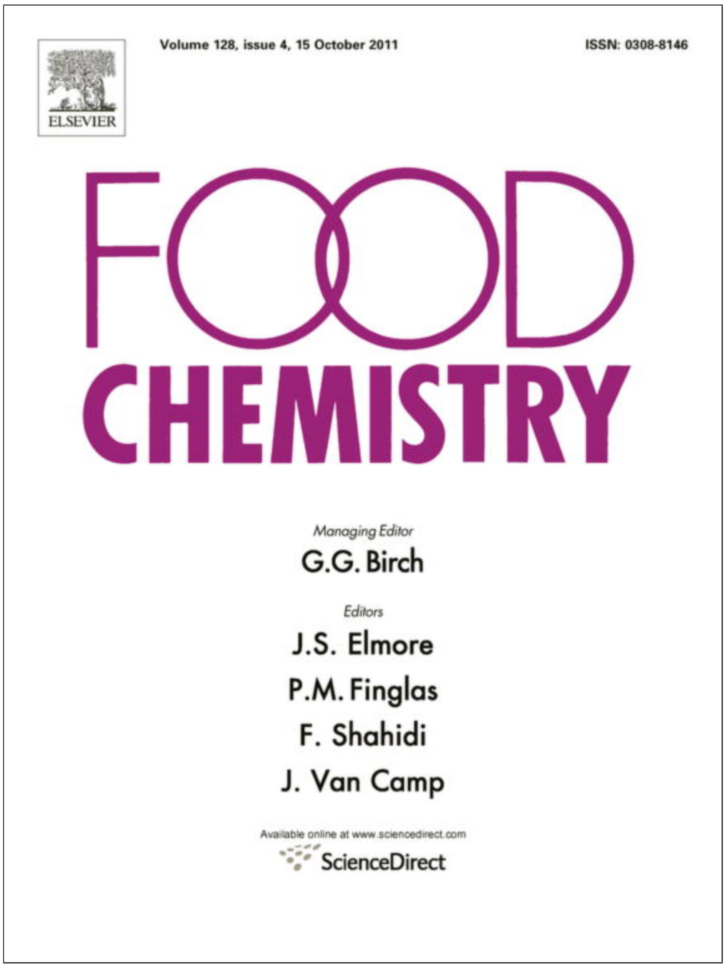
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Effects of the addition of wild legumes (*Lathyrus annuus* and *Lathyrus clymenum*) on the physical and nutritional properties of extruded products based on whole corn and brown rice

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ABSTRACT

The effects of the addition of wild legumes (*Lathyrus*) from the South of Spain on the physical and nutritional properties of extruded products based on whole corn and brown rice were studied. Samples were obtained with a Brabender single screw extruder. The physical characterisation of the expanded products was performed by the measurement of density, expansion, solubility and specific mechanical energy consumption (SMEC). Chemical composition, amino acids content, protein digestibility, total polyphenol content and potential availability of iron and zinc were determined.

Results showed that expansion, solubility and SMEC were higher for rice blends than for corn blends, while density followed an inverse trend. Addition of legumes produces a decrease of expansion and an increase in solubility in both rice-containing and corn-containing samples. With only 15% of legume replacement, a significant increase in protein content and quality, fibre, and mineral content was obtained. Protein digestibility was in the range of 82–84% and mineral availability in the 6.4–12.1% range for iron and 10–18.6% for zinc. The performance of each mixture during extrusion and the physical properties of the extruded products were considered to be in the range of those expected for snack type products.

1. Introduction

The utilisation of whole grains in food formulations is nowadays much recommended (Marquart, Wiemer, Jones, & Jacob, 2003). The beneficial effects of including whole grains in the diet have been demonstrated by several authors (Kelly, Summerbell, Brynes, Whittaker, & Frost, 2007; Venn & Mann, 2004). Whole grains are rich in nutritive, functional and phytochemical compounds (Slavin, 2003).

Moreover, extrusion cooking of starchy materials has become a very common technique to obtain a wide range of products, such as snacks, breakfast cereals, etc. (Bouzaza, Arhaliass, & Bouvier, 1996; Pansawat et al., 2008). The advantages of this cooking process are based mainly on the fact that it is an HTST process, which minimises the degradation of food nutrients by heat while improving digestibility by gelatinising starch, denaturing protein and deactivating undesirable compounds, such as enzymes and non-nutritional factors (Alonso, Aguirre, & Marzo, 2000; Shimelis & Rakshit, 2007).

Since maize (*Zea mays*) and rice (*Oryza sativa*) grits are widely used to formulate expanded products (Chaiyakul, Jangchud, Jangchud, Wuttijumnong, & Winger, 2009; Pérez-Navarrete, González, Chel-Guerrero, & Betancur-Ancona, 2006), there is a need to improve the nutritional value of this kind of food, particularly because cereal-based snack products are often consumed by children (Kasprzak & Rzedzicki, 2008; Onwulata, Konstance, Smith, & Holsinger, 2001; Onwulata, Smith, Konstance, & Hosinger, 2001; Rampersad, Badrie, & Comissing, 2003).

