LWT - Food Science and Technology 59 (2014) 794–799

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Effect of different oil extraction methods on proximate composition and protein characteristics of walnut (*Juglans regia* L.) flour

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ARTICLE INFO

Article history:
Received 12 February 2014
Received in revised form
4 June 2014
Accepted 16 June 2014
Available online 24 June 2014

Keywords:
Walnut flour
Oil extraction methods
Hydraulic press
Screw press
Proteins

ABSTRACT

The effect of different oil extraction methods on proximate composition and protein characteristics of walnut (*Juglans regia* L.) flour were evaluated. Treatments involving hydraulic press with further solvent extraction gave higher protein contents (38.7–38.8 g 100 g^{-1}) than those using screw-pressing at different temperatures (26.9–28.9 g 100 g^{-1}). The major polypeptide patterns from total soluble proteins and glutelin fractions showed minor variations between samples. However, low molecular weight globulins were strongly affected by treatment using hydraulic press followed by solvent extraction, whereas albumins were mostly influenced by temperature conditions used in screw-press extractions. Taking into account that globulins, the second most abundant protein fraction in walnut, is not affected by screw pressing at lower temperatures (25 and 50 °C) and that the flour obtained at 50 °C had the highest protein solubility and a good water holding capacity (3.3 g/g of flour), this process (screw-press at 50 °C) can be considered the most suitable for obtaining partially defatted walnut flour.

1. Introduction

The cultivated walnut (*Juglans regia* L.) is a nutritionally important and valuable crop both for domestic consumption and for trade. Walnut is a nutrient-dense food mainly owing to its lipid and protein profile. Typically, walnut oil extraction is carried out by pressing with either screw press or hydraulic press but extraction with solvent and CO_2 supercritical has been also reported (Martínez, Labuckas, Lamarque, & Maestri, 2010; Martínez, Mattea, & Maestri, 2008).

The oil-extraction residue is rich in proteins and it has been employed for formulation of various functional food products such as meat, bakery and dairy products (Ayo et al., 2007; Ayo, Carballo, Solas, & Jiménez-Colmenero, 2008; Cofrades, Serrano, Ayo, Carballo, & Jiménez-Colmenero, 2008). The characterization, stability and functional properties of walnut proteins have been extensively described for solvent-defatted flours (Labuckas, Maestri, & Lamarque, 2011; Labuckas, Maestri, Perelló, Martínez, & Lamarque, 2008; Mao & Hua, 2012; Sathe et al., 2009; Sze-Tao & Sathe, 2000). Walnut proteins are highly digestible and have a good balance of essential amino acids. The major protein fraction corresponds to glutelins (≅ 70 g 100 g^{-1}), followed by globulins (≅ 18 g 100 g^{-1}), albumins (≅ 7 g 100 g^{-1}) and prolamins (≅ 5 g 100 g^{-1}) (Sze-Tao & Sathe, 2000).

In addition to proteins, walnut cake obtained after oil extraction contains appreciable amounts of vitamins, minerals, phenolic compounds and melatonin (Arcan & Yemenicioglu, 2009; García-Parrilla, Cantos, & Troncoso, 2009; Pereira et al., 2008; Reiter, Manchester, & Tan, 2005) that provides several nutritional and health benefits.

The quantity, quality and functional properties of walnut proteins, may be affected by the process conditions employed for oil extraction. There is a paucity of information regarding protein recovery and quality from walnut flour defatted by pressing. This process involves the application of an external force that compresses, deforms and destroys the cell walls as well as the thin membrane surrounding the spherosomes causing release of the contained oil (Mattea, 1991). Although pressing recovers lower proportion of oil than that obtained by solvent extraction, it provides a simple methods to obtain oil from small batches of seeds or fruits with high lipid content (Singh, Wiesenborn, Tostenson, & Kangas, 2002; Wiesenborn, Doddapaneni, Tostenson, & Kangas, 2001; Zheng, Wiesenborn, Tostenson, & Kangas, 2003). Furthermore, requires more economical installations than solvent

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http://dx.doi.org/10.1016/j.lwt.2014.06.038
0023-6438/

extraction plants, implies safer processes and reduces the risk to the environment (Martínez et al., 2010; Mattea, 1991).

Considering the increasing demand of new sources of high quality vegetable proteins, this study evaluates the effect of different oil press-extraction conditions on the proximate composition and protein characteristics of partially defatted walnut flours.

2. Material and methods

Walnut fruits (*Juglans regia* L. cv. Franquette) were collected from commercial plantations at Belen location, Catamarca Province, Argentina. After cleaning, the fruits were dried at 30 ± 2 °C for 24 h and then were shelled manually.

