

Original article

Physical and nutritional properties of extruded products based on whole grain with the addition of wild legumes (*Vicia lutea* subsp. *lutea* var. *hirta* and *Vicia sativa* subsp. *sativa*)

Elena Pastor-Cavada,^{1*} Silvina R. Drago,² Rolando J. González,² Rocío Juan,³ Julio E. Pastor,³ Manuel Alaiz¹ & Javier Vioque¹

1 Instituto de la Grasa (C.S.I.C.), Avda Padre García Tejero 4, 41012 Sevilla, Spain

2 Instituto de Tecnología de Alimentos, FIQ, Universidad Nacional del Litoral, 1° de Mayo 3250, Santa Fe, 3000, Argentina

3 Departamento de Biología Vegetal y Ecología, Universidad de Sevilla, 41012, Sevilla, Spain

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Summary Flour blends based on whole corn and rice with two added wild legumes at 15% level of cereal replacement were extruded to produce whole grain snacks. Extrusion temperature was 175 °C, and the moisture content was adjusted to 14%. The extruded products were evaluated for their physical, chemical and nutritional properties. Results showed that the addition of legumes produces a decrease in expansion in rice-containing samples and an increase in solubility in corn-containing samples. With only 15% of legume added to cereal, a significant increase in protein content and quality was obtained. An increase in fibre, polyphenol, iron and zinc content was also obtained. Protein digestibility was in the range of 81.8–85.3%. Mineral availability ranged from 6.4 to 16.3% for iron and 10–16.3% for zinc. The performance of each mixture during extrusion and the physical properties of the extruded products were considered to be similar to those expected for snack-type products and described in the literature.

Keywords Amino acid, cereal, extrusion, mineral availability, nutritional properties, physical properties, protein, protein digestibility, snacks, wild legumes.

Introduction

Inclusion of legumes in the diet is nowadays highly recommended. Their value as food is related to their nutrient composition: protein, minerals, fibre, vitamins and polyphenols (Rocha-Guzmán *et al.*, 2007).

An important portion of phytodiversity has been lost in most parts of the world, as a consequence of the replacement of local varieties by new ones, mainly due to the fact that new varieties are genetically more uniform and produce higher yields.

Legumes are not excluded from this problem, and a significant reduction in the land used for their production has been noted in many countries, including Spain. The need to preserve biodiversity, together with the nutritional benefits obtained from legume consumption, make it important to promote local wild legume production and work on the chemical characterisation of wild legumes. In this study, wild legumes

directly harvested from Andalucía region have been used.

On the other hand, it is well known that the addition of legumes to cereals produces an increase in both the quantity and quality of the protein mix and also in some minerals, such as iron (Young, 1991; Pastor Cavada *et al.*, 2011). Moreover, this addition represents an economic way to improve the protein value of cereal-based foods (Messina, 1999).

Cooking extrusion of starchy materials has become a very commonly used technique to obtain a wide range of products, such as snacks, breakfast cereals. (Pérez *et al.*, 2008). Extrusion cooking is considered an HTST process, and as such, it is highly recommended for the processing of high protein content materials such as legumes (González *et al.*, 2002).

As corn and rice grits are widely used to elaborate expanded products, there is a need to improve the nutritional value of this kind of foods (Chaiyakul *et al.*, 2009; Brennan *et al.*, 2011).

Several authors have demonstrated the nutritional advantage obtained by the addition of legumes to extruded cereals (Veronica *et al.*, 2006). These

*Correspondent: Fax: +95 4616790; e-mails: epastor@ig.csic.es; epastor@cica.es

advantages can be increased even more using whole grains in each food formulation (Kelly *et al.*, 2007).

The use of wild legumes in snack formulations could also be interesting given that it can be a good way to add some utilities to commercial products, such as variety and novelty, which are important to modern consumers (Pastor Cavada *et al.*, 2011).

Not much information about the use of wild legume species has been found. There are only a few studies about *Vicia faba* (Masoero *et al.*, 2005; Díaz *et al.*, 2006). The aim of this study was to evaluate the effect of the addition of 15% of wild legumes (*Vicia lutea* subsp. *lutea* var. *hirta* y *V. sativa* subsp. *sativa*) on the physical and nutritional properties of extruded products based on whole corn and brown rice.

Materials and methods

Materials

Samples of *Vicia* seeds, *V. lutea* subsp. *lutea* var. *hirta* (yellow vetch) and *V. sativa* subsp. *sativa* (common vetch) were collected from wild populations along Andalucía, a region in southern Spain. They were harvested during summer (June to August). Voucher specimens of the populations studied are deposited in the Herbarium of the Department of Plant Biology and Ecology of the University of Seville.

