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Original article

Lipid and protein stability of partially defatted walnut flour (*Juglans regia* L.) during storage

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Summary Partially defatted walnut flour (WF) was stored (25 °C, 800 Lux) for an 8-month period and evaluated monthly in some protein properties and oil oxidative parameters. Transparent plastic-laminated (WFPL) and plastic-laminated, aluminium-coated (WFAC) packages were used. During the first 3 months, samples were more susceptible to endogenous hydrolysis; an increase in soluble peptide concentration was determined for both WFAC and WFPL. Minor changes between WFAC and WFPL were observed in protein electrophoretic patterns along storage period, but no differences were determined in total protein solubility. However, a progressive reduction in water-holding capacity (47% from time 0 to month 8) was observed for WFPL. A major effect of packaging material was found on lipid-quality parameters. Packaging materials' barrier to light effectively protect WF against polyunsaturated FA degradation and oil oxidation. Lipids from WF stored in plastic-laminated packages showed decreasing double-bond index values (from 1.71 at initial time, to 1.45 after 6 months of storage) and increasing oxidation rates along storage test. Aluminium-coated packages can be used to keep quality of WF for 8 months at room temperature.

Keywords Oil, oxidative stability, packaging, protein, walnut flour.

Introduction

Walnut (*Juglans regia* L.) has been characterised by a lipid and protein composition, which has been related to some health beneficial effects. Lipid content in walnut kernel is found to be 600–750 g kg^{-1}. More than 90% of the total fatty acids (FA) in walnut oil (WO) are reported to be unsaturated FA in which polyunsaturated FA (PUFA, linoleic and linolenic acids) are present in high amounts (Crews *et al.*, 2005; Martínez *et al.*, 2010). According to Simopoulos (2002), walnuts are unique because they have a perfect balance of n−6 and n−3 PUFA, a ratio of 4:1, which has been shown to decrease the incidence of cardiovascular diseases. The walnut kernel is also a good source of proteins (140–240 g kg^{-1}) composed mainly of NaOH-soluble and salt-soluble fractions (70% and 18% of total proteins, respectively) and a small proportion of albumin and prolamin (6.8% and 5.5% of total protein, respectively) (Sze-Tao & Sathe, 2000; Labuckas *et al.*, 2008; Sathe *et al.*, 2009). The amino acid composition is characterised by hydrophobic and acidic amino acid residues, with adequate amounts of all the essential amino acids for an adult. However, when compared to the FAO/WHO/UNU (1985) recommended essential amino acid amounts for 2- to 5-year-old child, lysine, threonine and methionine/cysteine are the essential limiting amino acids in walnut. Arginine is present at high concentration (134–138 g kg^{-1}) (Sze-Tao & Sathe, 2000; Venkatachalam & Sathe, 2006). Considering that arginine is a precursor of nitric acid – which acts as a potent vasodilator and can inhibit platelet adhesion and aggregation – walnut protein consumption has been associated with reduction in atherosclerosis development (Sabaté *et al.*, 2001).

In addition to the distinctive FA profile and high biological-value proteins, numerous studies have shown the contribution of other walnut micronutrients and phytochemical compounds in the prevention of cancer (Hardman & Ion, 2008; Carvalho *et al.*, 2010) and heart related diseases (Sabaté *et al.*, 2001; Albert *et al.*, 2002; Morgan *et al.*, 2002).

Walnut kernels are mainly consumed as a raw material in various forms such as whole, chopped, sliced or paste, and are used in many bakery products. They have also been used as a source to produce edible oil. The oil extraction can be done by pressing (Martínez &

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Maestri, 2008) giving a good-quality oil, and a press-cake residue [the walnut flour (WF)] which may be used as an ingredient for bakery products and for many frequently consumed foods (Ayo *et al*., 2008; Cofrades *et al*., 2008).