2.1. Flour preparation

Seeds were selected and ground in a stainless steel mill with variable aperture and sieved through serial stainless steel sieves to an optimum particle size for each treatment. Afterward, the lipid content was reduced to obtain walnut flour according to the methods described below.

Control: Oil extraction was performed with *n*-hexane by cross flow extraction at room temperature (25 °C) using a continuous lixiviation apparatus. Ground kernel (300 g) with a particle sizes between 0.5 and 1 mm were charged into the lixiviation vessel (66 cm high × 4 cm diameter) and 5.5 dm^3 of the solvent passed through the packed bed. The resulting meal was desolventized under reduced pressure at 30 °C. Walnut flour was obtained by grinding the walnut meal to fine particles, so that 900 g kg^{-1} of the product passed through a 0.21 mm-mesh screen.

Hydraulic press (HP): Seed material with particle size between 1 and 3 mm was used. Oil extraction was carried out using a pilot-plant hydraulic press (30 kg/cm^2) at 18 ± 2 °C.

HP and subsequent extraction with solvent: The material obtained by hydraulic pressing was ground and sieved again to reduce the particle size to 0.5–1 mm before passive solvent (*n*-hexane) extraction at room temperature for 24 h (HP + P) or by continuous solid–liquid extraction in a Soxhlet apparatus (HP + S) for 12 h.

Screw press (SP): The seed particle size used was between 2.4 and 4.8 mm, corresponding to the optimum values for feeding the press. Before pressing, seed particles were sprinkled with fresh water to reach 7–8 g 100 g^{-1} of moisture content (Singh & Bargale, 2000). The water-sprinkled samples were then packed in air-tight metal containers and stored for about 48 h for equilibration. The containers were shaken at regular intervals to distribute moisture uniformly throughout the sample. Pressing was carried out at three different temperatures (25, 50 and 70 °C). Oil extraction was carried out with a Komet screw press (Model CA 59 G, IBG Monforts, Mönchengladbach, Germany), with a 5 mm restriction die and a screw speed of 20 rpm. The temperature of the process was controlled using an electrical resistance-heating ring surrounding the press barrel. Running temperature was checked with a digital thermometer inserted into the restriction die. The resulting flours were encoded as SP 25°, SP 50° and SP 70°.

All flours obtained were stored at −18 °C until further use.

2.2. Proximate composition

Moisture, total fat, ash and protein contents of ground walnut seeds and flours were determined in triplicate according to standard AOAC (1998) methods. The total protein content was estimated by the Kjeldahl method (N × 5.3) (Venkatachalam & Sathe, 2006). Carbohydrate content was calculated by difference.

2.3. Colour

The colour of the flour samples was determined using a Minolta 508d (USA) spectrophotometer and expressed as Hunter system L^* (lightness/darkness), a^* (greenness/redness) and b^* (blueness/yellowness) values. Six measurements (three readings for each duplicate) were averaged and the total colour difference (ΔE) was calculated by the following equation (Veiga-Santos, Suzuki, Cereda, & Scamparini, 2005):

$$\Delta E = \left[(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2\right]^{1/2}$$

where ΔE is the total colour difference between the sample and the Control flour.

2.4. Protein fraction analysis

The following analyses were performed on the protein fraction from walnut flour samples obtained as described previously. The HP sample was not analysed due to its high oil content.

2.4.1. Solubility

Flour dispersion (1:10 w/v) in sodium phosphate buffer (0.2 mol dm^{-3}, pH 8) was mixed for 2 h at 25 °C using a magnetic stirrer before centrifugation at 9000 × *g* for 20 min at 4 °C. Protein content in the supernatant was determined according to Lowry, Rosebrough, Farr, and Randall (1951), using bovine serum albumin (BSA) as standard and was expressed as mg/g of flour.

2.4.2. Water absorption capacity

Water absorption capacity (WAC) was determined using the method of Lawal (2004) with minor modifications. One milligram of sample was homogenized with 1 cm^3 distilled water for 30 s. The samples were allowed to stand for 30 min at room temperature (25 °C) before centrifuging at 3000 × *g*, for 30 min. The supernatant was removed and the weight of the pellet was noted. WAC expressed as gram of water per gram of sample (g/g flour) was calculated by the following equation:

$$\mathrm{WAC(g/g\ flour)} = (\mathrm{m2} - \mathrm{m1})/(\mathrm{m1} \times \rho)$$

where m1 is the mass of the flour (g), m2 is the mass of the sediment (g) and ρ is the density of water.

