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Physicochemical characterization of lima bean (Phaseolus lunatus) and Jack bean (Canavalia ensiformis) fibrous residues

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Abstract

Some physicochemical, physiological and functional characteristics of Jack bean (Canavalia ensiformis) and lima bean (Phaseolus lunatus) fibrous residues, obtained as a by-product during the protein and starch extraction process were evaluated. The C. ensiformis residues had higher crude fibre (22.6%), total dietary fibre (55.8%) and insoluble dietary fibre (52.4%) contents than the P. lunatus (crude fibre, 12.8%; total dietary fibre, 29.4%; insoluble dietary fibre, 28.6%). Water-holding capacities in both legume fibrous residues were higher (C. ensiformis, 39.5%; P. lunatus, 26.5%) than their oil-holding capacities (23% and 18%, respectively). The P. lunatus residue had lower emulsifying activity $(8.6%)$ than the C. ensiformis residues $(49.3%)$ but higher emulsifying stability (P. lunatus, 100% versus C. ensiformis, 28.25%). Neither fibrous residue was capable of bonding calcium ions, but both did bind iron ions (C. ensiformis, 31.1%; P. lunatus, 28.4%). Similar antioxidant activity values were obtained for the Jack bean (39.4%) and lima bean (35.6%) residues.

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1. Introduction

A diet rich in plant materials may protect against a wide range of diseases (e.g. constipation, diverticular disease, colon-rectal diseases, diabetes, obesity, gall stones, colon cancer) [\(Oliveira, Reyes, Sgarbier, Areas,](#page-7-0) & Ramalho, 1991; Valiente, Esteban, Mollá, & López-Andreú, 1994). A major factor in this protection is the plant's high dietary fibre content, which highlights the benefits of a diet rich in dietary fibre. The definition of ''dietary fibre'' proposed by [Trowell et al. \(1976\)](#page-8-0) refers to the sum of the plant polysaccharides and lignin that are not digested by the endogenous secretions of the upper gastrointestinal tract. Dietary fibre is generally

from plant cell walls, which invariably contain other substances which are not hydrolyzed by digestive enzymes, such as cutin and suberin, gums (including b-glucans and galactomannans), mucilages or watersoluble polysaccharides, polyphenols and phenolic compounds, and inorganic salts of phytic acid (Asp, Schweizer, Southgate, & Theander, 1993; Saura-Calixto, 1998; Torre, Rodríguez, & Saura-Calixto, 1991). Resistant starch and resistant protein can also be included in the definition of dietary fibre [\(Cho, DeVries,](#page-7-0) [& Prosky, 1997; Englyst, Kingman, & Cummings,](#page-7-0) [1992\)](#page-7-0).

The physiological actions of dietary fibre are likely based on its physiological properties such as water and oil holding capacities [\(Leterme, Froidmont, Rossi, and](#page-7-0) Théwis, 1998; López et al., 1997; Oliveira et al., 1991), absorption of organic molecules ([Marfo, Wallace,](#page-7-0) [Timpo, & Simpson, 1990; Nishina, Scheneeman, &](#page-7-0) [Freeland, 1991\)](#page-7-0), bacterial degradation (Larrauri, Goñi, Martín-Carrón, Rupérez, & Saura-Calixto, 1996),

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cation-exchange capacity (Torre, Rodríguez, & Saura-[Calixto, 1992](#page-8-0)), and antioxidant activity (Larrauri, Sán[chez-Moreno, & Saura-Calixto, 1998; Lee, Howard, &](#page-7-0) Villalón, 1995; Yen & Chen, 1995).

Normal, daily dietary fibre intake is approximately 12–25 g [\(Cummings, 1993](#page-7-0)), and strong, continued recommendations have been made to double or even triple intake in most populations [\(Harrington, Flynn, &](#page-7-0) [Cashman, 2001](#page-7-0)). Increased intake of complex carbohydrates and dietary fibre has been recommended for the populations of Western countries because of the possible relationship between diets low in dietary fibre and illnesses such as hypertension, arteriosclerosis, diabetes [\(Torres et al., 1991\)](#page-7-0). As a result of these recommendations, materials rich in dietary fibre have gained popularity as beneficial food ingredients, yet relatively little is known about the effects of food processing on fibre and its components ([Camire, Viollette, Dougherty, &](#page-7-0) [McLaughlin, 1997](#page-7-0)).