Although FA composition of WO is considered to be nutritionally favourable, it results in a poor oxidative stability of the nut. Particularly, WO is highly susceptible to photo-oxidative degradation. Considering that WF obtained after pressing may contain a high proportion of oil (Vanhanen & Savage, 2006; Martínez & Maestri, 2008), it is important to determine how long it can be stored under shelf conditions (25 °C), without any deterioration. Using peroxide value as a measure of quality, Vanhanen & Savage (2006) found that WF could be preserved from oxidation for up to 26 weeks when stored below 23 °C in polypropylene plastic containers. However results from such experiments do not consider possible changes in other lipid- and protein-quality indicators. The present work is aimed to examine the effect of two types of containers, differing in their light-barrier properties, on some lipid and protein parameters of WF stored during the 8-month period.

Materials and methods

Flour preparation

Three independent samples (10 kg each) of unshelled walnuts (*Juglans regia* L. var. Franquette) were obtained from commercial plantations at Belén location, Catamarca Province, Argentina. After cleaning, walnuts were dried at 30 ± 2 °C for 24 h and then were shelled manually. Whole kernels were ground using a Braun Multiquick Professional Minipimer, and particles between 2.5 and 4.8 mm were selected using an automated screen. This material was conditioned to obtain a moisture content of 7.5% and then was screw-pressed at 50 °C. The resulting dry press-cake was ground in a roller-mill to 40 mesh size to produce partially defatted WF.

Dry matter, protein (N × 5.3), total oil content (Soxhlet, *n*-hexane, 12 h) and ash (furnace, 550 °C) of WF were determined using standard methods (AOAC, 1995). Determinations were made in triplicate.

Storage conditions and samplings

Walnut flour samples (12 g each) were packaged in two types of containers: transparent plastic-laminated (WFPL) and plastic-laminated, aluminium coated (WFAC) containers. Samples were stored for 8 months in a thermostatic and light controlled chamber set at 25 ± 1 °C and 800 lux. Furthermore, other WF samples stored in freezer (−18 °C) were used as controls. For each month, three individual samples from each treatment and controls were employed. Every month, each individual sample was withdrawn from the chamber for scheduled analyses. The flour obtained immediately after processing was considered a zero-time flour.

Total endogenous proteolytic activity and determination of TCA-protein solubility

Total endogenous proteolytic activity and TCA-protein solubility were determined using a modified form of the method described by Abugoch *et al*. (2009). Defatted WF (Soxhlet, *n*-hexane, 12 h) from each sample was dispersed at 1% (w/v) in 0.2 M phosphate buffer pH 8 and stirred in a bath at 37 °C for 90 min. Untreated samples (without heating) were used as controls. The reaction was stopped by the addition of 5% TCA (volume ratio TCA solution/WF dispersion, 1:1), and the sample was centrifuged at 4000 ***g*** for 5 min at 15 °C. Soluble peptides were determined in the supernatant according to Bradford (1976). Solubility was expressed as mg g^{-1} of total protein, using the following equation:

$$PS_{TCA\,t} = \frac{mgSP_{TCA\,t}}{gTP_t}$$

where PS is protein solubility; SP are soluble peptides; TP is the total protein and *t* stands for each storage time.

UV spectra

The UV spectra of defatted WF were determined according to Abugoch *et al*. (2009), with minor modification. Dispersions of WF (0.02 mg mL^{-1}) in 0.2 M phosphate buffer pH 8 were stirred gently for 1 h at room temperature and then centrifuged at 8500 ***g*** for 30 min at 15 °C. The concentration of the soluble protein fraction was normalised at 0.3 mg mL^{-1} by Kalckar method (Kalckar, 1947). UV spectra were measured with a Perkin Elmer Lambda 25 UV–Vis spectrometer at room temperature in the 250–350 nm wavelength range.

Electrophoresis

Defatted samples from each treatment were homogenised in 0.2 M phosphate buffer, pH 8, centrifuged at 8500 ***g*** for 30 min at 4 °C and then filtered. Total soluble proteins were precipitated with cold acetone.