The effect of extrusion variables on the properties of extruded cereals has been studied extensively (González, Torres, & Añón, 2000; Kokini, Chang, & Lai, 1992; Mason & Hoseney, 1986; Mitchell & Areas, 1992). Moreover, the texture of expanded cereal based snacks is determined mainly by extrusion conditions and their moisture content.

It is well known that the addition of legumes to cereals produces an increase in both the amount and quality of the protein mix (Young, 1991). This addition represents an economic way to improve the protein value of cereal-based foods (Messina, 1999).

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Among legumes, those most used in cereal food mixtures are: *Glycine max* (Solanas, Castrillo, Jover, & de Vega, 2008); *Cicer arietinum* (Brenes, Viveros, Centeno, Arija, & Marzo, 2008; Lazou, Michailidis, Thymi, Krokida, & Bisharat, 2007); *Lupinus albus* (Masoero, Pulimeno, & Rossi, 2005; Díaz et al., 2006); *Phaseolus vulgaris* and *Phaseolus lunatus* (Anton, Fulcher, & Arntfield, 2009; Pérez-Navarrete et al., 2006; Solanas et al., 2008). Not much information concerning the use of wild legume species has been found, although *Lathyrus sativus*, belonging to the tribe Fabeae, has been studied by Grela, Jensen, and Jakobsen (1999), and Kasprzak and Rzedzicki (2007). The use of wild legumes in snack formulations could also be interesting, as they could be a good way to add to commercial products some advantages, such as variety and novelty, which are important to modern consumers. There is only one problem concerning the use of genus *Lathyrus* seeds: β-*N*-oxalyl-L-α,β-diaminopropionic acid (ODAP). This is a non-protein amino acid responsible for the neurolathyrism syndrome in humans and animals, which is characterised by weakness of the hind limbs and paralysis or rigidity of the muscles, and it appears after the consumption for long periods of diets based on large amounts of seeds (Campbell et al., 1994). However, the content of ODAP is much lower or almost nonexistent in expanded products because extrusion is a fast and effective method for reducing anti-nutritional components of legumes (Shimelis & Rakshit, 2007).

The aim of this study was to evaluate the effect of the addition of wild legumes (*Lathyrus annuus and Lathyrus clymenum*) on physical and nutritional properties of extruded products based on whole corn and brown rice.

2. Materials and methods

2.1. Materials

Samples of *Lathyrus* seeds (*L. annuus* and *L. clymenum*) were taken from wild populations. Voucher specimens of the populations studied were deposited in the Herbarium of the Department of Plant Biology and Ecology of the University of Seville.

The beans were previously treated to inactivate lipoxygenase, by immersing them in boiling water for 2 min followed by immediate cooling with tap water (Fritz et al., 2006). This heat treatment was done to avoid the development of a beany flavour during the grinding step. Treated beans were dried in an oven, at 45 °C until they reached between 9% and 10% moisture. The dried beans were ground and converted into grits using a laboratory mill (Bühler AG, Braunschweig, Germany). The resulting grits had a particle size of between 210 and 570 μm.

Commercial samples of Fortuna rice (a low-amylose rice variety) and hard red corn, both from Molino Zacanini (Entre Rios, Argentina) were milled according to an experimental procedure developed previously (Robutti, Borras, González, Torres, & De Greef, 2002). Particle size of the rice and corn grits obtained was between 420 and 1119 μm.

2.2. Extruded samples

A Brabender 20 DN (Brabender GmbH & Co. KG, Duisberg, Germany) single screw extruder was used to produce extruded cereal–legume blend samples. The following conditions were selected based on preliminary work: screw compression ratio: 4:1; cylindrical die (diameter/length): 3/20-mm; screw speed: 150 rpm; and extrusion temperature: 175 °C (die and extruder barrel). One hour before each extrusion experiment, a grits blend sample containing 85% cereal (whole rice or corn) and 15% legume was prepared using a planetary mixer (Brabender) and the moisture content was adjusted to 14%, by adding tap water. This blend sample was kept in a plastic bag until the extrusion step. This moisture level was also selected as a result of preliminary work.

These extrusion conditions produce expanded products with a good expansion rate and texture. The extruder was fed at maximum rate ("full-capacity") and experimental samples were taken as soon as a stationary regime was reached. While each extruded sample was taken, torque and mass output were measured. The sample was then allowed to dry in the ambient air and kept in plastic bags.

2.3. Physical characterisation of extruded samples

The following details were determined for the expanded samples: (a) axial expansion was calculated as the ratio of extruded product diameter and die diameter, taking the average of 10 measurements at 10 different places along the sample; (b) density expressed as grams per cm^3 was calculated using the mass output coming from the extruder (in g per min, referred to as dry basis (db)) and the product volume, which is calculated from the length of product per minute and the product diameter; (c) specific mechanical energy consumption (SMEC), in joule/g, was determined according to González, Torres, and De Greef (2002), by using the following formula:

$$\text{SMEC} = (61.3 \times 10^{-3}) \times \text{torque (BU)} \times 150\ \text{rpm/Qa}\ (g/min),$$

Qa being, the mass output referred as feed grits moisture (14%); (d) product texture was evaluated by a trained panel (three judges), according to Fritz et al. (2006), using a hardness (*H*) 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.