The food industry is experiencing a constantly growing demand for new ingredients, especially in response to the current, very strong and visible public interest in food ingredients from natural sources ([Czuchajowska &](#page-7-0) [Pomeranz, 1994](#page-7-0)). This demand has drawn researchers' attention to legume components obtained from wet fractionation. The wet-fractionation process has been proposed as a detoxification method for lima bean (Phaseolus lunatus) and Jack bean (Canavalia ensiformis), and has also been proven to be a viable technology for the integral use of these seeds ([Betancur-](#page-7-0)Ancona, Camelo-Matos, Chel-Guerrero, & Dávila-Ortíz, 2001; Moguel-Ordoñez, Betancur-Ancona, & [Chel-Guerrero, 1996\)](#page-7-0). This process produces protein concentrates, starch and fibre-rich fractions ([Czucha](#page-7-0)[jowska, Otto, Paszczynska, & Baik, 1998](#page-7-0)). The potential usefulness of these legumes, however, also depends on their functional and physiological properties, which affect the nutritional and sensory characteristics of food, and play an important role in the physical behaviour of food or its ingredients during preparation, processing and storage. The objective of this study was to investigate the chemical composition, and functional and physiological properties of C. ensiformis and P. lunatus fibrous residues.

2. Materials and methods

2.1. Seeds and chemicals

Baby lima bean (Phaseolus lunatus) and Jack bean (Canavalia ensiformis) seeds were obtained from the February 2000 harvest in the state of Campeche, Mexico. All chemicals were reagent grade from J.T. Baker (Phillipsburg, NJ), and enzymes were Sigma (Sigma Co., St. Louis, MO, USA).

2.2. Flours

A total of 10 kg of seeds from each legume species were used in this study to produce the flours. First, all impurities and damaged seeds were discarded, then the remaining, sound seeds were milled in a Mykros impact mill, and the resulting flour passed through a 20-mesh screen.

2.3. Fibrous residues

The residues were processed using the wet-fractionation method reported by [Betancur-Ancona et al. \(2001\)](#page-7-0). Briefly, 1 kg of 20-mesh grade flour was suspended in distilled water at a 1:6 (w/v) ratio. The pH was adjusted to 11 with 1 N NaOH and the dispersion stirred for 1 h at 400 rpm with a mechanical agitator (Caframo Rz-1). Wet milling was then done in a Kitchen-Aid mill, separating the fibre solids from the starch and protein mix with 80- and 150-mesh sieves. The fibrous residue was washed 5 times, using a 1:3 solids to distilled water ratio. The resulting fibre fraction was dried at 60 \degree C in an air convection oven (Imperial V), and then weighed and milled in a Ciclotec mill, until it passed through a 20-mesh screen.

2.4. Particle size distribution

The fibrous residues (200 g) of P. lunatus and C. ensiformis were submitted to granulometry determination in a Rotap agitator (equipped with 45, 60, 80, 100, 200 and 250 mesh sieves) for 20 min. The material retained in each sieve was weighed and the percentage of each fraction calculated.

3. Chemical composition

3.1. Proximate composition

The nitrogen (method 954.01), fat (method 920.39), ash (method 923.03), crude fibre (method 962.09), and moisture (method 925.09) contents of the fibrous residues were determined according to official AOAC pro-cedures ([AOAC, 1997](#page-7-0)). Nitrogen content (N_2) was determined with a Kjeltec Digestion System (Tecator, Sweden), using cupric sulfate and potassium sulfate as catalysts. Protein content was calculated as nitrogen \times 6.25. Fat content was obtained from a 1 h hexane extraction. Ash content was calculated from the weight of the sample after burning at 550 \degree C for 2 h. Moisture content was measured, based on sample weight-loss after oven-drying at 110 \degree C for 2 h. Carbohydrate content was estimated as nitrogen-free extract (NFE).