SDS-PAGE and non-denaturing non-dissociating PAGE (NDND-PAGE) were performed according to Laemmli (1970). SDS-PAGE gels contained 14% (w/v) acrylamide (3% acrylamide stacking gels) and NDND-PAGE contained 5% (w/v) acrylamide (3% acrylamide stacking gels). The molecular mass of the polypeptides were

determined by simultaneous running standard molecular weight proteins (Bio-Rad Co., Hercules, CA, USA): myosin (198.08 kDa), β-galactosidase (113.58 kDa), bovine serum albumin (96.36 kDa), ovalbumin (52.98 kDa), carbonic anhydrase (35.96 kDa), soybean trypsin inhibitor (28.49 kDa), lysozyme (18.53 kDa) and aprotinin (5.73 kDa). Protein sample (0.1 mg) was added to 100 μL of sample loading buffer containing 0.16 M Tris–HCl (pH 6.8), 15% (v/v) glycerol, and for SDS-PAGE, 2% (w/v) SDS. For reducing conditions, 5% (v/v) β-mercaptoethanol (2-ME) was added and the sample was heated (100 °C, 2 min). Samples (40 μL) were loaded into each well. Except for the standard marker proteins, the protein loaded in each lane was 30 μg in NDND-PAGE and 40 μg in SDS-PAGE. Gels were run by using a minislab (28575-00 Model; Cole-Palmer, Vermon Hills, IL, USA). Electrophoresis was conducted at a constant current of 20 mA per gel. After electrophoresis, gels were fixed with MeOH/acetic acid/H_20 (2:2:1) for 45 min and stained with Colloidal Brilliant Blue G (Sigma-Aldrich, St Louis, MO, USA).

Solubility

WF was dispersed in distilled water at 1% w/v, stirred for 1 h at room temperature and centrifuged at 5500 ***g*** for 30 min at 15 °C. Soluble proteins (SP) in supernatant were determined by Bradford (1976) method and solubility (S) was calculated as percentage of total protein (TP), obtained for each time, as follows:

$$\%S = \frac{100 \times gSP_t}{gTP_t}$$

Water-holding capacity (WHC)

Water-holding capacity was determined by vortex mixing 0.1 g of sample and 1 mL of water for 30 s and allowed to stand for 30 min at room temperature (25 °C). The mixture was centrifuged (3000 ***g***, 20 min, 25 °C) and the supernatant was carefully retired and volume was noted. The volume (mL) of water absorbed per g of sample on a dry weight basis (dwb) was calculated using the following equation:

$$WHC = \frac{m_2 - (m_1 - m_3)}{m_1 - \delta}$$

where m_1 is the weight of the sample (g), m_2 is the weight of the sediment (g), m_3 is the weight of soluble protein from the supernatant (g) determined by Bradford (1976), and δ is the density of water (mL g^{-1}).

Oil analytical methods

Fatty acid composition

For FA composition, 50-μL oil aliquots were subjected to alkaline saponification (0.5 N KOH in MeOH). Unsaponifiable matter was extracted with n-hexane. Fatty acid methyl esters (FAME) were prepared by transmethylation with 400 μL of HCl/MeOH (4:1 v/v) solution as previously described by Scheuermann *et al.* (2002). The FAME were analysed using a CP-Wax 52 CB capillary column (30 m × 0.25 nm id × 0.25 μm film thickness), on a Perkin Elmer Clarus 500 gas–liquid chromatograph (Waltham, MA, USA) equipped with flame ionisation detector. Column temperature was programmed from 180 °C (held for 1 min) to 220 °C (2 °C min^{-1} rate). The carrier (Nitrogen) had a flow rate of 1 mL min^{-1}. The FAME were identified by comparing their relative retention times with those of authentic samples (Sigma-Aldrich). Determination of double-bond index (DBI) was used to describe oil unsaturation levels and was calculated according to Senanayake & Shahidi (2002).

Peroxide value and ultraviolet indices

Peroxide value (PV) (meq O_2/kg of oil), conjugated dienes (K_{232}) and conjugated trienes (K_{270}) determinations were performed following the analytical methods described in Regulations EEC/2568/91 and later modifications of the European Union Commission (EEC, 1991). All chemicals and solvents used were either of analytical or spectroscopic grade.

Statistical analyses

Analytical determinations were the average of triplicate measurements from three independent samples from each treatment. Statistical differences among all treatments were estimated by ANOVA test at the 95% level ($P < 0.05$) of significance for all parameters evaluated. Whenever ANOVA indicated a significant difference, a pairwise comparison of means by least significant difference was carried out. All statistical analyses were performed using InfoStat software, version 1.1 (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba).