2.4.3. Protein fractionation

Protein fractions were obtained from flour samples by mean of sequential extraction using deionised distilled water (albumin), 1.0 mol dm^{-3} NaCl (globulin), 700 cm^3 dm^{-3} aqueous ethanol (prolamin) and 0.1 mol dm^{-3} $Na_2B_4O_7$, pH 10 (glutelin), according to the method proposed by Sze-Tao and Sathe (2000) with some modifications. Extractions were made under constant magnetic stirring (2 h at 4 °C) using a sample-solvent ratio of 1:10 g:mL. Following each extraction, the slurry was centrifuged (9000 × *g*, 20 min, 4 °C), and the supernatant was vacuum filtered with Whatman filter paper #4 to remove insoluble particles. Residues from centrifugation and filtration steps were pooled and used for the next extraction.

An aliquot of each supernatant with soluble protein in phosphate buffer and with the different protein fractions was mixed with 4 volumes of cold acetone, incubated at −20 °C, for 2 h and centrifuged at 9000 × *g* for 15 min. The resulting precipitate was dried and reserved for electrophoretic analysis.

2.4.4. Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to Laemmli (1970), in the presence of β-mercaptoethanol (2-ME) as a reducing agent, using 0.12 g cm^{-3} separating gel and 0.03 g cm^{-3} stacking gel. The protein samples were solubilised in 0.2 mol dm^{-3} Tris–HCl buffer (pH 6.8) with 150 cm^3 dm^{-3} glycerol, 0.02 g cm^{-3} SDS, 50 cm^3 dm^{-3} 2-ME, 1 g dm^{-3} bromophenol blue, heated for 2 min in boiling water and centrifuged (5000 × *g*, at room temperature) before electrophoresis.

Pre-stained broad range SDS-PAGE protein standards (5.75–198.08 kDa) (Bio-Rad Co., Hercules, CA, USA) were used for molecular weight estimation. Electrophoresis was done in a mini-slab (28575-00 Model, Cole-Palmer, Vermin Hills, IL, USA) at a constant current of 20 mA per gel. After electrophoresis, gels were fixed in 25 cm^3 dm^{-3} acetic acid and dyed in MeOH/H_2O/acetic acid (5:5:2, mL:mL:mL) containing 5 cm^3 dm^{-3} Coomassie Brillant Blue G-250. Gels were discoloured in aqueous ethanol (300 cm^3 dm^{-3}) with 100 cm^3 dm^{-3} acetic acid.

2.5. Statistical analyses

Analytical determinations were done in duplicate or triplicate from two independent samples and data were reported as means ± standard deviation. When appropriate, statistical differences among treatments were estimated by ANOVA. Whenever ANOVA indicated a significant difference, a pairwise comparison of means by least significant difference was carried out at a significant level of 0.05. All statistical analyses were performed using InfoStat software, version 1.1 (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba).

3. Results and discussion

3.1. Proximate composition and colour

Proximate composition of walnut seeds and flour samples are shown in Table 1. Oil was the major component (72 g 100 g^{-1}) in walnut seed, followed by carbohydrates and proteins, which together accounted 26 g 100 g^{-1}. Values obtained for proteins and lipids were similar to those reported for the same variety grown in Portugal (Pereira et al., 2008), and were close to the published values for whole walnut from various origins and varieties (Colaric, Veberic, Solar, Hudina, & Stampar, 2005; Martínez et al., 2010). Ash and carbohydrates contents were close to values reported in literature (Fukuda, Ito, & Yoshida, 2003; Maguire, O'Sullivan, Galvin, O'Connor, & O'Brien, 2004).

Table 1
Proximate composition of walnut seeds and flours obtained by different treatments of oil extraction, expressed as g/100 g dry solid.

Sample	Protein	Oil	Ash	CH
Seed	10.7 ± 0.9	72.1 ± 2.3	1.4 ± 0.0	15.8
Control	38.1 ± 0.1^{d}	7.2 ± 0.1^{a}	5.2 ± 0.0^{c}	49.5
HP	24.2 ± 0.7^{a,A}	41.0 ± 2.1^{c,C}	3.7 ± 0.1^{ab,A}	31.2
HP + P	38.7 ± 0.1^{d,C}	6.0 ± 0.2^{a,A}	5.9 ± 0.02^{d,B}	49.4
HP + S	38.8 ± 0.1^{d,C}	4.6 ± 0.2^{a,A}	6.1 ± 0.03^{d,B}	50.5
SP 25°	28.7 ± 0.5^{c,B}	30.5 ± 2.5^{b,B}	3.7 ± 0.1^{a,A}	38.0
SP 50°	26.9 ± 1.4^{b,B}	31.1 ± 3.9^{b,B}	3.7 ± 0.1^{ab,A}	39.3
SP 70°	28.9 ± 1.2^{c,B}	27.7 ± 2.4^{b,B}	3.8 ± 0.1^{b,A}	39.6

Values are means ± standard deviations of triplicate determinations. Means followed by different lowercase letters in the same column indicates significant differences ($P < 0.05$) between treatments. Different capital letters indicates differences ($P < 0.05$) between treatments involving pressing.