3.2. Total (TDF), soluble (SDF) and insoluble (IDF) dietary fibre

The dietary fibre fractions were determined using the [Prosky, Asp, Schweizer, DeVries, and Furda \(1988\)](#page-7-0) method. Briefly, 1 g (d.b.) fibre samples were placed in four Erlenmeyer flasks (W_1) and weighed. To each of these samples, 50 ml of phosphate buffer (0.08 M, pH 6) were added, and the pH adjusted to 6 with 0.325 HCl N or 0.275 N NaOH. These were then placed in a water bath at 100 °C for 10 min. Then, 0.1 ml α -amylase (Sigma A-3306) was added to each and they were left to incubate at the same temperature for 15 min under constant agitation. The flasks were then cooled rapidly and the samples adjusted to pH 7.5. After this, they were placed in a water bath at 60° C for 10 min, 0.1 ml protease solution was added to each (Sigma P-3910, 50 mg in 1 ml phosphates buffer), and they were left to incubate at the same temperature for 30 min. The flasks were cooled and the samples adjusted to pH 4, and then returned to the water bath at 60° C until they reached this temperature. After adding 0.3 ml amyloglucosidase (Sigma A-9913) to each, the samples were left to incubate for 30 min under constant agitation. Then, 95% ethanol at the same temperature was added at a 1:4 sample/ethanol ratio, and the mixture left in the water bath for 1 h. The samples were filtered at a constant weight into crucibles for fibre into which a 1 g cap of Celite had been previously placed. The flasks were rinsed three times with 20 ml of 78% ethanol, twice with 10 ml 95% ethanol, and twice with 10 ml acetone. The crucibles were then placed in a stove at 130° C for 1.5 h, and weighed (W_2) . Two of the crucibles were placed in a furnace at 550 °C for 4 h (W_3) , and crude protein was determined using the contents of the remaining two (W_4) . Calculations were done using the formula:

 $\%TDF = (W_2 - W_3 - W_4 - W_5) \times 100/W_1$

Where W_5 is the reagent weight (blank).

3.2.1. Insoluble (IDF) and soluble dietary fibre (SDF)

The same [Prosky, Asp, Schweizer, De Vries, and](#page-7-0) [Furda \(1988\)](#page-7-0) method was used to quantify IDF, the only difference being that alcohol was not added to precipitate IDF. Calculation of IDF percentage in the samples was the same as for TDF. The SDF was calculated by subtracting the IDF value from the TDF value.

3.3. Functional and physiological properties

3.3.1. Water-holding (WHC) and oil-holding capacity (OHC)

These holding capacities were determined using the technique described by [Chau, Cheung, and Wong](#page-7-0) [\(1997\)](#page-7-0). Briefly, 1 g (d.b.) of sample was weighed and

then stirred into 10 ml of distilled water or corn oil (Mazola, CPI International) for one minute. These fibrous suspensions were then centrifuged at $2200 \times g$ for 30 min and the supernatant volume measured. Waterholding capacity was expressed as g of water held per g of sample, and oil-holding capacity was expressed as g of oil held per g of fibre. Corn oil density was 0.92 g/ml.

3.3.2. Emulsifying activity (AE) and emulsion stability (ES)

These properties were evaluated using the method of [Chau et al., \(1997\)](#page-7-0). Samples of 100 ml of 2% (w/v) fibrous suspension were homogenized using a Caframo RZ-1 homogenizer at 2000 rpm for 2 min. Then, 100 ml of corn oil (Mazola, CPI International) were added to each sample and homogenized for 1 min. The emulsions were then centrifuged in 15 ml, graduated centrifuge tubes at $1200 \times g$ for 5 min, and the emulsion volume measured. Emulsifying activity was expressed as percentage of the emulsified layer volume of the entire layer in the centrifuge tube. To determine emulsion stability, the prepared emulsions were heated at 80 \degree C for 30 min, cooled at room temperature and centrifuged at $1200 \times g$ for 5 min. Emulsion stability was expressed as percentage of the remaining emulsified layer volume of the original emulsion volume.