The beans were previously treated to inactivate lipoxigenase, by immersing them in boiling water for 2 min followed by immediate cooling with tap water (Fritz *et al.*, 2006). This heat treatment was carried out to avoid the development of a beany flavour during the grinding stage. Treated beans were dried in an oven, at 45 °C until they reached between 9% and 10% moisture. Whole seed was processed in a conical grooved grinding mill Bühler–Miag MLI 204 (Germany) using progressive and successive reduction in distance between the two grooved surfaces. Final particle size was between 420 and 250 µm.

Commercial Fortuna variety rice (a low-amylose rice: 16%) and commercial hard red corn, both from Molino Zacanini (Entre Rios, Argentina) were used. Maize endosperm hardness showed a coarse-to-fine particle ratio of 4.6 and NIR (near infrared reflectance) index of 450, corresponding to a hard endosperm maize (Robutti, 1995). Whole grains (rice and corn) were ground with a roller mill (Vario Miag, Bad Homburg, Germany) in such a manner to avoid the production of too many fine particles (less 250 µm). A progressive and successive reduction in distance between rollers from 1 to 0.5 mm was used. After each milling, the flour was screened through 1.114 mm (14 ASTM mesh). The thicker particles (> 1.114 mm) were remilled until the whole sample was ground.

Extruded samples

A Brabender 20 DN (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 r.p.m.; and extrusion temperature: 175 °C. While the extruder feeding section was maintained cool by circulating water through the jacketed device, the barrel (intermediate and metering) and die sections were both kept at 175 °C using the heat control device of the extruder. 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, an accessory of Brabender DO-Corder (Brabender P600; Duisburg, Germany), and the moisture content was adjusted to 14%, by adding tap water. This blend sample was kept in a plastic bag until the extrusion stage. 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. Single-screw extruder works at full capacity, and in this condition, feeding rate depends on the other extrusion conditions (grits moisture content, r.p.m. or die diameter). However, mass output is the consequence of the feeding rate, and it is taken as extrusion response. 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.

Physical characterisation of extruded samples

The following determinations were done for expanded samples: (i) radial expansion was calculated as the ratio of extruded product diameter and die diameter, taking the average of 10 measurements at ten different places along the sample; (ii) density expressed as grams per cm³ 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; (iii) specific mechanical energy consumption (SMEC), in Joule g⁻¹, was determined according to González *et al.* (2002), using the following formula: $SMEC = 61.3 \times 10^{-3} \times \text{Torque (BU)} \times 150 \text{ r.p.m.} \times Qa^{-1} \text{ (g min}^{-1})$. Mass output (Qa), in g min⁻¹ (at the feed moisture basis), was calculated by weighing the amount of sample coming out in 1 min and determining the moisture content after it was stabilised at room temperature. The 1-min sample was referred to 14% moisture; (iv) product texture was evaluated by a trained panel, 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) where ground samples were used. 100 g of each sample were first ground with a laboratory hammer mill (Retsch-Muhle, Germany) with a 2-mm sieve and then with a Cicoltec mill (UD Corp Boulder Colorado – USA) using a 1-mm sieve. Water solubility was calculated as soluble solids per 100 g of flour (db). 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.

Chemical composition

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 (Juliano, 1985). Total fibre was determined according to the procedure described by Lee *et al.* (1992). Lipids associated with the flour and protein isolates were extracted and measured following the method of Nash *et al.* (1967). Soluble sugars and polyphenols were measured using standard curves of glucose (Dubois *et al.*, 1956) and catechin (Mazza *et al.*, 1999), respectively.

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 *et al.* (1992), using D, L- α -aminobutyric acid as an internal standard. Tryptophan was determined after basic hydrolysis according to Yust *et al.* (2004).

In vitro protein digestibility

In vitro protein digestibility (IVPD) was determined according to the method of Hsu *et al.* (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: % digestible protein = $210.464 - 18.103 \times \text{pH}$.

Determination of mineral dialyzability (DFe%, DZn%)

The method according to Drago *et al.* (2005) 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 0.15 M PIPES [piperazine-N,N'-bis (2-ethane-sulphonic acid) disodium salt] buffer (pH 7; 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 NaHCO₃) 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 dialyzability was calculated from the amount of each dialysed mineral expressed as a percentage of the total amount present in each sample.

Dialyzable Mineral (%) = $\text{DM\%} = [\text{D}/(\text{W} \times \text{A})] \times 100$; 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⁻¹).

The daily requirement supply (DRS) which is supplied by a 30 g ration of the extruded products was calculated using the following formula: %DRS = $100 \times \text{Mineral Content} \times \text{DM} \times 30 \text{ g/DR}$; where DR is 2.2 and 1.8 mg day⁻¹ for zinc and iron, respectively (Galán *et al.*, 2012).