Water solubility determinations were carried out according to González et al. (2002) and the sample as flour was used. One hundred gram of each sample were first ground with a laboratory hammer mill (Retsch GmbH & Co. KG, Haan, Germany) with a 2-mm sieve, and then with a Cyclotec (FOSS, Hillerød, Denmark) mill through a 1-mm sieve. Water solubility was calculated as soluble solids per 100 g of flour (d.b). This was done by dispersing 2.5 g of flour in 50 mL water, shaking for 30 min and centrifuging at 2000*g*; soluble solids were obtained after evaporation in an oven at 105 °C.

2.4. Analytical methods

Moisture and ash contents were determined using AOAC (1999) 945.39 and 942.05 approved methods, respectively. Total nitrogen was determined by the micro-Kjeldahl method according to AOAC (1999) 960.52 approved method. Crude protein content was estimated using a conversion factor of 6.25 for legumes and corn, and 5.95 for rice (according to Juliano, 1985). Total fibre was determined according to the procedure described by Lee, Prosky, and De Vries (1992). Lipids associated with the flour and protein isolates were extracted and measured following the method of Nash, Eldridge, and Woolf (1967). Soluble sugars and polyphenols were measured using standard curves of glucose (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and catechin (Mazza, Fukumoto, Delaquis, Girard, & Ewert, 1999), respectively.

2.5. Amino acid analysis

Samples (10 mg) were hydrolysed with 4 mL of 6 N HCl. The solutions were sealed in tubes under nitrogen and incubated in an oven at 110 °C for 24 h. Amino acids were determined after derivatisation with diethyl ethoxymethylenemalonate by high-performance liquid chromatography (HPLC), according to the method of Alaiz, Navarro, Giron, and Vioque (1992), using DL-α-aminobutyric acid as an internal standard. Tryptophan was determined after basic hydrolysis (Yust et al., 2004).

2.6. In-vitro protein digestibility (IVPD)

In-vitro protein digestibility was determined according to the method of Hsu, Vavak, Satterlee, and Miller (1977). Samples containing 62.5 mg of protein were suspended in 10 mL of water and the pH was adjusted to 8.0. An enzymatic solution containing 1.6 mg trypsin (17.7 BAEE U mg^{-1}), 3.1 mg α-chymotrypsin (43 U mg^{-1}) and 1.3 mg peptidase (50 U g^{-1}) per mL was added to the protein suspension in a 1:10 v/v ratio. The pH of the mixture was measured after 10 min, and the in-vitro digestibility was calculated as a percentage of digestible protein using the equation:

$$\%\text{digestible protein} = 210.464 - (18.103 \times \text{pH}).$$

2.7. Determination of mineral dialysability

(*D*Fe%, *D*Zn%) A modification of the widespread in-vitro Miller, Schricker, Rasmussen, and Van-Campen (1981) method, according to Wolfgor, Drago, Rodríguez, Pellegrino, and Valencia (2002) was followed. The samples were prepared to 10% (w/w) of solids concentration using deionised water. Aliquots (25 g) of homogenised samples were adjusted to pH 2.0 with 6 N HCl and after the addition of 0.8 mL pepsin digestion mixture (16% pepsin (Sigma P-7000) solutions in 0.1 N HCl), were incubated at 37 °C for 2 h in a shaking water bath. At the end of the pepsin digestion, dialysis bags containing 20 mL 0.19 M PIPES (piperazine-*N*,*N*′-bis[2-ethanesulfonic 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 2.5% bile (Sigma B-8631), 0.4% pancreatin (Sigma P-1750) solution in 0.1 N $NaHCO_3$) was then added to each flask and the incubation continued for another 2 h. The bag contents were then weighed and analysed for mineral content by flame atomic absorption spectroscopy (AAS). Assessment of minerals in samples was made by AAS after dry ashing (AOAC, 1999).

Mineral dialysability was calculated from the amount of each dialysed mineral expressed as a percentage of the total amount present in each sample.