3.3.3. Antioxidant activity

Antioxidant activity was determined using the modified ferric thiocyanide method of [Kikuzaki and Naka](#page-7-0)[tani \(1993\),](#page-7-0) mentioned by [Larrauri et al. \(1996\)](#page-7-0). First, the samples were prepared, extracting 500 mg of solid sample in a sequence with 40 ml of methanol/water (50:50, v/v), and 40 ml of acetone/water (70:30 v/v) at room temperature for 60 min. These were then centrifuged at $15,000 \times g$ for 30 min, the supernatants of the previous extractions were mixed in and volume adjusted to 100 ml with distilled water. The extracts were concentrated in a rotational evaporator with vacuum at $75 \degree C$, lyophilized and dissolved in absolute ethanol.

To determine antioxidant activity, a mixture of 0.5 ml of a weighed sample (providing a final concentration of 0.5 g/l of mixture) in absolute alcohol, 0.5 ml of 25.1 g/l of linoleic acid in 995 ml/l of ethanol, 1 ml sodium phosphate buffer 0.05M, pH 7, and 0.5 ml distilled water, was placed in a threaded-cover tube, agitated and placed in an oven at 40 \degree C in darkness. A control mixture without sample was also used. To 0.1 ml of this solution were added 9.7 ml of 750 ml/l ethanol and 0.1 ml of 300 g/l ammonium tiocyanide. Exactly 3 minutes after addition of 0.1 ml of 0.02 M ferric chloride in 35 g/ l hydrochloric acid to the reacting mixture, absorbancy was measured by comparison to a blank at 500 nm, and then every 24 h (t) until one day after the blank reached its maximum. The oxidation index (OI) and antioxidant activity (AA) were calculated as follows:

$$
OI = \frac{Absorbarcy \ t = 72h}{Absorbarcy \ t = 0}
$$

AA =
$$
\frac{OI \ of \ the \ extracted \ product \ 72 \ h}{OI \ blank \ 72 \ h} \times 100
$$

3.3.4. Mineral binding

Mineral binding capacity was determined according to the modified method proposed by Torre, Rodriguez, [and Saura-Calixto \(1995\).](#page-8-0) It was measured by mixing the fibre residue with a known concentration solution of the ion $(Ca^{+2}$ and Fe⁺², respectively) at pH 2.5 until reaching equilibrium. The solution was then centrifuged in order to determine the soluble metallic cation concentration in the supernatant. The initial concentrations of the analyzed solutions were 3, 5, 7, 9 and 11 ppm. Then, 100 ± 0.1 mg of sample were placed in a 50 ml centrifugal tube, and 25 ml of metallic solution added. The suspension was agitated at room temperature for 3 h at 150 rpm. The suspension was then centrifuged at $1200 \times g$ for 20 min, taking an aliquot of the supernatant for metallic concentration determination. To estimate the contribution of endogen minerals, a blank test was carried out omitting the metallic solution and substituting its volume with bidistilled water. The absorbed metal percentage was obtained with the formula:

Absorbed metal% =
$$
\frac{\text{ppm of absorbed metal}}{\text{ppm of known solution}} \times 100
$$

3.3.5. Statistical analysis

All determinations were done in triplicate, and a statistical study done to determine central tendency and results dispersion. Data were analyzed using a one-way variance analysis and Duncan's multiple range test according to [Montgomery \(1991\),](#page-7-0) using a 5% significance level (Statgraphics plus 2.1 statistics computer software).

4. Results and discussions

4.1. Fibrous residues recovery

Milling of the seeds produced 0.952 kg of flour per kilogramme of C. ensiformis and 0.933 kg of flour per kilogramme of P. lunatus. After processing a kilogramme of each flour separately to obtain the fibrous residues, the fibre yields were 33.74% for C. ensiformis and 35.43% for P. lunatus. These yield values were both lower than those reported by [Moguel et al. \(1996\)](#page-7-0), probably due to the fact that extraction for the present study was done via soaking (pH 12, soaking 36 h, 32 °C). Therefore, the method, using one hour of agitation, has a clear advantage over soaking.