The proximate composition of flour samples showed that the HP treatment was the least efficient to extract the oil from the seeds; the amount of oil retained in the flour was 41 g 100 g^{-1}. As a result of the double extraction (HP plus solvent) protein content increased compared to walnut seeds and HP flour. Samples from HP + P and HP + S did not differ significantly in their proximate composition. Likewise, flours obtained by screw-pressing (SP 25°, SP 50° and SP 70°) showed no differences in both protein content and on the residual oil retained. The protein content of flours from screw-pressing resulted significantly higher than that from HP treatment, but lower than those from treatments involving solvent extraction (Control, HP + P, HP + S).

For the extraction conditions employed in this study, the different temperature levels did not affect protein recovery. There were no variations among flour samples obtained by lixiviation (*n*-hexane, ambient temperature) or HP followed by solvent (*n*-hexane) extraction at ambient temperature or at boiling point. Similarly, there were no significant variations in protein recovery from screw-pressed samples at different temperature conditions.

3.2. Colour

The flour samples obtained were light beige to brown coloured (Fig. 1). The colours of the samples were heterogeneous due to the presence of the seed coat. Flour samples from both HP + P and HP + S treatments were similar in appearance to that obtained from the Control treatment. The highest oil content in samples subjected only to press (hydraulic or screw-press) resulted in a darker flours, particularly that obtained from SP 70° treatment.

Colour parameters confirm the visual perception of the samples (Table 2). Lighter flours (lower values of ΔE) were HP + P and HP + S, similar ($P < 0.05$) to the Control flour in lightness (L^*) and chromaticity (a^*, b^*). The flours obtained only by pressing (HP and SP 25°, 50° or 70°) showed lower values in L^* parameter, resulting the SP 70° significantly darker ($L^* = 52.4$) than the others. The a^* and b^* values, indicatives of greenness/redness and blueness/yellowness, respectively, were consistent with the flour colour appearance.

The flour sample obtained by screw-pressing at 70 °C (SP 70°) had higher ΔE values than those obtained at lesser temperature (SP 25° and SP 50° treatments). The effect of high pressing temperature (70 °C) could lead to oxidation of phenolic compounds and interactions between reducing sugars and amino groups of proteins in Maillard reactions. The compounds that are generated are coloured, resulting in darker flour.

3.3. Protein solubility and water holding capacity

Table 3 shows results of protein solubility (PS) and water absorption capacity (WAC) of flour samples. The lowest solubility values were found from HP samples with further solvent extraction (HP + P and HP + S), with no significant variation between them, suggesting that solvent temperature did not affect this physicochemical property. Flour samples from screw-pressing at 50 °C showed the highest protein solubility values. Differences in protein solubility between flours can be attributed to different levels of protein denaturation caused either by the effect of mechanical stress, temperature and/or the intensity of the treatment used for oil removal.

Once denatured, protein is unfolded, exposes its internal hydrophobic groups, and acquires a random conformation which depends on the intensity of the treatment applied as well as the forces that stabilize the structure. Generally, the exposure of hydrophobic residues reduces protein solubility.

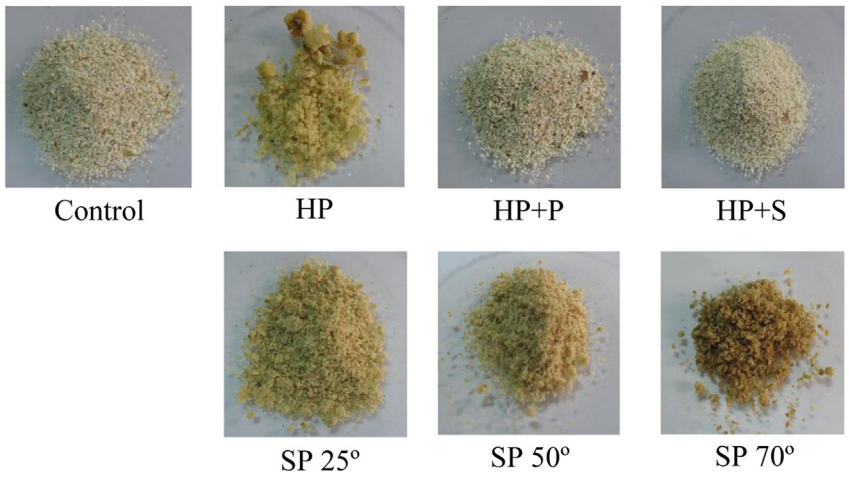


Fig. 1. Colour of the flours obtained by different treatments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Colour parameters of flours obtained by different treatments of oil extraction and total colour differences (ΔE) between each sample and Control flour.

