Physicochemical and bioactive properties of the fibrous fraction of hard-to-cook black bean (*Phaseolus vulgaris* L. var. Jamapa)

MUKTHAR SANDOVAL-PERAZA – DAVID BETANCUR-ANCONA – LUIS CHEL-GUERRERO

SUMMARY

An evaluation was done of physicochemical properties of a fibre-rich fraction (FRF) obtained by wet fractionation of hard-to-cook black bean (*Phaseolus vulgaris*). The results show that the quantity of total dietary fibre, insoluble dietary fibre and soluble dietary fibre in the FRF of hardened beans were 431.20 g·kg^{-1}, 352.10 g·kg^{-1} and 79.10 g·kg^{-1}, respectively. The water-holding capacity, water-adsorption capacity and water-absorption capacity were 7.30 kg·kg^{-1}, 0.29 kg·kg^{-1} and 3.45 kg·kg^{-1}, respectively. The oil-holding capacity, emulsifying activity and emulsion stability values were 1.53 kg·kg^{-1}, 79.59 g·kg^{-1} and 580.40 g·kg^{-1}, respectively. The bioactive properties showed values of 7.70 g·kg^{-1} for the organic molecule absorption capacity and 795.06 mol·kg^{-1} of Trolox ((6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent antioxidant coefficient for the antioxidant activity. These data on the fibrous fraction of hard-to-cook black bean suggest that it can be used as an additive to functional foods, and their consumption could reduce the risks of chronic degenerative diseases.

Keywords

hard-to-cook; dietary fibre; soluble fibre; insoluble fibre; functional properties; bioactive properties; *Phaseolus vulgaris*

The common bean (*Phaseolus vulgaris* L.) is the most widely consumed legume in the world, and in Latin America is a basic component of the diet. Countries such as Mexico, Honduras, Nicaragua, Guatemala and El Salvador reported a per capita consumption of 16 kg, 13 kg, 14 kg, 10 kg and 13.5 kg per year, respectively [1]. The common bean is valuable as food because it can provide 16–33% of protein, vitamins (rivoboflavin, thiamine, niacin, folic acid, etc.), dietary fibre (14–19%, especially soluble dietary fibre), minerals (Ca, Fe, Cu, Zn, P, K, Mg), and free unsaturated fatty acids [2].

Poor handling and storage after the harvest induces the hardening process in the grain. According to the method dictated in the applicable Mexican regulations NMX-FF-038-SCFI-2002 [3], hardened grain is that for which cooking time has increased significantly in relation to cooking freshly harvested grain. The hardening process in the grain is caused by the effects of aging or grain storage under conditions of high relative humidity in combination with high temperatures (temperature > 25 °C and relative humidity > 65%). Beans are considered hard when their cooking time is over 55 min [3]. This phenomenon is characterized by the difficulty of the cotyledons to soften during cooking. Some mechanisms have been proposed to explain the effect of hardening, which include lipid oxidation to oxygenated polymers, formation of insoluble pectates, lignification, hydrolysis and denaturing of proteins; these reactions occur mostly in the cotyledon [4, 5].

Despite the deterioration reactions that may occur in the grain, portions such as the fibre can still be used. Peraza-Mercado [6] reported the use of fibrous residues of *Canavalia ensiformis* L. and *Phaseolus lunatus* L. in the production of cookies. The sensorial evaluation showed the acceptance of the cookies with fibre added compared to conventional wheat cookies. Fibrous fractions are used because of their functional properties such as retention, absorption and adsorption of water, oil-holding capacity, viscosity, antioxidant capacity, organic molecule absorption capacity, etc. Therefore, the food industry uses this fibre

Mukthar Sandoval-Peraza, David Betancur-Ancona, Luis Chel-Guerrero, Laboratory of Food Science, Faculty of Chemical Engineering, Autonomous University of Yucatan, Campus of Exact Sciences and Engineering, Periférico norte km 33.5, Tablaje catastral 13615 Col Chuburná de Hidalgo Inn, C.P. 97203, Mérida, Yucatán, Mexico.

Correspondence author:
Luis Chel-Guerrero, e-mail: cguerrer@uady.mx

in many products such as pasta, soups, beverages, meat and bread [7].

Besides the functional properties of the fibre, its consumption also has a beneficial effect for the consumer. Recent studies indicate that dietary fibre may be protective against cardiovascular diseases, diabetes, obesity, colon cancer and diverticular diseases, among others [2]. It has also been reported that the amount of indigestible saccharides that reach the colon can be degraded by fermentation by intestinal bacteria. The metabolites produced by this degradation repair the colonic mucosa, reduce cholesterol levels in the blood, eliminate carcinogenic agents and have vasodilator effects [8]. The objective of this study was to evaluate the physicochemical and bioactive properties of a fibre-rich fraction extracted from hardened *P. vulgaris* L.