4.2. Particle size distribution

Fig. 1 shows the particle size distribution of the fibrous residues. The analysis of both legume residues indicated a minor particle diameter of $500 \mu m$, which coincides with that proposed by [Fuertes, Ayala,](#page-7-0) [Ledesma, Zumbado, and Benedito \(1993\)](#page-7-0), and indicates an ideal particle size range of $50-500 \mu m$. This physical parameter is important because it will directly affect the functional properties, such as water and oil retention, as a result of the particle surface contact area with these food components ([Oliveira et al., 1991](#page-7-0)).

The C. ensiformis fibrous residue had a higher amount of sample (60.13%) coming from the 80 mesh, while the P. lunatus residue had the highest proportion (50.19%) of same-size particles from the 200 mesh. Consequently, the P. lunatus fibrous residue contained more refined particles, which may cause a decrease in oil-holding capacity, as reported by López et al. (1997). This might also be the same for holding capacity, physiologically affecting intestinal traffic when the fecal mass descends. [Dreher \(1999\)](#page-7-0) reported similar behaviour in wheat bran when particle size was diminished.

4.3. Chemical composition

4.3.1. Proximate composition

The proximate compositions of C. ensiformis and P. lunatus fibrous residues are shown on [Table 1.](#page-4-0) The C. ensiformis residue had a low moisture content (4.52%), which was almost the same as that of P . lunatus (4.72%). There was also a difference in ash values

Fig. 1. Particle size distribution of C. ensiformis (a) and P. lunatus (b) fibrous residues.

(C. ensiformis, 2.56%; P. lunatus, 1.77%) even though it was too low in both cases. In comparison, [Dreher](#page-7-0) [\(1999\)](#page-7-0) reports wheat bran moisture content as 9.7% and ash content as 6.1%, notably higher than for the studied legumes. [Chang and Morris \(1997\),](#page-7-0) indicated similar leves of ash (3.63%) in soybean fibre. Protein content was higher for P. lunatus (11.1%) than for C. ensiformis (7.38%), a difference that may be due to the different natures of the samples, though both are still low in comparison to the 16.0% content of wheat bran [\(Dre](#page-7-0)[her, 1999](#page-7-0)) and 21.4% content of soybean fibrous residues ([Chang & Morris, 1997](#page-7-0)). Fat content was notably higher for C. *ensiformis* (1.32%) than for P. *lunatus* (0.66%), though again these are insignificant amounts when compared to wheat bran (5.9%) and similar to soybean fibre (1.73%). Crude fibre content for C. ensiformis (22.68%) was much higher than for P. lunatus (12.88%), and both the legume crude fibre contents were far above that of wheat bran (4.9%) [\(Dreher,](#page-7-0) [1999\)](#page-7-0).

Both C. ensiformis and P. lunatus residues had a high nitrogen-free extract (NFE) content (60.0 and 68.2%, respectively). This occurs because NFE represents part of the cellulose, hemicellulose, pectins and other carbohydrates not included in the crude fibre content as a result of the imprecise methods used. Therefore, a more adequate determination for this estimate would be total dietary fibre together with the Van Soest fractions, which would avoid this high error percentage.

In comparing the fibrous residues of the studied legumes to rice husk ([Dreher, 1999\)](#page-7-0), the latter has higher values in all aspects: moisture 10.0% , ash 8.5% , protein 14.0% and fat 19.0%. When compared to wheat bran (Duque, Gallardo, Santoyo, $&$ Sánchez, 1998) the wheat bran also has higher values: moisture 7.3%, crude protein 17%, ash 5.3% and crude fat 3.9. These contents are important because food can be considered low quality when crude protein content is less than 7.0%, which leads to a decrease in consumption due to protein deficiency ([Mendoza, 1997\)](#page-7-0).

4.3.2. Total (TDF), soluble (SDF) and insoluble (IDF) dietary fibre

Total dietary fibre (TDF) had a higher value in C. ensiformis (55.88%) than in P . lunatus (29.42%) (Fig. 2). Insoluble dietary fibre (IDF) also showed a clear difference between samples, with C. ensiformis (52.49%) having levels almost double that of P. *lunatus* (28.64%). There was also a clear difference between samples for soluble dietary fibre (FDS), with 3.38% for C. ensiformis and 0.77% for P. lunatus. The results obtained by [Duque et al. \(1998\)](#page-7-0) for wheat bran were 16.0% (TDF), 15.4% (IDF) and 0.6% (SDF), notably lower in comparison to the two studied legume fibrous residues.