	Control	HP	HP + P	HP + S	SP 25°	SP 50°	SP 70°
L^*	82.1 ± 0.9^{d}	64.3 ± 1.2^{c}	84.2 ± 1.0^{e}	82.5 ± 0.6^{d,e}	62.9 ± 0.5^{b,c}	61.5 ± 0.2^{b}	52.5 ± 0.2^{a}
a^*	0.8 ± 0.2^{a}	3.7 ± 0.5^{b}	0.7 ± 0.4^{a}	1.0 ± 0.1^{a}	4.1 ± 0.04^{b}	4.9 ± 0.2^{c}	5.6 ± 0.2^{d}
b^*	14.3 ± 0.6^{a}	22.4 ± 1.1^{b}	14.5 ± 0.1^{a}	13.6 ± 0.4^{a}	23.2 ± 1.1^{b}	21.8 ± 0.1^{b}	21.9 ± 0.2^{b}
ΔE	–	19.7 ± 1.8^{b}	2.2 ± 0.1^{a}	1.0 ± 0.8^{a}	21.4 ± 1.1^{b,c}	22.2 ± 0.9^{c}	30.9 ± 0.8^{d}

Values are means ± standard deviations of six measurements. Means followed by different letters in the same row indicates significant differences ($P < 0.05$) between treatments.

On the other hand, the WAC is the ability of a food material to retain water, during the application of centrifugal forces. This property depends primarily on the content and level of hydration of the insoluble fraction of a matrix. The high proportion of water insoluble glutelins and carbohydrates in walnut residue and the degree of denaturation of protein during oil extraction largely determines its WAC. The highest WAC was found in samples obtained by hydraulic pressing and subsequent solvent extraction (Table 3). All samples presented values higher than 3 g g^{-1} of flour, similar to those reported for isolates, concentrates and partial and totally defatted flours obtained from various sources (Abugoch et al., 2009; Adebowale, Adeyemi, & Oshodi, 2005; Mao & Hua, 2012; Tang, 2007).

Looking at the total protein contents, protein solubility values and WAC, it is possible that HP combined with solvent extraction causes higher protein denaturation than screw press extraction, thus resulting in the lower protein solubility and higher WAC observed in HP + P and HP + S.

Table 3
Protein solubility (PS) and water absorption capacity (WAC) of flours obtained by different oil extraction methods.

Sample	PS (mg/g flour)	WAC (g/g flour)
Control	22.3 ± 0.9^{c}	4.1 ± 0.2^{b,c,d}
HP + P	13.4 ± 1.1^{a}	4.2 ± 0.0^{c,d}
HP + S	15.8 ± 1.1^{a}	4.4 ± 0.1^{d}
SP 25°	25.2 ± 1.1^{d}	3.7 ± 0.0^{a,b,c}
SP 50°	32.8 ± 1.1^{e}	3.3 ± 0.3^{a}
SP 70°	19.0 ± 1.0^{b}	3.6 ± 0.3^{a,b}