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 $P \leq 0.05$ degree of significance.

Results and discussion

Extrusion process and physical evaluation

Table S1 shows the values of the physical evaluation obtained from extruded samples corresponding to rice, corn and their respective blends with 15% wild legume (*V. lutea* subsp. *lutea* var. *hirta* and *V. sativa* subsp. *sativa*). It is well known that expansion and density

are the best properties to described product porosity (Asare *et al.*, 2004). In Table S1, it is observed that density values followed an inverse trend to that of expansion, as is expected (Anton *et al.*, 2009; Chaiyakul *et al.*, 2009).

It is observed that axial expansion values corresponding to rice-containing samples are higher than those corresponding to corn-containing samples ($P < 0.001$). 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 & Hosney, 1982). During the extrusion process, fat components act as lubricants, reducing the degree of cooking and consequently the expansion ratio (Bhattacharya & Hanna, 1988). However, the addition of legumes did not affect significantly the expansion, neither for rice nor for corn.

On the other hand, extrudate density was affected by addition of legume but only in the case of *V. lutea* subsp. *lutea* var. *hirta* added to corn was it significant ($P < 0.001$). It seems that 15% of cereal replacement by legume is not high enough to affect significantly the extrudate density. Several authors have shown that extrudate density is increased as addition of legume to cereal increases (Pérez-Navarrete *et al.*, 2006). Another aspect to consider is fibre content, because according to some authors, addition of fibre could increase extrudate density of starchy materials (Onwulata *et al.*, 2001; Veronica *et al.*, 2006), and legume addition increases the fibre content of extruded products.

Regarding solubility, it is well known that its value can be used as an indicator of starch degradation or degree of cooking during extrusion (González *et al.*, 2002). Table S1 shows that solubility values corresponding to corn-containing samples are lower than those of rice ones ($P < 0.001$). This can be explained by taking into account the higher oil content of corn, which would reduce the friction levels during extrusion, and consequently, a lower degree of cooking is obtained. The same consideration can be made for the case of SMEC (corn SMEC values are lower than those for rice) because this magnitude is directly related to the level of friction generated inside the extruder.

Moreover, the addition of legume caused an additional reduction of SMEC in rice samples, while in the case of corn, no significant effect was observed. The SMEC reduction effect has been discussed by Pérez-Navarrete *et al.* (2006), by considering that the lower hardness of legume particles in comparison with corn or rice ones would reduce the friction level inside the extruder. In the case of corn samples, the reduction of oil content of the blend could increase friction levels and consequently overcome the 'hardness effect'.

The higher SMEC is the higher gelatinisation degree is caused by intermolecular hydrogen bonds break-up (Gropper *et al.*, 2002).

The gelatinisation degree is directly related to product expansion (Díaz, 2003). In this way, the snack with the highest SMEC value ($P < 0.001$) has the highest expansion value too (Table S1). Ruiz-Ruiz *et al.* (2008) observed the same in *Phaseolus vulgaris* and corn expanded products.

Nutritional evaluation

Chemical composition

Table S2 shows the chemical composition corresponding to extruded samples. Moisture content is in the expected range (between 7 and 9%). Ash content corresponding to corn-containing samples tends to be higher than those of rice, although only corn samples containing *V. lutea* subsp. *lutea* var. *hirta* showed a significant difference compared with rice samples. It seems that the addition of legumes at the level used in this study does not have much effect on the ash content of cereal extrudates. In the case of fat (%), rice samples showed lower values than those of corn, and this was reduced when legumes were added to corn, while the opposite effect occurs with rice. This is because the lipid content of legumes is between those of maize and rice.

Regarding fibre and protein, the addition of legumes significantly increased their content in both corn and rice samples, as was expected. Furthermore, rice showed significant lower values in protein ($P < 0.05$) and fibre ($P < 0.001$) than corn.

These results confirm the beneficial effects of adding legumes to cereal products.

Amino acid profile

Table S3 shows the results of amino acid (AA) composition (g/100 g of protein) corresponding to extruded samples. When legumes were added to corn, leucine content was decreased ($P < 0.001$), but no significant change occurred with rice. Nevertheless, the leucine level was higher than that recommended by FAO/WHO/UNU (1985). On the other hand, the addition of legumes increased lysine content ($P < 0.001$) for both cases, but the recommended level (FAO/WHO/UNU, 1985) was not reached, indicating that a higher level of cereal replacement than 15% should be used. In the case of tryptophan, it was observed that its content in rice is higher than that in corn ($P < 0.05$) and that the addition of legumes tended to reduce the content of this amino acid, this effect being significant only for rice-containing samples. Although cereal samples satisfied the recommended level of FAO/WHO/UNU (1985), the blends with legumes reach more than 80% of this limit.