The *P. lunatus*, in the present study, had similar values (TDF, 29.4%; IDF, 28.64% and SDF, 0.775%) to those reported by Herrera, González, and Romero (1998) for P. vulgaris (TDF, 28.9%; IDF, 26.5%; SDF, 2.4%). Only SDF exhibited slight variation, though its value is still deficient in both the studied seeds, because they are legumes. These results are therefore adequate for this sample type since legumes contain higher quantities of insoluble dietary fibre such as cellulose, hemicellulose and lignin. This high IDF content could affect mineral binding in a way similar to the effect of IDF upon iron ions reported by Periago, Ros, López, and Martinez (1995) for pea fibrous residues. This high fibrous content may also be beneficial, as demonstrated in past studies in which wheat bran diets rich in TDF and IDF increase fecal bulk weight 5–9% [\(Duque et al.,](#page-7-0) [1998\)](#page-7-0). Given this, incorporation of C. ensiformis and P. lunatus fibrous residues could be used to aid in treatment of constipation, by reducing intestinal transit time through an increase in peristaltic movements.

4.4. Functional and physiological properties

4.4.1. Water holding capacity (WHC)

The water-holding capacities of C. *ensiformis* and P. lunatus fibrous residues (39.5 and 26.5%, respectively)

□C. ensiformis ■P. lunatus

Different letters on the same row indicate statistical difference $(P < 0.05)$.

Fig. 2. Dietary fibre fractions of C. ensiformis and P. lunatus fibrous residues (% d.b.). a–b Different letters on the same block pair indicate statistical difference $(P<0.05)$.

were low, due to the low quantity of soluble fibre in the samples (Table 2). Of the two studied legumes, C. ensiformis had higher WHC because of its 3.38% SDF content, as compared to the 0.77% SDF of P. lunatus. High WHC has been reported for cauliflower fibre (48.1%), which results from its high soluble fibre content (19.2%). This kind of fibre increased the yield of a hamburger-type product by 10%, and served as a partial substitute for purified gums in sauces [\(Femenia,](#page-7-0) [Lefebvre, Thebaudin, Robertson, & Bourgeois, 1997\)](#page-7-0). Despite its lower SDF, similar behaviour can be expected from C. ensiformis fibrous residue. It is important to remember that WHC reflects the fibre source, is related to polysaccharide structure, and can be influenced by porosity, particle size, ionic strength, pH, temperature and fibre type ([Femenia et al., 1997](#page-7-0)).

Both the studied legumes had a low SDF to IDF content ratio. According to [Oliveira et al. \(1991\)](#page-7-0), this, along with pectin and mucilage, is partially responsible for WHC. Physiologically, this would be reflected in decreased use of glucose and lipid metabolism, because decreased postprandial glucemia is related to lower glucose intestinal absorption speed [\(Wolever, 1989](#page-8-0)), and lipid absorption decreases as bulk viscosity increases, decreasing lipids reabsorption in the ileum (López et al., [1997\)](#page-7-0). Another physiological effect is that high WHC can lead to endogen protein excretion, producing nitrogen retention [\(Leterme et al., 1998\)](#page-7-0). This would be a concern for C. ensiformis fibrous residue because of its high WHC.

4.5. Oil holding capacity (OHC)

Oil-holding capacity for the C. ensiformis and P. lunatus fibrous residues (23 and 18%, respectively) was similarly low because of the low SDF content of both (Table 2). This is confirmed by [Rosado \(1989\),](#page-7-0) who reports that SDF is responsible for adsorption of organic compounds, such as biliary acids. This could also be explained as a function of the high cellulose content (12.28% for P. lunatus) in the samples, which slows oil adsorption. [Femenia et al. \(1997\)](#page-7-0), for example,

Table 2

Functional and physiological properties of C. ensiformis and P. luna-			
<i>tus</i> fibrous residues $(\%$ d.b.)			