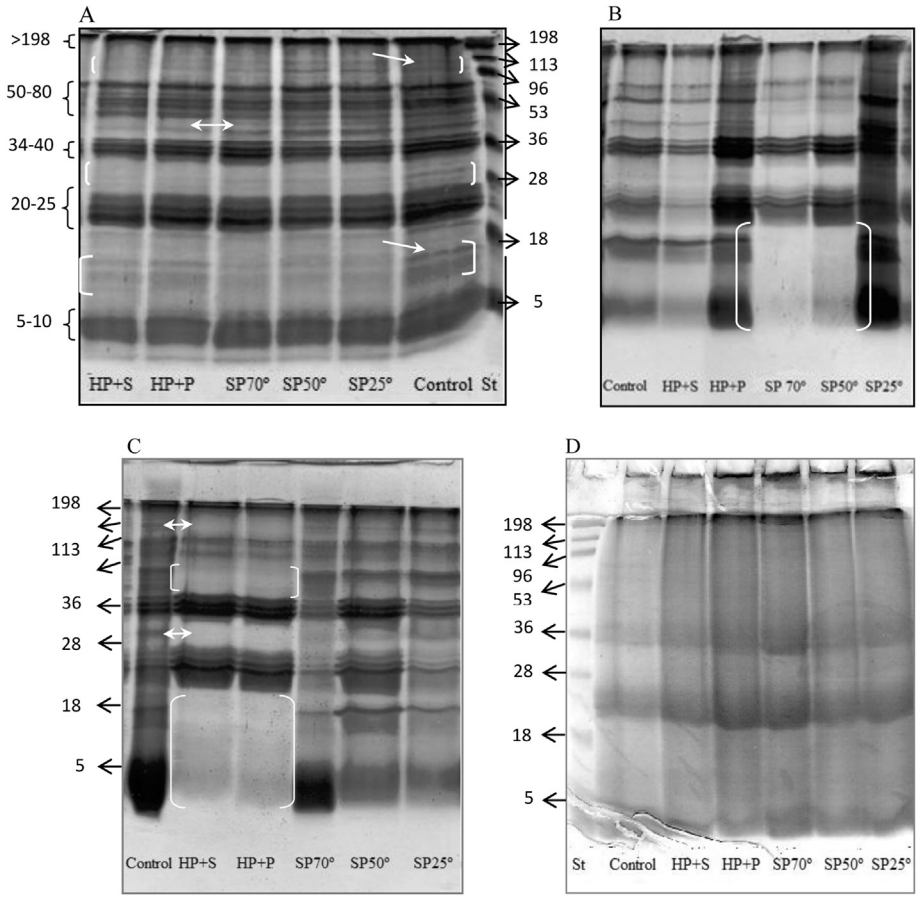
HP: Hydraulic Press; P: passive; S: Soxhlet; SP: Screw Press at different temperatures (25°, 50° y 70 °C). Values are means ± standard deviations of triplicate determination. Means followed by different letters are significantly different ($P < 0.05$).

3.4. Electrophoresis results

Fig. 2-A shows the electrophoretic profile of total soluble proteins in phosphate buffer. All samples showed the presence of five groups of intense bands. Four of them correspond to polypeptide complexes with molecular weight between 5 and 10 kDa, 20–25 kDa, 34–40 kDa and 50–80 kDa, respectively, and the remaining with greater protein mass (>198 kDa) retained at the top of the separating gel. In general, the major polypeptide band patterns did not differ between the different flour samples. In contrast, some minor polypeptides in the molecular weight range of 10–18 kDa, 28–32 kDa and 96–113 kDa occurred with lower intensities in samples from both HP + P and HP + S treatments (marked with brackets in Fig. 2-A). Homogeneity in the profiles of major polypeptide bands indicates that total soluble proteins were not affected by the pressing treatments (hydraulic or screw pressing), or by the temperature applied in each treatment (Fig. 2-A).

On the contrary, the process conditions used to obtain the flour samples strongly affected the water soluble protein profile (Fig. 2-B) which is mainly composed by albumins (Sze-Tao & Sathe, 2000). In general, flour samples obtained at ambient temperature conditions showed more complex and intense polypeptide band patterns. Post-pressing temperature during solvent extraction in Soxlhet apparatus (HP + S) caused a decrease in albumin solubility, probably due to protein denaturation by heat and exposure of hydrophobic residues. This effect implied a more tenuous profile than that from HP + P flour.

The protein pattern obtained from both SP 50° and SP 70° flour samples showed lighter and diffuse bands than that from SP 25° treatment, thus suggesting an effect of temperature applied during



HP: Hydraulic Press; P: passive; S: Soxhlet; SP: Screw press at different temperatures (25, 50 and 70 ºC), St: Molecular weight standards

Fig. 2. SDS-PAGE profiles of walnut protein solubilised in 0.2 mol dm^{-3} sodium phosphate buffer (A); deionised distilled water (B); 1.0 mol dm^{-3} NaCl (C) and 0.1 mol dm^{-3} $Na_2B_4O_7$ (D).

the pressing process. Differences were stronger at low molecular weight polypeptide regions (<20 kDa) where heavy stained bands were found in flour samples obtained at the lowest temperature.

The aqueous NaCl soluble protein bands also showed a treatment-dependent effect (Fig. 2-C). Samples HP + P and HP + S were similar to each other and different to the Control treatment, with smaller number of bands, especially in the ranges of < 5–20 kDa and 40–50 kDa. Moreover, polypeptides at 30 and 100 kDa (indicated by arrows in the Control sample) were not observed in samples obtained by hydraulic pressing. These results indicate that hydraulic pressing affected the globulin fraction; however, based on staining intensity and bandwidth an effect of the solvent temperature (25 °C or boiling point of the solvent) could not be seen.