MATERIALS AND METHODS

Seeds and chemicals

Twenty-five kilograms of common black beans (*P. vulgaris* L.) var. Jamapa were used. Two lots (1 kg) were taken to measure cooking time and hardness following Mexican official methods [3]. The use of a fibrous residue extracted from fresh beans was implemented as a control. All chemicals were of reagent grade or better and were acquired from Sigma Chemical (St. Louis, Missouri, USA).

Degree of hardness

Degree of hardness for freshly harvested and hard-to-cook (HTC) beans was determined using a 300 g sample for each type. The respective samples were soaked in water for 12 h, this soak water discarded, fresh water added and the beans put to boil. Cooking was stopped at 55 min, according to the method dictated in applicable Mexican regulations [3]. Hardness was measured per bean type using 30 cooked beans (10 g) in Instron 4411 Universal Testing Machine (Instron, Norwood, Massachusetts, USA) at 10 mm·min^{-1} compression speed, 8 mm diameter probe and 5 kg load cell, according to the method described in Mexican regulation [3].

Flour preparation

Selected grains were ground in a disk mill (Quaker model 4-E, Mill Straub, Philadelphia, USA) and then sifted through 4.76 mm and 2.38 mm screens in order to remove the smallest particles before the air classification. Hulls were removed with a fluidizing air bed and the flour resulted was milled in a Cyclotec mill (Tecator, Höganas, Sweden) and then passed through a 0.841 mm screen.

Fibrous residue

The fibrous residues of fresh and HTC beans were processed using the wet-fractionation method described by Betancur-Ancona et al. [9]. Briefly, 1 kg of 0.841 mm screen-grade flour was suspended in distilled water at a 1:6 (w:v) ratio. The pH was adjusted to 11 with 1 mol·l^{-1} NaOH and the dispersion was stirred for 1 h at 6.66 Hz with a mechanical agitator Caframo Rz-1 (Heildolph, Schwabach, Germany). Wet milling was then done in a mill (Kitchen-Aid, St. Joseph, Michigan, USA), separating the fibre solids from the starch and protein mix with 0.173 mm and 0.104 mm screen. The fibrous residue was washed 5 times, using a 1:3 ratio of solids to distilled water. The resulting fibre fraction was dried at 60 °C in an air convection oven Imperial V (Lab-Line, San Diego, California, USA), and then weighed and milled in a Cyclotec mill, and finally passed through a 0.841 mm screen.

Proximate composition

The nitrogen (method 976.05), fat (method 2003.06), ash (method 942.05), crude fibre (method 962.09), and moisture (method 2001.12) contents of the fibrous residues were determined according to official AOAC procedures [10]. Nitrogen content (N_2) was determined with a Kjeltec Digestion System (FOSS Tecator, Hillerřd, Denmark), using cupric sulfate and potassium sulfate as catalysts. Protein content was calculated as nitrogen × 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 °C for 2 h. Moisture content was measured on the basis of weight loss after oven-drying at 110 °C for 2 h. Saccharide content was estimated as nitrogen-free extract (NFE).

Total dietary fibre

The dietary fibre fractions were determined using the method of Prosky et al. [11].

Briefly, 1 g on dry basis (d.b.) fibre samples were placed in four Erlenmeyer flasks (marked as W_1) and weighed. To each of these samples, 50 ml of phosphate buffer (0.08 mol·l^{-1}, pH 6) were added, and pH was adjusted to 6 with 0.325 mol·l^{-1} HCl or 0.275 mol·l^{-1} NaOH. These were then placed in a water bath at 100 °C for 10 min. Then, 0.1 ml α-amylase E.C. 3.2.1.1. (Sigma A-3306) was added to each and they were left to incubate at the same temperature for 15 min under 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 E.C. 3.4.21.62. 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 E.C. 3.2.1.3 (Sigma A-9913) to each, the samples were left to incubate for 30 min under agitation. Then, 95% ethanol at the same temperature was added at a 1:4 sample:ethanol ratio, and the mixture was 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 (Sigma) 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 (marked as 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 equation 1:

$$TDF = \frac{(W_2 - W_3 - W_4 - W_5)}{W_1} \times 100 \qquad (1)$$

where W_1–W_4 are weights of dietary fibre fractions and W_5 is the reagent weight (blank). Total dietary fibre (*TDF*) is expressed in percent.

Insoluble and soluble dietary fibre

The same method [11] was used to quantify insoluble dietary fibre (*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*.

Soluble dietary fibre (*SDF*) was calculated by subtracting the *IDF* value from the *TDF* value.

Functional and bioactive properties

Water-holding and oil-holding capacity

Water-holding capacity (*WHC*) and oil-holding capacity (*OHC*) were determined following the method of Chau et al. [12].