Results and discussion

Initial proximate composition of WF showed 332.97 g kg^{-1} protein, 270.27 g kg^{-1} oil and 57.3 g kg^{-1} ash (on a dwb). Protein content was comparable to those reported for totally defatted macadamia flour (Jitngarmkusol *et al.*, 2008) and lupin flour (Lqari *et al.*, 2002). Oil content of WF obtained here was significantly higher than that from WF obtained from kernels extracted with hexane (47 g kg^{-1}, dwb), using a continuous lixiviation process (Labuckas *et al.*, 2008). Ash content was similar to that found in solvent-defatted flour (Labuckas *et al.*, 2008), indicating that the method employed for oil extraction has no apparent effect on mineral content.

Table 1 Effect of storage time and package on endogenous proteolytic activity of WF

	WFAC		WFPL	
Months	**Nh (mg g^{-1} protein)**	**h (mg g^{-1} protein)**	**Nh (mg g^{-1} protein)**	**h (mg g^{-1} protein)**
0	68.18 ± 3.2^{a}	104.53 ± 4.2^{b}	67.94 ± 4.81^{a}	95.47 ± 5.12^{b}
1	58.62 ± 4.8^{a}	101.69 ± 4.6^{b}	79.26 ± 12.2^{a}	87.35 ± 16.3^{b}
2	56.52 ± 9.8^{a}	82.77 ± 2.9^{b}	70.00 ± 5.6^{a}	105.74 ± 9.6^{b}
3	74.67 ± 7.3^{a}	84.61 ± 10.9^{a}	85.79 ± 1.1^{a}	85.77 ± 7.9^{a}
4	77.04 ± 5.0^{a}	77.18 ± 12.1^{a}	68.31 ± 1.8^{a}	71.37 ± 1.5^{a}
5	81.08 ± 4.6^{a}	87.89 ± 1.2^{a}	73.19 ± 1.6^{a}	73.94 ± 1.3^{a}
6	60.58 ± 3.9^{a}	83.20 ± 7.5^{a}	24.43 ± 0.9^{a}	24.90 ± 0.2^{a}
7	84.67 ± 3.9^{a}	89.56 ± 6.2^{a}	16.74 ± 1.9^{a}	19.11 ± 0.4^{a}
8	76.06 ± 5.8^{a}	84.57 ± 0.2^{a}	11.75 ± 3.3^{a}	18.78 ± 4.0^{a}

Nh, untreated samples; h, treated samples (37 °C, 90 min).
Means with different superscripts letters in the same row for each package condition are statistically different ($P < 0.05$).

Total endogenous proteolytic activity and TCA-protein solubility

Data on endogeneous proteolytic activity in WFPL and WFAC are shown in Table 1. During the first 3 months of storage, samples were more susceptible to endogenous hydrolysis; thus, an increase in soluble peptide concentration was observed for both treated WFAC and WFPL. After that time, there was no difference between treated (h) and untreated (Nh) samples ($P > 0.05$). The large drop in protein solubility in WFPL samples from month 6 occurs simultaneously when fatty acid and oxidative oil indices become to show lipid degradation.

UV spectra

UV spectra of the supernatants of WFPL and WFAC at storage time 0, 1, 3, 5, 7 and 8 are presented in Fig. 1. For all the storage period, WFPL showed a lower UV absorbance than WFAC. For UV spectra determinations, normalised concentrations of the soluble protein fraction from each WF sample were used. Until month 7, the total protein solubility did not show a clear tendency (increasing or decreasing) along the storage period. Furthermore, there were no significant correlations between protein solubility and UV intensity values at both 260 and 280 nm wavelengths. Hence, a possible effect of protein solubility on UV-absorption intensity data should be discarded.