The protein profiles of samples obtained by screw pressing at 25 °C and 50 °C were similar to the Control treatment, while pressing at the highest temperature (SP 70°) caused a decrease in the intensity of bands between 15 and 40 kDa and an increase in the intensity of low molecular weight peptides (<10 kDa).

The bands pattern of the glutelin fraction, which is the most abundant protein in walnut (Labuckas et al., 2008; Sathe et al., 2009; Sze-Tao & Sathe, 2000) is shown in Fig. 2-D. Electrophoretic profiles showed the presence of three polypeptide groups of about 5, 20 and 36 kDa, similar to those observed from total protein profile (Fig. 2-A). Moreover, a high molecular weight polypeptide was also detected. It did not penetrate the separating gel, despite the denaturing and reducing conditions applied to the samples.

Owing to their relatively low concentration (2.3–2.6 g/100 g of total protein content) prolamins could not be detected electrophoretically under the condition used in the present work.

4. Conclusion

The treatments applied to reduce the lipid content of walnut seeds modified the proximate composition and colour of the flours obtained. Treatments involving hydraulic pressing and solvent extraction generate flours with higher protein content and lighter colour than those obtained by screw pressing; however, the protein solubility was significantly lower and the WAC was higher, in the former, indicating a greater proteins denaturation.

Electrophoretic profiles confirm the effect of different treatments mainly on albumin and globulin fractions. While albumins were mostly affected by temperature conditions used in the screw-pressing treatments, globulins were strongly influenced in treatments using hydraulic press with further solvent extraction. The major polypeptide band patterns from both total soluble proteins and glutelin fraction showed minor variations between samples obtained under the different treatments employed.

In view of present results and taking into account that: a) globulin, (the second most abundant protein fraction in walnut) is

not affected by screw pressing at lower temperatures (25 and 50 °C), and b) flour from screw pressing at 50 °C, showed the highest proteins solubility value and a good WAC, it can be concluded that this process is the most suitable for obtaining partially defatted walnut flour.

Acknowledgements

This research was supported by grant from Secretaría de Ciencia y Tecnología – Universidad Nacional de Córdoba (SECyT-UNC) (26/11). The assistance of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and the Universidad Nacional de Córdoba, both of which support facilities for this investigation is also acknowledge.

References

Abugoch, L., Castro, E., Tapia, C., Añón, M. C., Gajardo, P., & Villarroel, A. (2009). Stability of quinoa flour proteins (*Chenopodium quinoa* Willd.) during storage. *International Journal of Food Science & Technology, 44*, 2013–2020.

Adebowale, Y. A., Adeyemi, A. A., & Oshodi, A. A. (2005). Functional and physicochemical properties of flours of six *Mucuna* species. *African Journal of Biotechnology, 4*, 1461–1468.

AOAC (Ed.). (1998). *Official methods and recommended practice of the American Oil Chemists' Society*. Champaign, USA: AOCS Press.

Arcan, I., & Yemenicioglu, A. (2009). Antioxidant activity and phenolic content of fresh and dry nuts with or without the seed coat. *Journal of Food Composition and Analysis, 22*, 184–188.

Ayo, J., Carballo, J., Serrano, J., Olmedilla-Alonso, B., Ruiz-Capilla, C., & Jiménez-Colomero, F. (2007). Effect of total replacement of pork backfat with walnut on the nutritional profile of frankfurters. *Meat Science, 77*, 173–181.

Ayo, J., Carballo, J., Solas, M. T., & Jiménez-Colmenero, F. (2008). Physicochemical and sensory properties of healthier frankfurters as affected by walnut and fat content. *Food Chemistry, 107*, 1547–1552.

Cofrades, S., Serrano, A., Ayo, J., Carballo, J., & Jiménez-Colmenero, F. (2008). Characteristics of meat batters with added native and preheated defatted walnut. *Food Chemistry, 107*, 1506–1514.

Colaric, M., Veberic, R., Solar, A., Hudina, M., & Stampar, F. (2005). Phenolic acids, syringaldehyde, and juglone in fruits of different cultivars of *Juglans regia* L. *Journal of Agricultural and Food Chemistry, 53*, 6390–6396.

Fukuda, T., Ito, H., & Yoshida, T. (2003). Antioxidative polyphenols from walnut (*Juglans regia* L.). *Phytochemistry, 63*, 795–801.