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

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

The percentage of recommended daily intake (RDI) which is supplied by a 30-g ration of the extruded products was calculated using the following formula:

$$\%\text{RDI} = \text{Mineral Content} \times DM \times 30.$$

2.8. β-N-oxalyl-L-αβ-diaminopropionic acid (ODAP) determination

The ODAP content of extruded products was determined according to Hussain, Chowdhurry, Haque, Wouters, and Campbell (1994) with modifications. Samples (50 mg) were extracted twice by stirring with 500 μL of 60% ethanol for 1 h. The samples were centrifuged at 11600*g* for 10 min. Supernatants were recovered and the volumes made up to 1 mL. Extract (200 μL) mixed with 400 μL of 3 M KOH was hydrolysed in boiling water for 30 min and then cooled. An aliquot (25 μL) of the hydrolysed extract was mixed with 75 μL of distilled water and 200 μL of a reagent made of 10 mg of OPT 97%, 100 μL of 95% ethanol and 20 mL of 2-mercaptoethanol dissolved in 10 mL potassium tetraborate buffer 0.5 M pH 9.9. The absorbance was measured at 425 nm after 30 min The procedure includes three blanks: 25 μL non-hydrolysed extract + 75 μL of distilled water + 200 μL of reagent OPT (OPT blank); 25 μL non-hydrolysed extract + 75 μL of distilled water + 200 μL tetraborate buffer (sample blank); 25 μL of hydrolysed extract + 75 μL of distilled water + 200 μL tetraborate buffer (buffer blank). The final absorbance was given as:

$$A = (A_{\text{sample}} - A_{\text{buffer blank}}) - 1/3(A_{\text{OPT blank}} - A_{\text{sample blank}}).$$

The calibration curve ($y = 0.5146x + 0.0741$) was made with a solution of DL-2,3-diaminopropionic acid HCl (DAP) as standard and converted to ODAP by using a factor of 1.69 (Aletor, El-Monein, & Goodchild, 1994). ODAP content was expressed as mg of ODAP/100 mg of sample (%).

2.9. Statistical analysis

Analysis of variance was carried out using the software Statgraphics Plus 5.0, and the statistical differences among samples were determined using Tukey's test and $p \leqslant 0.05$ degree of significance.

3. Results and discussion

3.1. Extrusion process and physical evaluation

Table 1 shows values obtained from the physical evaluation of the extruded samples corresponding to rice, corn and the respective blends with 15% wild legume added (*L. annuus* and *L. clymenum*). It is observed that axial expansion values corresponding to rice-containing samples are higher than those containing corn. This difference may be due to the higher oil content of whole corn compared with brown rice (4.72% and 2.38%, respectively). The effect of oil on expansion rate has been studied by several authors and its magnitude could depend on the type and amount of oil (Faubion & Hoseney, 1982). During the extrusion process fat components act as lubricants, reducing the degree of cooking and consequently the expansion ratio (Bhattacharya & Hanna, 1988). It was also observed that the addition of legumes produces a decrease of expansion in both rice- and corn-containing samples. In the case of rice samples, significant reduction ($p < 0.05$) was caused by both legume species, while for corn, only the blend with *L. clymenum* showed a significant effect. These results are in agreement with other authors working with mixtures of cereals and several other

Table 1
Physical properties of extruded samples.

Sample	Expansion	Density (g/cm3)	Torque (gf. cm)	Qa (g/min)	Sol. (%)	TS	SMEC (J/g)
Rice	3.46 ± 0.09^{d}	0.143 ± 0.01^{ab}	6330 ± 90^{b}	84.4 ± 0.5^{c}	39.3 ± 0.22^{c}	7	689.7 ± 11^{c}
R + *LA*	3.20 ± 0.06^{c}	0.147 ± 0.01^{b}	5980 ± 70^{b}	89.9 ± 0.6^{d}	40.6 ± 0.41^{cd}	8	611.4 ± 7^{bc}
R + *LC*	3.22 ± 0.04^{c}	0.146 ± 0.00^{b}	5930 ± 80^{b}	94.4 ± 0.7^{d}	42.5 ± 0.10^{d}	8	577.5 ± 8^{ab}
Corn	2.84 ± 0.14^{b}	0.131 ± 0.01^{ab}	4000 ± 40^{a}	69.8 ± 0.3^{a}	19.3 ± 0.80^{a}	8	526.3 ± 6^{a}
C + *LA*	2.76 ± 0.11^{ab}	0.142 ± 0.01^{ab}	4270 ± 30^{a}	78.2 ± 0.3^{b}	22.6 ± 0.21^{b}	8	502.4 ± 3^{a}
C + *LC*	2.59 ± 0.06^{a}	0.126 ± 0.00^{a}	4520 ± 50^{a}	80.6 ± 0.4^{bc}	21.2 ± 0.58^{ab}	8	515.9 ± 7^{a}

Qa, feed caudal; Sol, water solubility; TS, product texture; SMEC, specific mechanical energy consumption; R, rice; *LA, L. annuus*; *LC, L. clymenum*; C, corn. Mean values ± standard deviation ($x \pm$ SD).
[abcd]Different letter superscripts in the same column indicate statistical differences among samples ($p < 0.05$), Tukey's test.

Table 2
Chemical composition corresponding to each extruded sample.