On the other hand, valine content was higher than FAO recommendation in all cases. Valine content was

dependent on the cereal, being higher for rice-containing samples.

In the case of threonine and aromatic amino acids (phenylalanine and tyrosine), their content in all samples satisfied FAO recommendations. Sulphur-containing amino acids (methionine and cysteine) also satisfied that recommendation, except for *V. sativa* subsp. *sativa* with rice sample. Other cases not satisfying FAO recommendations were histidine content of rice extrudate, and isoleucine content of corn samples.

Regarding nonessential amino acid, some significant differences were found. When legumes are added, alanine content is decreased ($P < 0.001$). However, when legumes are added, this content is increased ($P < 0.001$).

In vitro protein digestibility

Protein digestibility is one of the most important factors determining protein quality (FAO/WHO/UNU, 1985). Table S4 shows the values of *in vitro* protein digestibility corresponding to extruded samples. Values corresponding to rice-containing samples were higher than those of maize. Addition of legumes did not affect digestibility, because no significant differences were observed among samples, neither for rice nor for corn. These results are in agreement with this of other work (Pérez-Navarrete *et al.*, 2006).

Total phenolics contents

Table S5 shows polyphenol contents corresponding to extruded samples. Corn contains much higher polyphenol content than rice, and the addition of legume did not produce significant changes.

Mineral content and potential availability

Table S6 shows the results of iron and zinc content, their potential availability (%) and the % of recommended daily intake (RDI) supplied by a 30 g serving, corresponding to extruded samples.

Iron

It is observed that iron content increased with legume addition to corn and rice. Legumes are considered iron-rich materials, and other researchers have observed a positive effect of the addition of legumes to cereals (Lombardi-Boccia *et al.*, 1991). In general, iron availability increased by adding the legume to rice, although the only significant difference was observed in the expanded *V. lutea* subsp. *lutea* var. *hirta* with rice, which presented the highest value for this parameter ($P < 0.05$).

It is estimated that it is necessary to absorb 1.8 mg of Fe daily to meet the needs of 80-90% of adult women and adolescents of both sexes (Monsen, 1978). Taking into account, the values of iron potential supply, maize/rice extruded products and their blends with legumes provide from 1.67 to 5.74% of these requirements

per 30 g serving. In general, iron daily requirement supply increased by adding the legume to cereal.

Zinc

Table S6 shows that corn samples contain higher amount of Zn than those of rice ($P < 0.001$). With the addition of legumes, Zn content significantly increased ($P < 0.001$). Regarding DZn (%), the value corresponding to corn was higher than that of rice ($P < 0.01$), and when legumes were added, DZn (%) tended to increase, but the differences were not significant.

In the case of zinc, the daily requirements for adults are 2.2 mg day⁻¹ (Martín de Portela, 1993). A portion of maize/rice snacks and their legumes blends supply from 2.21 to 5.87% of those recommendations. Values corresponding to corn-containing samples were higher than those of rice. When legumes are added, the daily requirement supply is increased.

The recommended daily intake (RDI) of a nutrient is always above its actual requirement, because the nutritional recommendation is calculated taking into consideration environmental factors, individual variability and the bioavailability of such nutrients in the diet (Ziegler & Filer, 1990).

Conclusions

The present study revealed that a blend of whole corn flour or brown rice flour supplemented with 15% wild legume flour (*V. lutea* subsp. *lutea* var. *hirta* and *V. sativa* subsp. *sativa*) could be extruded with promising characteristics for use as a snack product with acceptable physical and nutritional properties. The chemical composition of extrudates based on legume addition was improved with respect mainly to protein and fibre content, in comparison with extrudates based on cereal alone. Protein quality was also improved, taking into account the increase in sulphur-containing amino acids, lysine and tryptophan.

Extruded products based on whole corn or rice with the addition of wild legumes offer three main advantages: they are whole grain food grade; they have a better nutritional quality than a traditional extrudate; and they are made with wild legumes, which make them a novel product.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Physical properties corresponding to extruded samples.

Table S2. Chemical composition corresponding to each extruded sample

Table S3. Amino acid composition (g/100 g of protein) corresponding to extruded samples. Mean values \pm standard deviation ($\bar{x} \pm SD$) of three analyses and FAO recommendation

Table S4. In vitro protein digestibility (PD) of extruded samples.

Table S5. Polyphenol content of extruded samples

Table S6. Fe and Zn content (ppm); potential availability (%) and daily requirements, which are supplied by a 30 g portion, corresponding to extruded samples