Briefly, 1 g (d.b.) of sample was weighed and then stirred into 10 ml of distilled water or corn oil (Mazola, CPI International, México D.F., México) for one minute. These fibrous suspensions were then centrifuged at 2200 ×*g* for 30 min and the supernatant volume was measured. *WHC* was expressed as kilograms of water held per kilogram of sample, and *OHC* was expressed as kilograms of oil held per kilogram of fibre. Corn oil density was 0.92 g·ml^{-1}.

Water-absorption capacity

Water-absorption capacity (*WAbC*) was determined according to AACC method 88-04 [13]. Approximate water absorption capacity was first determined by weighing out 2 g (d.b.) of sample, adding water until saturation (approx. 40 ml) and centrifuging at 2000 ×*g* for 10 min in a Beckman GS-15R centrifuge (Beckman Coulter, Indianapolis, Indiana, USA). Excess water was discarded and the residue weighed. Approximate *WAbC* was calculated by dividing the increase in sample weight (in grams) by the quantity of water needed to complete original sample weight (2 g d.b.) to 15 g. *WAbC* was then determined by placing samples in four tubes, adding different quantities of water to bracket the measurement (1.5 ml and 0.5 ml water above original weight, and 1.5 ml and 0.5 ml water below; one in each tube), agitating vigorously in a vortex for 2 min, and centrifuging at 2000 ×*g* for 10 min in a Beckman GS-15R centrifuge. The supernatant was discarded and the residue weighed. Average water absorbed was calculated and the *WAbC* expressed as kilograms of water absorbed per kilogram of sample.

Water-adsorption capacity

Water-adsorption capacity (*WAdC*) was determined according to Chen et al. [14].

Briefly, 1 g (d.b.) of sample was placed in an equilibrium micro-environment at 98% relative humidity, generated by placing 20 ml of saturated potassium sulfate saline solution in tightly sealed glass flasks and placing these in desiccators at 25 °C. The sample was left in the micro-environment until reaching constant weight (72 h). *WAdC* was expressed as kilograms of water per kilogram of sample.

Emulsifying activity and emulsion stability

Emulsifying activity (*EA*) and emulsion stability (*ES*) were evaluated according to Chau et al. [12].

Briefly, 100 ml of fibrous suspension (2 g of fibrous residues in 100 ml of distilled water) were homogenized using a Caframo RZ-1 homogenizer (Heidolph Schwabach, Germany) at 33.33 Hz for 2 min. Then, 100 ml of corn oil (Mazola) were added to each sample and homogenized for 1 min. The emulsions were then centrifuged in 15 ml, graduated centrifuge tubes at 1200 ×*g* for 5 min, and the emulsion volume measured. *EA* was expressed as percentage of the emulsified layer volume of the entire layer in the centrifuge tube.

To determine *ES*, the prepared emulsions were heated at 80 °C for 30 min, cooled at room temperature and centrifuged at 1200 ×*g* for 5 min. *ES* was expressed as percentage of the remaining emulsified layer volume of the original emulsion volume.

Organic molecule absorption capacity

Organic molecule absorption capacity (*OMAC*) was determined according to Zambrano et al. [15]. An amount of 3 g (d.b.) of sample was placed in an excess quantity of corn oil (approx. 10 ml) for 24 h at 25 °C, and then centrifuged at 2000 ×*g* and 25 °C for 15 min in a Beckman GS-15R centrifuge. *OMAC* was expressed as the absorbed hydrophobic component and calculated in terms of sample weight gain (expressed in kilograms of oil per kilogram of sample).

Antioxidant activity

Phenolic compounds were extracted according to Iqbal et al. [16]. A 5 g (d.b.) sample was dissolved in 20 ml of 800 ml·l^{-1} methanolic solution and then incubated for 3 h at 25 °C at stirring. It was then centrifuged at 2500 ×*g* for 15 min, and the sediment submitted to a second extraction under the same conditions. Both resulting extracts were centrifuged again at 2500 ×*g* for 15 min, the two supernatants mixed and filtered through Whatman 41 paper (G. E. Whatman, Piscataway, New Jersey, USA).

Antioxidant activity in this filtrate (approx. 17 ml) was quantified with an ABTS$^+$ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid, Sigma 11557-1G) decolorization assay according to Sánchez-González et al. [17]. The ABTS$^+$ radical cation was produced by reacting the ABTS with potassium persulfate. The stock solution was prepared by dissolving 2 mmol·l^{-1} ABTS in 50 ml phosphate buffered saline (PBS) prepared from 8.1816 g NaCl, 0.2694 g KH_2PO4, 1.4196 g Na_2HPO_4 and 0.1498 g KCl dissolved in 1 l ultrapure water. If lower than 7.4, pH was adjusted with NaOH. The ABTS$^+$ radical cation was produced by reacting 20 ml ABTS stock solution with 80 μl of $K_2S_4O_8$ solution (prepared with 70 mmol·l^{-1} $K_2S_4O_8$ solution in ultrapure water) and allowing the mixture to stand at room temperature in darkness for 16 h before use. The radical remained stable in this form for more than 48 h when stored at room temperature in darkness. Antioxidant compounds were measured by diluting the ABTS$^+$ solution with PBS to an absorbance of (0.800 + 0.030) AU at 734 nm; this value was considered blank absorbance (A_0).