Sample	Moisture (%)*	Ash (%)2***	Fat (%)2**	Fibre (%)2**	Protein (%)2*	Soluble sugars (%)2***	Carbohydrate (%)1,2***
R	8.2 ± 0.03[c]	1.19 ± 0.01[a]	0.53 ± 0.15[a]	3.81 ± 0.15[a]	7.38 ± 0.10[a]	0.23 ± 0[a]	86.86 ± 0.13[c]
R + LA	8.1 ± 0.11[c]	1.42 ± 0.02[b]	0.54 ± 0.14[a]	7.51 ± 0.16[b]	10.50 ± 0.53[b]	0.32 ± 0[b]	79.70 ± 0.49[b]
R + LC	7.9 ± 0.07[bc]	1.44 ± 0.01[b]	0.81 ± 0.00[a]	7.96 ± 0.01[b]	10.09 ± 0.16[b]	0.31 ± 0[b]	79.38 ± 0.14[b]
C	9.1 ± 0.34[d]	1.46 ± 0.02[b]	2.18 ± 0.02[c]	7.15 ± 0.18[ab]	10.23 ± 0.66[b]	0.44 ± 0[c]	78.54 ± 0.89[ab]
C + LA	7.1 ± 0.17[a]	1.65 ± 0.01[c]	1.65 ± 0.09[bc]	11.95 ± 0.13[c]	11.25 ± 0.36[bc]	0.50 ± 0.01[d]	72.99 ± 0.42[a]
C + LC	7.3 ± 0.05[ab]	1.67 ± 0.02[c]	1.12 ± 0.15[ab]	11.86 ± 1.53[c]	12.13 ± 0.007[c]	0.31 ± 0[b]	72.89 ± 1.40[a]

Samples: R, rice; C, corn; R + LA, rice + *L. annuus*; *R* + LC, rice + *L. clymenum*; C + LA, corn + *L. annuus*; C-LC, corn + *L. clymenum*. 1, Calculated as 100 – (moisture + ash + fat + fibre + protein + soluble sugars). 2 = Dry basis. Mean values ± standard deviation.
[abcd]Different letter superscripts in the same column indicate statistical differences.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$, Tukey's test.

legumes, such as *Lens culinaris*, *P. vulgaris*, *P. lunatus* and *C. arietinum*, (Anton et al., 2009; Patil, Berrios, Tang, & Swanson, 2007; Pérez-Navarrete et al., 2006; Shirani & Ganesharanee, 2009). They observed that as the degree of cereal replacement by legume is increased, expansion rate decreases, mainly as a consequence of the lower content of starch present in legumes.

It is well known that expansion and density are the best properties to describe product porosity (Asare, Sefa-Dedeh, Sakyi-Dawson, & Afoakwa, 2004). In Table 1 it is observed that density values followed an inverse trend to that of expansion, as is expected (Ahmed, 1999; Anton et al., 2009; Chaiyakul et al., 2009). Moreover Onwulata, Konstance et al. (2001), Onwulata, Smith et al. (2001b) and Veronica, Olusola, and Adebowale (2006) observed that as fibre and protein-rich materials are added to starchy materials, the density of expanded product is increased, although in these studies the density value variations are not significant.

Solubility is an important indicator of the degree of cooking (González, Torres, De Greef, & Bonaldo, 2006), because starch granule degradation leads to the production of soluble fragments (Colonna, Tayeb, & Mercier, 1989). In Table 1 it is observed that solubility values corresponding to corn samples are lower than those of rice ($p < 0.05$). Again, this can be attributed to the reduction of friction during extrusion caused by higher oil content of corn with respect to rice, which in its turn caused a reduction in the degree of cooking. On the other hand the addition of a legume caused an increase in solubility depending on the sample. In the case of rice-containing samples this increase was significant ($p < 0.05$) only for the *L. clymenum* blend sample and in the case of corn samples, only *L. annuus* blend showed significant difference.

Regarding the SMEC results, Table 1 shows that values corresponding to rice samples are higher than those of corn (except the blend of rice with *L. clymenum* and only corn extruded, where there are not significant differences), as is expected according to the explanation concerning the oil's effect on the degree of friction. Ahmed (1999) obtained similar results working with linseed–corn extruded mixtures. On the other hand, when legumes are added to cereal, SMEC values also decrease (in the present study there are only significant differences between samples containing only rice extruded and rice blended with *L. clymenum*). Pérez-Navarrete et al. (2006) observed a similar effect by extruding a corn–*P. lunatus* mixture. This effect could be attributed to the lower hardness of legume cotyledons in comparison to the cereal endosperm hardness, which could reduce the degree of friction in the extruder.

3.2. Nutritional evaluation

3.2.1. Chemical composition

Table 2 shows the chemical composition corresponding to extruded samples. Moisture content is in the expected range (between 7% and 79%). Ash content increased significantly ($p < 0.001$) with the addition of legumes. Ash content in corn-containing samples is higher ($p < 0.001$) than in those containing rice. Regarding fat content, rice-containing samples have lower amounts ($p < 0.01$) than those containing corn and there were no significant differences between rice and its legume blends. On the other hand, the addition of legumes reduced fat content of corn blends, the differences being significant in the case of *L. clymenum.*

Fibre content increased significantly ($p < 0.01$) with the addition of legume for both cereal samples, the highest values being those corresponding to corn and its legume blends. Protein content also increased significantly ($p < 0.05$) with the addition of legume, except in the case of corn–*L. annuus* blend. It also observed that the protein content of extruded corn is higher ($p < 0.05$) than that of rice. Regarding soluble sugars, results depended on the type of blend. In the case of rice-containing samples, soluble sugars increased with the addition of legume. In the case of corn-containing samples, the addition of *L. clymenum* diminished soluble sugar content ($p < 0.001$), while an opposite effect is observed when *L. annuus* is added, in which case soluble sugars increased ($p < 0.001$). Furthermore, the soluble sugar content of corn samples is higher ($p < 0.001$) than those of rice.