García-Parrilla, M. C., Cantos, E., & Troncoso, A. M. (2009). Analysis of melatonin in foods. *Journal of Food Composition and Analysis, 22*, 177–183.

Labuckas, D., Maestri, D., & Lamarque, A. (2011). Lipid and protein stability of partially defatted walnut flour (*Juglans regia* L.) during storage. *International Journal of Food Science & Technology, 46*, 1388–1397.

Labuckas, D., Maestri, D., Perelló, M., Martínez, M., & Lamarque, A. (2008). Phenolic from walnut (*Juglans regia* L.) kernels: antioxidant activity and interaction with proteins. *Food Chemistry, 107*, 607–612.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head bacteriophage T4. *Nature, 227*, 680–685.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with Folin phenol reagent. *Journal of Biological Chemistry, 193*, 265–275.

Maguire, L. S., O'Sullivan, S. M., Galvin, K., O'Connor, T. P., & O'Brien, N. M. (2004). Fatty acid profile, tocopherol, squalene and phytosterol content of walnut, almond, peanut, hazelnuts and the macadamia nut. *International Journal of Food Science International Journal of Food Sciences and Nutritionand Nutrition, 55*, 171–178.

Mao, X., & Hua, Y. (2012). Composition, structure and functional properties of protein concentrates and isolates produced from walnut (*Juglans regia* L.). *International Journal of Molecular Sciences, 13*, 1561–1581.

Martínez, M. L., Labuckas, D. O., Lamarque, A. L., & Maestri, D. M. (2010). Walnut (*Juglans regia* L.): genetic resources, chemistry, by products. *Journal of the Science of Food and Agriculture, 90*, 1959–1967.

Martínez, M. L., Mattea, M. A., & Maestri, D. (2008). Pressing and supercritical carbon dioxide extraction of walnut oil. *Journal of Food Engineering, 88*, 399–404.

Mattea, M. A. (1991). Fundamentos sobre el prensado de semillas oleaginosas. *Aceites y Grasas, 2*, 31–37.

Pereira, J. A., Oliveira, I., Sousa, A., Ferreira, I. C. F. R., Bento, A., & Estevinho, L. (2008). Bioactive properties and chemical composition of six walnut (*Juglans regia* L.) cultivars. *Food and Chemical Toxicology, 46*, 2103–2111.

Reiter, R. J., Manchester, L. C., & Tan, D. (2005). Melatonin in walnut: influence on levels of melatonin and total antioxidant capacity of blood. *Nutrition, 21*, 920–924.

Sathe, S. K., Venkatachalam, M., Sharama, G. M., Kshirsagar, H. H., Teuber, S. S., & Roux, K. H. (2009). Solubilization and electrophoretic characterization of select edible nut seed proteins. *Journal of Agricultural and Food Chemistry, 57*, 7846–7856.

Singh, J., & Bargale, P. C. (2000). Development of a small capacity double stage compression screw press for oil expression. *Journal of Food Engineering, 43*, 75–82.

Singh, K. K., Wiesenborn, D. P., Tostenson, K., & Kangas, N. (2002). Influence of moisture content and cooking on screw pressing of crambe seed. *Journal of the American Oil Chemist's Society, 79*, 165–170.

Sze-Tao, K. W. C., & Sathe, S. K. (2000). Walnut (*Juglans regia* L.): proximate composition, protein solubility, protein amino acid composition and protein in vitro digestibility. *Journal of the Science of Food and Agriculture, 80*, 1393–1401.

Tang, C.-H. (2007). Functional properties and in vitro digestibility of buckwheat protein products: influence of processing. *Journal of Food Engineering, 82*, 568–576.

Veiga-Santos, P., Suzuki, C. K., Cereda, M. P., & Scamparini, A. R. P. (2005). Microstructure and color of starch-gum films: effect of gum deacetylation and additives. Part 2. *Food Hydrocolloids, 19*, 1064–1073.

Venkatachalam, M., & Sathe, S. K. (2006). Chemical composition of selected edible nut seeds. *Journal of Agricultural and Food Chemistry, 54*, 4705–4714.

Wiesenborn, D., Doddapaneni, R., Tostenson, K., & Kangas, N. (2001). Cooking indices to predict screw-press performance for crambe seed. *Journal of the American Oil Chemist's Society, 78*, 467–471.

Zheng, Y., Wiesenborn, D. P., Tostenson, K., & Kangas, N. (2003). Screw pressing of whole and dehulled flaxseed for organic oil. *Journal of the American Oil Chemist's Society, 80*, 1039–1045.