Sequential dilutions of 4 mmol·l^{-1} Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Sigma) stock solution (10 mg of Trolox in 800 ml·l^{-1} methanolic solution) were done to produce concentrations of 3.5 mmol·l^{-1}, 2.5 mmol·l^{-1}, 1.5 mmol·l^{-1}, 1.0 mmol·l^{-1} and 0.5 mmol·l^{-1}. After addition of 990 μl diluted ABTS$^+$ solution to 10 μl Trolox standard, absorbance (734 nm) was measured at room temperature exactly 6 min after initial mixing. The appropriate solvent blanks were run in each assay. All determinations were done in triplicate.

The same procedure was repeated, mixing 990 μl ABTS$^+$ solution + 10 μl sample solution, and the percentage decrease in absorbance at 734 nm was calculated and plotted as a function of Trolox concentration for the standard reference data (Eq. 2).

$$I = \frac{A_0 - A_S}{A_S} \times 100 \quad (2)$$

where I is percentage of inhibition, A_0 is ABTS$^+$ blank absorbance and A_S is absorbance of sample.

The Trolox equivalent antioxidant coefficient (*TEAC*) of sample was calculated by comparing the slope of the absorbance inhibition percentage plot to the antioxidant concentration divided by the slope of the Trolox plot.

Statistical analysis

All determinations were done in triplicate. Statistical analysis was done to determine the central tendency of the data and standard deviation. The differences between fibre-rich fraction from fresh and hardened beans were established according to Montgomery [18] using Student t-test by the Statgraphics plus 5.1 computer software (Statgraphics Technologies, Warrenton, Virginia, USA).

RESULTS AND DISCUSSION

Degree of hardness

Cooking time and hardness were compared between fresh and hard beans. The fresh bean had values of 50 min of cooking and 0.38 kg of compression of texture. The hard bean had 70 min of cooking and 0.76 kg of compression of texture. The latter values exceeded acceptable values and were indicative of the development of the HTC phenomenon (NMX-FF-038-SCFI-2002; ≤55 min of cooking and ≤ 0.5 kg of compression of texture) [3]. The increase in cooking time of hardened beans may be caused by the difficulty of the cotyledons to soften; due to the insolubilization of pectin and cell wall lignification, water takes longer to

penetrate into the grain interior [19]. Another explanation suggests that the grain can undergo false germination, which is responsible for the mobilization of the molecules present in the grain. However, inability to progress to germination leads to re-arrangement of the compounds, resulting in difficulties to the water to penetrate the grain, which is reflected by an increase in the values of cooking and compression [20].

Proximate composition

The proximate composition of fibre-rich fraction (FRF) from fresh and hard beans is shown in Tab. 1. The high value of protein found in both cases may be caused by the process applied, when the grains were peeled leaving only the cotyledon, which contains the highest amount of protein. FRF from hard bean had a higher content of protein (159.30 g·kg^{-1}) in comparison with FRF from fresh bean (122.50 g·kg^{-1}). This can be explained by some hydrolysis of storage proteins taking place during the hardening process. This hydrolysis produces reactive peptides that can react with FRF; this link may be responsible for the insolubilization of proteins from the FRF. The hardening process may be responsible for high protein values despite the fact that solubilization was done at alkaline pH. Ribeiro et al. [21] reported similar observation – they induced hardening of *P. vulgaris* variety IAPAR 44 and noticed that, as hardening process took place, the protein insolubilization reached 50%.

In the case of crude fibre, FRF from fresh bean had a higher value (56.80 g·kg^{-1}) than FRF from hard bean (46.20 g·kg^{-1}). The samples of FRF from fresh and hard bean used in the present study were extracted from dehulled seeds, which could explain the lower content of crude fibre in FRF from hard bean in comparison with FRF from fresh bean because, in the hardening process, some compounds present in the cotyledon may react with the hull of the seed [5] and this part of the seed was removed. Ruiz-Ruiz et al. [22] reported the same decrease in the amount of crude fibre in the fibrous residue from fresh and hardened *P. vulgaris* L. var. Jamapa beans.