Finally, carbohydrate content diminished with the addition of legumes, these differences being significant ($p < 0.001$) only in the case of rice-containing samples; also the carbohydrate content of corn–legume blends is lower than their rice-containing equivalents.

3.2.2. Amino acid profile

Table 3 shows the results of amino acid (AA) composition (g/100 g of protein) for extruded samples. It is observed that some changes in AA profile occurred with the addition of legumes. Cysteine contents decreased with the addition of legumes, but only in the case of corn–*L. clymenun* blend is the decrease significant ($p < 0.01$), compared with corn alone. Nevertheless, the sulphur amino acid content (Cys + Met) of all blends is higher than that recommended by FAO/WHO/UNU (1985). It is also observed that in all samples, Phe + Tyr content is higher than that of FAO recommendations.

Changes in leucine and valine contents depended on the type of cereal. Corn samples have a higher content ($p < 0.001$) of leucine than the rice samples, but the addition of legume did not significantly affect this value. All samples showed higher leucine content than that of FAO recommendations. In the case of valine, rice samples showed higher ($p < 0.05$) content than those of corn and the addition of legume did not significantly affect valine contents. Again, all samples showed higher valine content than that of FAO recommendations.

Table 3
Amino acid composition (g/100 g of protein) corresponding to extruded samples. Mean values ± standard deviation and FAO recommendation.

		Rice	Rice + *LA*	Rice + *LC*	Corn	Corn + *LA*	Corn + *LC*	FAO
Essential amino acids	Cys**	2.0 ± 0.1[ab]	1.3 ± 0.4[a]	1.3 ± 0.0[a]	2.3 ± 0.1[b]	2.1 ± 0.0[ab]	1.5[a]	
	Phe	5.0 ± 0.1	4.9 ± 0.4	4.6 ± 0.1	4.8 ± 0.0	5.0 ± 0.0	5.0	
	His	1.7 ± 0.1	2.2 ± 0.3	1.8 ± 0.0	2.3 ± 0.4	2.5 ± 0.1	2.3	1.9
	Ile	2.8 ± 0.0	3.7 ± 1.1	2.9 ± 0.0	2.3 ± 0.0	2.6 ± 0.0	2.5	2.8
	Leu***	8.9 ± 0.0[a]	8.4 ± 0.5[a]	8.9 ± 0.1[a]	13.0 ± 0.2[b]	11.3 ± 0.0[b]	11.6[b]	6.6
	Lys**	3.3 ± 0.3[b]	4.4 ± 0.1[de]	4.7 ± 0.1[e]	2.6 ± 0.0[a]	3.9 ± 0.2[c]	4.2[cd]	5.8
	Met	1.6 ± 0.0	1.1 ± 0.2	1.0 ± 0.0	0.7 ± 0.8	1.7 ± 0.0	1.1	2.5♠
	Tyr**	3.2 ± 0.1[b]	2.9 ± 0.1[ab]	3.0 ± 0.1[ab]	3.0 ± 0.1[ab]	2.8 ± 0.0[a]	2.8	6.3♠♠
	Thr	3.9 ± 0.1	4.4 ± 0.3	4.0 ± 0.0	4.3 ± 0.2	4.3 ± 0.2	4.1	3.4
	Trp**	1.5 ± 0.1[b]	1.0 ± 0.0[a]	1.1 ± 0.0[a]	1.1 ± 0.1[a]	0.9 ± 0.0[a]	0.9[a]	1.1
	Val*	4.5 ± 0.2[b]	4.4 ± 0.4[b]	4.1 ± 0.0[ab]	3.6 ± 0.1[a]	3.9 ± 0.0[ab]	3.8[ab]	3.5
Non-essential amino acids	Ala**	6.3 ± 0.1[ab]	6.6 ± 1.1[ab]	5.7 ± 0.1[a]	8.5 ± 0.1[b]	7.6 ± 0.1[ab]	7.4[ab]	
	Arg***	8.2 ± 0.1[b]	8.5 ± 0.3[b]	8.4 ± 0.1[b]	4.9 ± 0.0[a]	6.0 ± 0.1[a]	6.0[a]	
	Asp**	10.7 ± 0.2[ab]	11.0 ± 0.4[ab]	11.6 ± 0.2[b]	9.4 ± 0.5[a]	9.1 ± 0.5[a]	9.5[ab]	
	Gly*	5.2 ± 0.0[b]	5.2 ± 0.0[ab]	5.1 ± 0.0[ab]	5.1 ± 0.4[ab]	4.6 ± 0.1[a]	4.8[ab]	
	Glu	20.8 ± 0.0	20.4 ± 1.7	21.0 ± 0.2	22.6 ± 1.1	21.9 ± 0.4	21.8	
	Pro	4.8 ± 0.4	3.8 ± 0.2	4.8 ± 1.3	3.6 ± 0.5	4.0 ± 0.1	4.6	
	Ser	5.7 ± 0.3	5.8 ± 0.1	6.0 ± 0.0	5.9 ± 0.1	5.8 ± 0.0	6.1	