Different letters on the same row indicate statistical difference $(P<0.05)$.

report a low 2.7% OHC value for cauliflower fibre, largely due to its cellulose (38.1%) and hemicellulose (11.4%) content, the latter being only partially responsible. This occurs because oil is trapped on the fibre surface, with lignin- and hemicellulose-rich sources having more oil-holding capacity and larger particle size. Though the oil adsorption mechanism is unknown, it has been suggested that the surface properties and hydrophobic nature of the particles may be important for fibre incorporation into food.

The fibrous residues of the two studied legumes may have other benefits. [Marfo et al. \(1990\)](#page-7-0) demonstrated that C. ensiformis protein- and fibre-rich diets decreased cholesterol and total lipids in plasma. They fed adult rats with a hypercholesterolemic diet (2% cholesterol) rich in C. *ensiformis* protein (15%) and fibre (10%) . They obtained a decrease in plasma cholesterol levels, followed by a cholesterol level decrease in the liver, kidney and heart, as well as low serum lipid levels. This same behaviour can be expected of the C. ensiformis and P. lunatus fibrous residues as they contain protein (C. ensiformis, 7.56% ; P. lunatus 11.1%) and have high TDFs (C. ensiformis, 55.88; P. lunatus, 29.42%).

To increase these properties, particle size could be reduced to augment contact surface area between the fibre and water or oil (López et al., 1997), making their use possible in baked products to impart freshness and softness [\(Fuertes et al., 1993\)](#page-7-0). Another option is to incorporate them into fried products as their low OHC would provide a non-greasy sensation.

4.5.1. Emulsifying activity (AE) and emulsion stability (ES)

For emulsifying activity (EA), P. lunatus has a high percentage (49.3%) in comparison to C. ensiformis (8.6%), though the former also has the inconvenience of lower emulsion stability (28.25%) (Table 2). This behaviour is directly related to fibre type and the percentage of the soluble and insoluble fibre fractions. As a result, use in and incorporation of these two legume fibrous fractions into food will depend on product type. For products in which long shelf life is required, and thus higher emulsifying stability (ES), C. ensiformis should be used because it has 100% ES, whereas P. lunatus, with higher emulsifying activity (49.3%) but less stability (28.25%), might not be so appropriate.

Phaseolus lunatus, however, may have other benefits. Because emulsifying properties are related to oil adsorption, [Kahlon, Edwards, and Chow \(1998\)](#page-7-0) demonstrated that diets with 10% cellulose and low oil adsorption decrease cholesterol. Given the characteristics of the studied samples, P. lunatus, with 12.28% cellulose [\(Peraza, 2000](#page-7-0)) and 18.0% OHC, may behave in this way, and thus be useful in hypercholesterolemic patients. Emulsification is positively associated with the fact that dietary fibre can bind biliary acids, which limits

absorption in the small intestine, allowing excretion in the feces and reducing blood cholesterol levels. The hypercholesteremic effect of dietary fibre is based on the fact that biliary salts bind to fibre through mycelia formation, are eliminated as less soluble compounds in the feces and are replaced by the organism via new biliary acid synthesis initiated with cholesterol, causing a cholesterol decrease in the organism (López et al., [1997\)](#page-7-0).

4.5.2. Antioxidant activity

Antioxidant activity is currently seen as one of the most important properties of dietary fibre. Dietary fibre acts as a free metal trap in the body, forming pro-oxidant metal complexes which act as reducing agents, and a simple oxygen-formed chelating agent. Antioxidants protect the organism from degenerative diseases, such as cancer and reduce oxidation of low density lipoproteins, avoiding arteriosclerosis [\(Emmons & Peterson, 1999\)](#page-7-0). Antioxidant activity results for C. ensiformis and P. lunatus fibrous residues at 72 h ([Table 2](#page-5-0)) were 39.4 and 35.6%, respectively. These are intermediate values when compared to those reported by [Larrauri et al. \(1996\)](#page-7-0) for lemon (50.0%) and mango (14.0%) peel. The sample values are comparable to α -tocopherol or vitamin E (35%), which is a natural preservative or free radical inhibitor present in vegetables oils and especially in wheat seedling oil. Another antioxidant is 2-terbuty-4 hydroxyanisols (BHA), a synthetic phenol with low antioxidant activity (4%) that is widely used as a food preservative. High antioxidant activity has been reported for grape peel, indicating high concentrations of phenolic compounds [\(Saura-Calixto, 1998](#page-7-0)). This has been verified using oat husk, which has 30.3% antioxidant activity because of its phenolic compounds [\(Emmons & Peterson, 1999](#page-7-0)). Given the above, the C. ensiformis and P. lunatus fibrous residues antioxidant properties may be attributed to high phenolic compounds contents.