Total, soluble and insoluble dietary fibre

Chemical composition of FRF from fresh and hard beans is presented in Tab. 2. FRF from hard bean had higher content of *TDF*. In both cases, the highest portion of fibre was in the insoluble fraction. However, FRF from hard bean had a higher content of *SDF*. Similar amounts of fibre were found by Ruiz-Ruiz et al. [22] in fibrous residue from hard *P. vulgaris* L. var. Jamapa beans, with 421 g·kg^{-1} of *TDF*, 341 g·kg^{-1} of *IDF* and 79 g·kg^{-1} of *SDF*, which are similar values for FRF from hard bean as those obtained in this study. Betancur-Ancona et al. [23] reported the values of *TDF, IDF* and *SDF* in FRF from *P. lunatus*, which were 294 g·kg^{-1}, 286 g·kg^{-1} and 7 g·kg^{-1}, respectively, i.e. the content of fibre in *P. vulgaris* was higher. These values indicate that FRF from hard bean is a good source of *SDF*. This portion aids in the decrease of cholesterol levels in blood, control of glucose in the bloodstream in the case of diabetics, and this fraction can be an ingredient in diet food products against obesity. The insoluble residue is linked to the ability to decrease the incidence of colon/rectal cancer and diverticulitis [2, 7, 8].

Functional and bioactive properties

Water-holding and oil-holding capacity

A higher *WHC* was shown for FRF from hard bean in comparison with FRF from fresh bean (Fig. 1). This may be because FRF from hard bean has a higher value of *SDF* (79.10 g·kg^{-1}),

Tab. 1. Proximate composition of fibre-rich fraction from fresh and hard *P. vulgaris* beans.

Component	Fibre-rich fraction of *P. vulgaris*	
	Fresh	Hard
Moisture [g·kg^{-1}]	104.30 ± 0.014^{a}	63.04 ± 0.08^{b}
Protein [g·kg^{-1}]	122.50 ± 0.174^{a}	159.30 ± 0.065^{b}
Fat [g·kg^{-1}]	19.20 ± 0.082^{a}	17.40 ± 0.22^{a}
Crude fibre [g·kg^{-1}]	56.80 ± 0.049^{a}	46.20 ± 0.09^{b}
Ash [g·kg^{-1}]	16.60 ± 0.017^{a}	25.50 ± 0.42^{a}
Nitrogen-free extract [g·kg^{-1}]	783.70 ± 0.006^{a}	752.50 ± 0.58^{b}

Values are expressed per kilogram on dry basis.
a, b – different superscript letter in the same row indicates statistical difference ($p < 0.05$).

Tab. 2. Total, insoluble and soluble dietary fibre in fibre-rich fraction from fresh and hard *P. vulgaris* beans.

Component	Fibre-rich fraction of *P. vulgaris*	
	Fresh	Hard
TDF [g·kg^{-1}]	405.20 ± 0.66^{a}	431.20 ± 0.46^{b}
IDF [g·kg^{-1}]	379.10 ± 0.6^{a}	352.10 ± 0.46^{b}
SDF [g·kg^{-1}]	26.00 ± 0.56^{a}	79.10 ± 0.28^{b}

Values are expressed per kilogram on dry basis.
TDF – total dietary fibre, *IDF* – insoluble dietary fibre, *SDF* – soluble dietary fibre.
a, b – different superscript in the same row indicates statistical difference ($p < 0.05$).

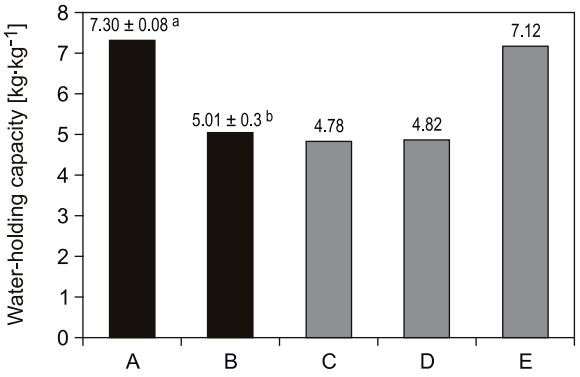

Fig. 1. Water-holding capacity of fibre-rich fraction from hard and fresh bean, compared to other fibre sources.

A – fibre-rich fraction hard, B – fibre-rich fraction fresh, C – extruded hard bean flour [24], D – extruded fresh bean flour [24], E – extruded mixed flour [24].
a, b – different superscript in the bars indicates statistical difference ($p < 0.05$).