Cys, cysteine; Phe, phenylalanine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Tyr, tyrosine; Thr, threonine; Trp, tryptophan; Val, valine; Ala, alanine; Arg, arginine; Asp, aspartic acid; Gly, glycine; Glu, glutamic acid; Pro, proline; Ser, serine; ♠ = Met + Cys, ♠♠ = Phe + Tyr; *LA*, *L. annuus*; *LC*, *L. clymenum*.
[abcd] Different letter superscripts in the same row indicate statistical differences ($p < 0.05$), Tukey's test.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

The changes in lysine contents depended on both the type of cereal and legume. Rice samples showed higher lysine values ($p < 0.01$) than those for corn samples and the addition of legume significantly increased ($p < 0.01$) lysine content. However, none of the samples showed enough lysine content to satisfy FAO recommendations, indicating that the percentage of legume addition should be higher if FAO recommendations are to be satisfied. Tryptophan content in rice samples is higher ($p < 0.01$) than in those of corn, and the addition of legume reduced it significantly ($p < 0.01$) only in the case of rice-containing samples. Regarding FAO recommendations for tryptophan content, it is observed that almost all samples reach the minimum recommended level, the highest value being for rice samples.

Regarding the content of other amino acids, such as isoleucine, histidine, methionine and threonine it is observed that no significant differences were observed among samples. All samples satisfied FAO recommendations for threonine content. However, corn-containing samples did not satisfy the requirements for isoleucine. Finally, all samples satisfy FAO recommendations for histidine, with the exception of rice and rice–*L. clymenum* samples.

Regarding non-essential AA, significant differences among extruded samples were found for alanine, aspartic acid, arginine and glycine. The most noticeable difference corresponded to arginine, due to the fact that its content in rice is much higher than that in corn.

3.2.3. In-vitro protein digestibility

Protein digestibility is one of the most important factors determining protein quality (FAO, 1985). Table 4 show values of in-vitro protein digestibility corresponding to extruded samples. Addition of legumes did not affect digestibility, since no significant differences were observed among samples. These results are in agreement with those of other workers (Alonso et al., 2000; Balandrán-Quintana, Barbosa-Cánovas, Zazueta-Morales, Anzaldúa-Morales, & Quintero-Ramos, 1998; Pérez-Navarrete et al., 2006).

3.2.4. Total phenolics contents

Table 5 shows polyphenol contents corresponding to extruded samples. Corn contains a much higher polyphenol content than rice and the addition of legume did not produce significant changes.

Table 4
In-vitro protein digestibility (PD) of extruded samples.

Sample	PD (%)
Rice	84.1 ± 0.5
Rice + *L. annuus*	82.5 ± 1.7
Rice + *L. clymenum*	82.8 ± 0.2
Corn	81.8 ± 0.2
Corn + *L. annuus*	81.8 ± 0.3
Corn + *L. clymenum*	82.5 ± 0.0

Mean values ± standard deviation.

Table 5
Polyphenol content of extruded samples.

Sample	Polyphenol (mg/g of sample)
Rice	0.36 ± 0.02[a]
Rice + *L. annuus*	0.28 ± 0.02[a]
Rice + *L. clymenum*	0.47 ± 0.10[a]
Corn	1.20 ± 0.03[b]
Corn + *L. annuus*	1.06 ± 0.06[b]
Corn + L. clymenum	1.04 ± 0.09[b]

Mean values ± standard deviation.
[ab]Different letter superscripts indicate statistical differences ($p < 0.05$), Tukey's test.

3.2.5. Mineral contents and potential availability

Table 6 shows the results of iron and zinc content, their potential availability (%) and the % of recommended daily intake (RDI) which is supplied, corresponding to extruded samples.

3.2.5.1. Iron. It is observed that iron content increased with the addition of legumes, although the values were not always significantly different. Legumes are considered iron-rich materials and several researchers have observed a positive effect of the addition of legumes to cereals (Hazell & Johnson, 1989; Lombardi-Boccia, Dilullo, & Carnovale, 1991).

Table 6
Fe and Zn content (ppm); potential availability (%) and the percentage of recommended daily intake which is supplied by a 30-g ration, corresponding to extruded samples.