Physiologically, antioxidant activity is beneficial in cardiovascular diseases and cancer because it prevents the tissue damage caused by free radicals through the formation of relatively stable and non-reactive free radicals ([Saura-Calixto, 1998](#page-7-0)). This property is

Table 3

 $Fe⁺²$ ion (%) binding capacity of C. ensiformis and P. lunatus fibrous residues (% d.b.)

Known concentration solution	C. ensiformis	P. lunatus		
3 ppm	31.1 ± 0.88 ^a	28.4 ± 0.12^a		
5 ppm	11.1 ± 0.18^a	11.8 ± 0.39 ^a		
7 ppm	11.4 ± 0.42^a	$15.7 \pm 0.04^{\text{a}}$		
9 ppm	14.8 ± 0.76 ^a	9.19 ± 0.49^a		
11 ppm	15.6 ± 0.46^a	$8.14 \pm 0.02^{\text{a}}$		

Different letters on the same row indicate statistical difference $(P<0.05)$.

increasingly important as the search for natural antioxidant sources intensifies in an effort to replace synthetic antioxidants, since the latter have been shown to be carcinogenic and teratogenic ([Larrauri et al., 1998\)](#page-7-0).

4.6. Mineral binding

Before determining the mineral binding capacity of the C. ensiformis and P. lunatus fibrous residues, their calcium and iron contents were evaluated. Both residues had notably high calcium contents (C. ensiformis, 25.5 ppm; P. lunatus, 11.1 ppm), though iron content was similarly low for both (C. *ensiformis*, 0.19 ppm; P. lunatus, 0.20 ppm). The high calcium contents rebounded positively because the fibrous residues are saturated with metals and thus, instead of binding ions, they liberate them into the environment. As a result, the residues could be consumed without causing deficiencies and avoiding decalcification and circulatory problems.

Affinity for iron was determined by applying known iron concentrations to the samples. When the lowest iron concentration (3 ppm) was applied, the highest absorption rate was obtained for C. ensiformis (31.1 ppm) and P. lunatus (28.4 ppm) (Table 3). Linear behaviour could not be specified for this because metal reception was extremely variable. P. lunatus had a comparable iron affinity (12.28%) to pea fibrous residues, which have an iron affinity of 12% due to high cellulose content [\(Periago et al., 1995\)](#page-7-0). Even though this absorption is through complex formation [\(Torre et al., 1995\)](#page-8-0), it is apparently a weak bond in this case, its rupture being susceptible to the presence of other food compounds. Given this, the studied legume fibrous residues are not harmful to health. They may fix iron to a certain degree, but over the long-term they do not prevent iron bioavailability as the bond formed with the fibrous residue is weak.

5. Conclusions

Higher proportions of total, insoluble and soluble dietary fibre were found in C. ensiformis fibrous residue $(55.88, 52.59 \text{ and } 3.38\%$, respectively) than in *P. lunatus* (29.42, 28.64 and 0.77%, respectively) fibrous residue. Low oil holding capacity values for both legumes were probably due to their deficient lignin contents. High emulsifying activity was exhibited by *P. lunatus* fibrous residue (49.3%), compared to C. ensiformis (8.6%) fibrous residue, though the latter had 100% emulsification stability. Antioxidant activity was 39.4% for P. lunatus fibrous residue and 35.4% for C. ensiformis fibrous residue, both having similarly high levels due to high phenolic concentrations. In terms of the mineral binding capacity of the two studied fibrous residues, calcium could not be bound because the samples were already calcium saturated, though iron bound in similar quantities to the C . ensiformis (31.1 ppm) and P . lunatus (29.4 ppm) fibrous residues. This binding of iron, however, is not considered harmful because it bonds weakly to the residues, and is released by other food components.

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