0.58^{a}	3.23 ± 0.29^{b}
Mineral bio-accessibility (g 100 g^{-1})			
Zn	11.46 ± 0.52^{c}	13.76 ± 0.82^{a}	12.61 ± 0.73^{b}
Fe	11.21 ± 1.15^{c}	14.47 ± 0.62^{b}	16.24 ± 0.45^{a}

Mean values ± SD ($n = 3$); Different letters in the same column mean significant differences between samples ($p < 0.05$).

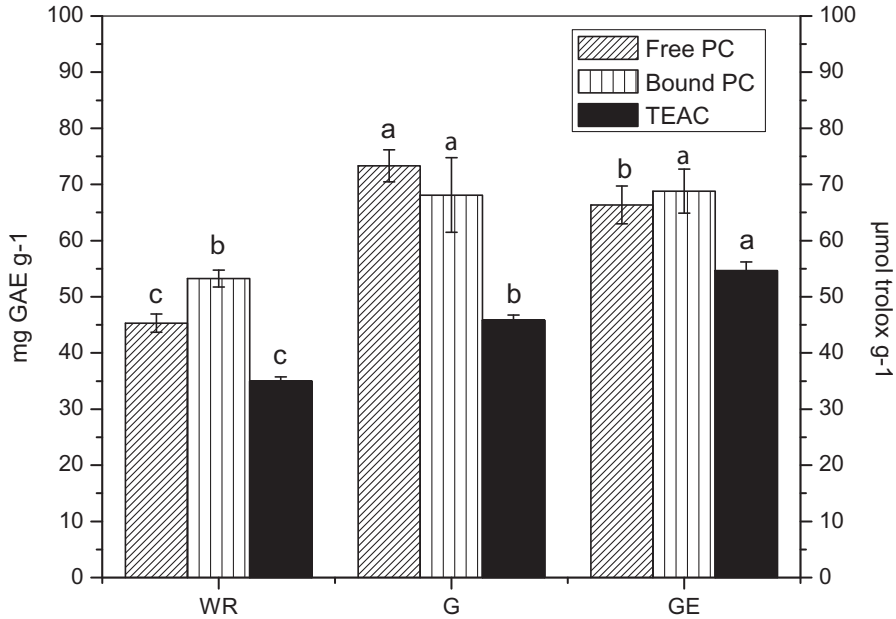

Figure 5. FPC, BPC and TEAC of the WR, G and GE. Data are expressed as mean ± SD. Different letters indicate significant differences at $p < 0.05$ level.

the extrusion and also the starch–lipid and protein–lipid complexes produced, which are not extractable by non-polar solvents, are involved in the decrease in ether extract content of extruded samples (Asp & Björck, 1989; Ho & Izzo, 1992).

Dietary fiber content did not change with germination process, but extruded–germinated rice had lower content (~43%) than the other samples. During extrusion, some components of fiber, such as pectin polymers and cellulose, would undergo degradation reactions, and their fragments, having low-molecular-weight, may not be determined as fiber with the methodology used (Pastor-Cavada et al., 2014).

Regarding mineral bio-accessibility, germination increased %DZn and %DFe. The higher mineral bio-accessibility could be due to the reduction of PA, which is an inhibitor of Fe and Zn availability. Extrusion further increased %DFe. Hazell & Johnson (1989) indicated that the reaction products of depolymerization under the high temperature and shear of extrusion processes might change the chemical form of iron and make it more soluble, which can increase its bio-accessibility. However, %DZn lightly decreased after extrusion process, this effect was observed in other studies (Watzke, 1998).

Figure 5 shows significant changes in bioactive compounds. In comparison with WR without treatment, germination increased FPC and BPC ~62 and 28%, respectively. These values reached 46 and 28%, after extrusion. Total phenolics content enhanced from 98.54 mg GAE 100 g^{-1} (d.b) to 141.44 and 135.16 mg GAE 100 g^{-1} (d.b) for WR without treatment, G and GE, respectively. These results indicated that even though extrusion reduced FPC of GE, total phenolics content increased when compared with WR. Several researchers noted that there was no clear trend in the effect of extrusion conditions on the content of phenolics, since they observed no change (Yang et al., 2014), a decrease (Altan et al., 2009) or an increase (Zielínski et al., 2001) depending on the studied cereal, the extrusion conditions and the methods of phenolic analysis.

Also, TEAC was improved ~30% for germination, and extrusion process further increased 56% respect to WR without treatments. This indicates that both germination and combined GE enhanced bioactive compounds of WR products.

Conclusion

Nutritional and clinical studies indicate that WG may have advantages over refined flours due to the presence of various bioactive compounds. Appropriate conditions of germination and extrusion for WR were determined. Considering the results of the physicochemical, nutritional properties and the sensory evaluation, the most suitable conditions for obtaining an expanded germinated WR product were 175 °C and 14 g 100 g^{-1} of moisture. A reduction of ~38% of PA content, an increase of 37% of total phenolic content and 2-folds increase in antioxidant capacity were obtained. Germinated rice and its extruded flours could be used to elaborate bread, breakfast cereals, noodles and

derivates with gluten-free characteristics. Thus, the extrusion cooking of germinated WR would be a good processing method to produce functional foods and enhance consumption of whole grain products.

Declaration of interest

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