Sample	Fe (ppm)*	*D*Fe (%)*	RDI Fe (%)**	Zn ppm***	*D*Zn (%)**	RDI Zn (%)***
Rice	14.8 ± 1.5a	7.4 ± 0.0[ab]	0.17 ± 0.0[a]	17.7 ± 0.0[a]	10.0 ± 1.3[a]	0.32 ± 0.0[a]
Corn	23.9 ± 5.6[ab]	6.4 ± 0.2[a]	0.23 ± 0.0[a]	24.3 ± 0.1[d]	14.5 ± 0.1[b]	0.65 ± 0.0[c]
LA + rice	22.5 ± 0.0[ab]	9.8 ± 1.1[bc]	0.32 ± 0.0[ab]	19.4 ± 0.1[b]	14.6 ± 0.4[b]	0.52 ± 0.0[b]
LC + rice	17.2 ± 0.0[a]	10.0 ± 0.5[bc]	0.26 ± 0.0[ab]	20.1 ± 0.0[c]	14.0 ± 0.1[b]	0.51 ± 0.0[b]
LA + corn	29.5 ± 3.3[b]	7.1 ± 0.2[ab]	0.30 ± 0.0[ab]	26.1 ± 0.1[e]	16.1 ± 0.0[bc]	0.79 ± 0.0[d]
LC + corn	24.4 ± 2.7[ab]	12.1 ± 1.3[c]	0.45 ± 0.0[b]	25.5 ± 0.0[e]	18.6 ± 0.6[c]	0.91 ± 0.1[e]

LA, *L. annuus*; LC, *L. clymenum*; %*D*Zn and %*D*Fe. Potential availability of Zn and Fe. RDI Zn (%) and RDI Fe: % of recommended daily intake for Zn and Fe.
Mean values ± standard deviation.
[abcde]Different letter superscripts in the same column indicate statistical differences.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$, Tukey's test.

Table 7
ODAP (%) studied expanded contents. Data are expressed as the averages (2 determinations) ± standard deviation. Different superscript small letters indicate significant differences between values in the same column (Tukey's test, $p < 0.05$).

Expanded	% ODAP
L. annuus + rice	0.0002 ± 0.0[a]
L. clymenum + rice	0.112 ± 0.006[b]
L. annuus + corn	0.0001 ± 0.0[a]
L. clymenum + corn	0.119 ± 0.02[b]

On the other hand, Fe dialysability increased with the addition of legumes in all cases, but significant differences ($p < 0.05$) were only observed between corn–*L. clymenum* and corn alone. Drago et al. (2010) also observed an improvement in iron dialysability when dehulled *P. vulgaris* was added to whole corn flour in a 50:50 ratio (11.6% *vs.* 10.5%).

Table 6 also shows the percentage of the recommended daily intake (RDI) of iron given by a 30-g ration of expanded products. It is noted that adding the wild legume increases the contributions of this mineral, especially for rice–*L. annuus* (1.9 times compared with rice) and corn–*L. clymenum* (1.96 times compared with corn). Even though these values are not high, the RDI for iron (18 mg/day) takes into account the value of bioavailability, and thus the percentage covered by this ration of these expanded products may be higher, since the value we reported is the amount of potentially bioavailable mineral.

3.2.5.2. Zinc. Table 6 shows that corn samples contain higher ($p < 0.001$) amounts of Zn than those containing rice, and that the addition of legumes increased the Zn content of both cereal samples. It is also observed that Zn potential availability of corn-containing samples is higher than that of samples containing rice, and that it is also improved by the addition of legumes, although in the case of corn alone and corn–*L. annuus* the difference is not significant.

'With respect to the percentage of the recommended daily intake (RDI) for zinc supplied by a 30-g ration of expanded products, it is observed that for corn-containing samples it is higher than those of rice ($p < 0.001$) and the addition of wild legumes increases this by 1.2–1.4 times for corn and 1.6 times for rice ($p < 0.001$). The RDI (15 mg/day) takes into account a zinc bioavailability of 20% (Olivares, Martínez, López, & Ros, 2001), and thus the percentage covered by the ration of these expanded products may be higher, since the value we reported is the amount of potentially bioavailable zinc.

3.2.6. ODAP contents

Table 7 shows ODAP contents (%) of extruded samples. ODAP content in extruded products is very low, which is interesting for human consumption. Grela (1998) and Singh, Azeem, and Singh (2003) also noted that the extrusion process results in decreased levels of ODAP in *L. sativus.* Table 7 shows that blends containing *L. clymenum* have higher ODAP contents than blends containing *L. annuus* ($p < 0.05$).

4. Conclusions

Extruded products based on whole corn or rice with the addition of wild legumes offer four main advantages: they are whole grain food grade; they have a better nutritional quality than a traditional extrudate, they are made with grains with such a small amount of gluten that they can almost be considered "free from gluten" cereals and they have very low amounts of antinutritional components.

Corn–legume samples show a better chemical composition than those of rice, since they have a higher content of proteins, fibre and minerals. Finally, the fact that they are made with wild legumes makes them innovative products.

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