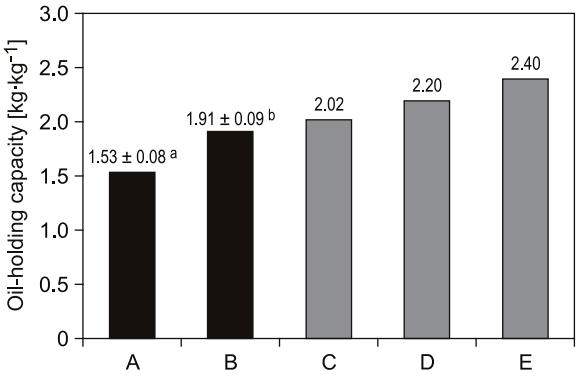

Fig. 2. Oil-holding capacity of fibre-rich fraction from hard and fresh beans, compared to other fibre sources and flours of common bean (*P. vulgaris*).

A – fibre-rich fraction hard, B – fibre-rich fraction fresh, C – *Salvia hispanica* [26], D – flour of fresh common bean [27], E – flour of germinated common bean [27].
a, b – different superscript in the bars indicates statistical difference ($p < 0.05$).

which is the portion responsible in great part for *WHC*. Steel et al. [24] reported the values of *WHC* for extruded flours from *P. vulgaris* fresh, hard and mixed flours of hard bean with rice to be 4.82 kg·kg^{-1}, 4.78 kg·kg^{-1} and 7.12 kg·kg^{-1}, respectively. *WHC* values reported by Zambrano et al. [15] for maize hulls (2.32 kg·kg^{-1}), wheat hulls (2.48 kg·kg^{-1}) and soybean hulls (1.27 kg·kg^{-1}) were lower than *WHC* for FRF from hard bean in this study. This may be caused by several factors like the chemical composition of the fibre, its source, the ratio of insoluble/soluble fibre present in the dietary fibre, as well as the particle size [25].

The fibre with a high *WHC* can be an advantage because, physiologically, the fibre can form viscous solutions in the small intestine decreasing the transit time of nutrients. It also increases the thickness of water that has to transfer the solutes to reach the intestinal membrane, which produces a decrease in the absorption of glucose, lipids and amino acids. Likewise, there will be a decrease in the absorption of bile acids, and so these acids will be synthesized from cholesterol thus decreasing the cholesterol levels in the blood [25]. In the manufacturing of foods, the water-holding capacity is very important because this ability is related to the formulation of low calorie foods [7].

Regarding the *OHC* (Fig. 2), FRF from fresh bean had a higher value (1.91 kg·kg^{-1}) than for FRF from hard bean (1.53 kg·kg^{-1}). The *OHC* depends in part on *SDF*, because this fraction is responsible for adsorption of organic compounds. The hemicellulose and lignin have the ability to trap oil on the surface of their fibres [23]. These facts could explain the lower value of *OHC* for FRF from hard bean, as hemicellulose and lignin react with the hull during the hardening process and these compounds were removed in the dehulling process. Possibly, the *OHC* may be bound to lignin and hemicellulose contents, despite the higher content of *SDF* in FRF from hard bean. Betancur-Ancona et al. [23] reported the *OHC* in the fibrous fraction extracted from *P. lunatus* and *Canavalia ensiformis* to be 180 g·kg^{-1} and 230 g·kg^{-1}, respectively. These values were higher than the results of our study. Vázquez-Ovando et al. [26] reported 2.02 kg·kg^{-1} for *OHC* in the fibrous fraction extracted from *Salvia hispanica,* which was a higher value than the result of our study. Sangronis et al. [27] obtained values of 2.20 kg·kg^{-1} and 2.40 kg·kg^{-1} of *OHC* in flours from fresh and germinated common black *P. vulgaris*, respectively. López et al. [28] mentioned that the ability of the fibre to interact with oil compounds depended in part on the surface characteristics, particle thickness of the compound, and the amount of lignin present in the fibre. Taking into consideration the enzymatic and molecular reactions occurring in the hardening process, it is possible that these reactions may produce chemical and physical changes in the fibre affecting the retention of oil compounds.

Water-absorption and water-adsorption capacities

In the *WAbC* (Fig. 3), FRF from hard bean had a slightly higher value but was statistically different ($p < 0.05$) with respect to FRF from fresh bean. Zambrano et al. [15] reported *WAbC* of maize

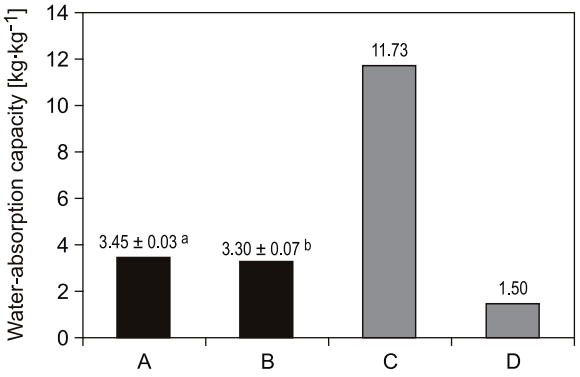

Fig. 3. Water-absorption capacity of fibre-rich fraction from hard and fresh beans, compared to other fibre sources and flour of *Tylosema esculentum*.

A – fibre-rich fraction hard, B – fibre-rich fraction fresh, C – *Salvia hispanica* [26], D – flour of *Tylosema esculentum* [29]. a, b – different superscript in the bars indicates statistical difference ($p < 0.05$).

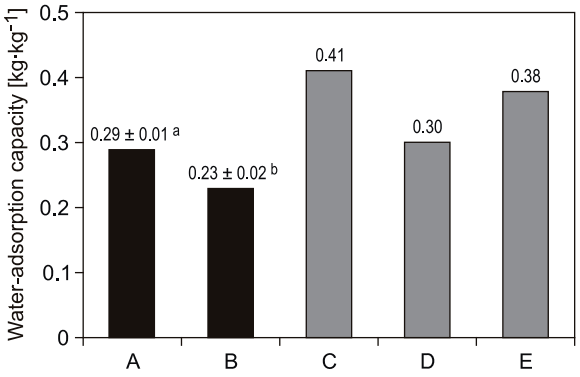

Fig. 4. Water-adsorption capacity of fibre-rich fraction from hard and fresh beans compared to other fibre sources.

A – fibre-rich fraction hard, B – fibre-rich fraction fresh, C – maize hulls [15], D – wheat hulls [15], E – soybean hulls [15]. a, b – different superscript in the bars indicates statistical difference ($p < 0.05$).

hulls (3.17 kg·kg^{-1}), wheat hulls (2.91 kg·kg^{-1}) and soybean fibre (1.42 kg·kg^{-1}), showing that *WAbC* of FRF from hard bean was mostly higher in comparison with all materials mentioned. Gaamangwe et al. [29] reported *WAbC* in dehulled flours from *Tylosema esculentum* (1.5 kg·kg^{-1}) and observed that FRF from hard bean showed a higher *WAbC*. Vázquez-Ovando et al. [26] reported 11.73 kg·kg^{-1} of *WAbC* in fibrous residues of *Salvia hispanica,* which was higher compared to this study. This could be caused by the chemical composition of the fibre, since it can form a lattice trapping the water of the medium, or can associate with the structural water matrix [25].

Regarding the *WAdC* (Fig. 4), FRF from hard bean had a higher value than FRF from fresh bean. Vázquez-Ovando et al. [26] reported the values of *WAdC* for the fibrous residue extracted from *Salvia hispanica* as 0.3 kg·kg^{-1}, which were similar to FRF from hard bean (0.29 kg·kg^{-1}) of this study. Zambrano et al. [15] reported *WAdC* for maize hulls, wheat hulls and soybean fibre, which were 0.41 kg·kg^{-1}, 0.30 kg·kg^{-1} and 0.38 kg·kg^{-1}, respectively. The value of *WAdC* for wheat hulls was very similar to *WAdC* for FRF from hard bean but lower than for the other materials. The determination of this parameter is important, considering its relationship to stability and deteriorative changes in the source of fibre during storage, where molecular changes can be produced by water because it may act as a plasticizer modifying the physiological function of the dietetic fibre [15]. Physiologically, *WAdC* has a very important role, as it has been demonstrated that compounds present in the fibre have the capacity to absorb organic molecules like acids and biliary salts. López et al. [28] mentioned that the adsorption process in the fibres limits the absorption of bile acid in the intestine, forcing the organism to synthesize new bile acids from cholesterol, which results in reduced levels of this compound in the blood.

Emulsifying activity and emulsion stability

EC is the ability of a molecule to act as an agent that facilitates solubilization or dispersion of two immiscible liquids, and *ES* is the ability to maintain an emulsion and to resist rupture. FRF from hard bean showed higher *EA* and *ES* values than FRF from fresh bean (Tab. 3). This behaviour is directly related to fibre type and the contents of soluble and insoluble fibre fractions. In this case, FRF from hard bean had higher values of *IDF* (352.10 g·kg^{-1}) and *SDF* (79.10 g·kg^{-1}) in comparison to FRF from fresh bean (379.10 g·kg^{-1} of *IDF* and 26 g·kg^{-1} of *SDF*), thus indicating that the compounds of the soluble fraction present in FRF from hard bean may be responsible for *ES*. Betan-

Tab. 3. Emulsifying activity and emulsion stability of fibre-rich fraction from fresh and hard *P. vulgaris* beans.

	Fibre-rich fraction of *P. vulgaris*	
	Fresh	Hard
EA [g·kg^{-1}]	47.90 ± 0.05^{a}	79.50 ± 0.58^{b}
ES [g·kg^{-1}]	491.10 ± 0.59^{a}	580.40 ± 0.78^{b}

Values are expressed per kilogram on dry basis.
EA – emulsifying activity, *ES* – emulsion stability.
a, b – different superscript in the same row indicates statistical difference ($p < 0.05$).

CUR-ANCONA et al. [23] reported *EA* and *ES* of the fibrous residue from *P. lunatus* to be 493 g·kg^{-1} of *EA* and 282 g·kg^{-1} of *ES*, i.e. the fibrous residue of *P. lunatus* had higher *EA* in comparison with FRF from hard bean in this study. However, FRF from the hardened beans had higher *ES* than *P. lunatus* fibrous residue.

Organic molecule absorption capacity and antioxidant activity

Tab. 4 shows the *OMAC* and antioxidant activity for FRF from *P. vulgaris*. VÁZQUEZ-OVANDO et al. [26] reported the value of *OMAC* for the fibrous residue of *Salvia hispanica* to be 10 g·kg^{-1}. ZAMBRANO et al. [15] found values of *OMAC* for wheat hulls, maize hulls and soybean fibre to be 19 g·kg^{-1}, 16 g·kg^{-1} and 6 g·kg^{-1}, respectively; these values except for the soybean were higher than FRF from hard bean obtained in our study. It was proposed that lignin and the soluble fraction in the fibre are responsible for *OMAC* [26]. An explanation of the low value of *OMAC* for FRF from hard bean (7.70 g·kg^{-1}) would be that, during the process of obtaining FRF, the seeds were dehulled. As this grain already underwent the hardening process, it is probable that the interaction between the cell walls and lignin already took place. During the dehulling process, a great amount of lignin was removed, affecting the capacity to absorb organic molecules. This fact was reported by DE LEÓN et al. [30] who found the highest amount of lignin in the hull and there the lignification process took place during hardening.

Quantification of this parameter indicates indirectly the amount of compounds with a hydrophobic nature that can be absorbed by the fibre. Various studies have shown that dietary fibre alters the lipolysis process due to the ability of the soluble dietary fibre to form emulsions and viscous solutions, reducing the amount of fat available for the biochemical process mentioned above. Dietary fibre may also bind bile acids through the formation of small micelles. However, these features do not guarantee that the fibre is able to absorb bile acids in the digestive system. HUR et al. [31] reported the digestion in vitro of lipids in meat cakes added with fibre, finding that the addition of fibre in this food helps to reduce the amount of lipids digested in vitro.

Regarding to the antioxidant activity, FRF from hard bean showed a higher *TEAC* than FRF from fresh bean. VÁZQUEZ-OVANDO et al. [26] reported the *TEAC* of fibrous residue from *Salvia hispanica* to be 488 mmol·kg^{-1}. The value of *TEAC* obtained for the fibrous residue from hard bean was 795 mol·kg^{-1}, which was higher than that obtained for the aforementioned chia residue. LECUMBERRI et al. [32] reported the *TEAC* from a high-fibre product of cocoa to be 7.73 mmol·kg^{-1}, and this result was also lower than the values obtained in this study. BETANCUR-ANCONA et al. [23] reported 39.4% and 35.6% of antioxidant capacity for *Canavalia ensiformis* and *P. lunatus* respectively; the percentage of antioxidant capacity (Eq. 2) in FRF from hardened bean was 62.4%, and this value was higher than those reported for *Canavalia ensiformis* and *P. lunatus*. The fact that this residue has an antioxidant activity is an advantage because phenolic compounds present in various legumes help reduce oxidative stress levels, which are produced by reactive oxygen species, hydroxyl radicals and peroxides, among others, connected to chronic degenerative diseases.

Tab. 4. Organic molecule absorption capacity and antioxidant activity of fibre-rich fraction from fresh and hard *P. vulgaris* beans.

	Fibre-rich fraction of *P. vulgaris*	
	Fresh	Hard
OMAC [g·kg^{-1}]	12.40a ± 0.06^{a}	7.70b ± 0.04^{b}
TEAC [mol·kg^{-1}]	564.74a^{a}	795.06^{b}

OMAC – organic molecule absorption capacity expressed per kilogram on dry basis, *TEAC* – Trolox equivalent antioxidant coefficient.
a, b – different superscript in the same row indicates statistical difference ($p < 0.05$).

CONCLUSION

In the fibrous residue of hard bean (*P. vulgaris*), 431.20 g·kg^{-1} of *TDF* was found. This contained 352.10 g·kg^{-1} of the *IDF* and 79.10 g·kg^{-1} of the *SDF*. It was observed that this residue had comparatively high retention, absorption and adsorption of water, but comparatively low retention of oil, emulsifying capacity and emulsion stability. However, the combination of these functional properties makes possible the use of this residue in the formulation of functional foods (breads, pasta, soups, meats, etc.). The physiological properties shown by the fibrous residue would provide beneficial effects to human health and can be used in the prevention and treatment of chronic degenerative diseases.

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