Most proteins show a distinct light absorption maximum at 280 nm, mainly because of the presence of tyrosine, tryptophane and at 260 nm because of phenylalanine. These aromatic amino acids are present in WF at relatively high amounts (Sze-Tao & Sathe, 2000). It has been suggested that aromatic amino acids are sensitive markers of the protein environment (Chen & Barkley, 1998). In the present work, reductions in UV-absorption spectra were observed along the storage period. One possible explanation may focus on protein

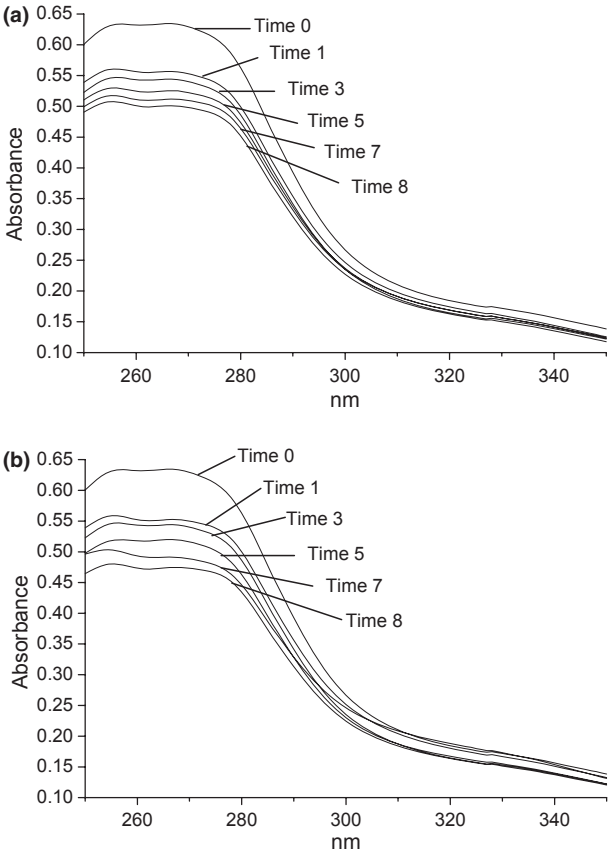

Figure 1 Effect of storage time and package on UV spectrum of soluble protein fraction. (a) WFAC and (b) WFPL.

denaturation, which has been reported to occur in long-term storage flour proteins (Abugoch *et al.*, 2009) and during thermal treatment (Avanza & Añón, 2007). As a result of protein denaturation, residues of the aromatic

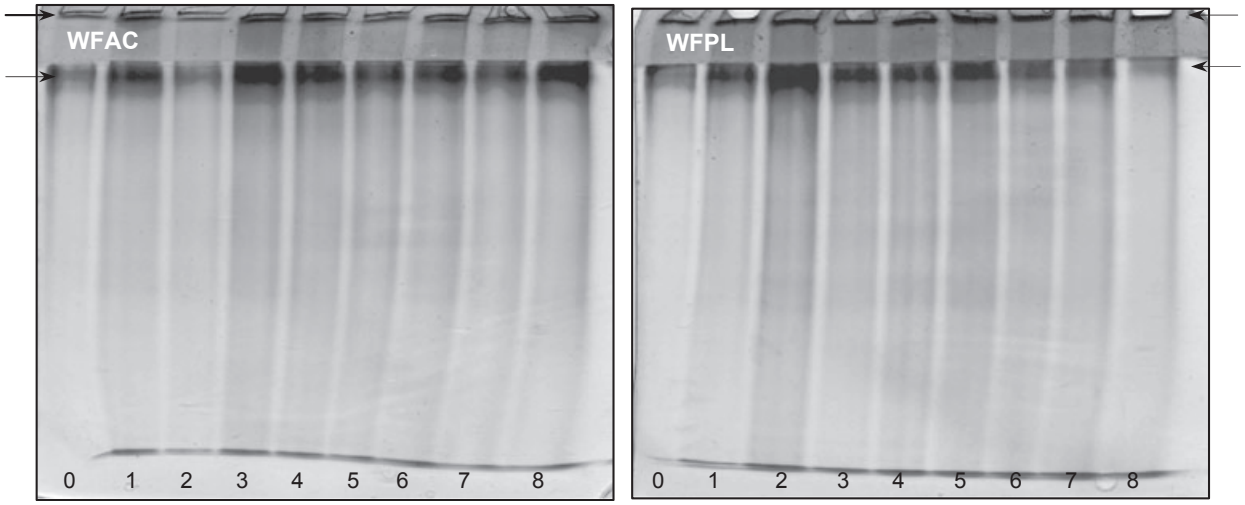

Figure 2 Electrophoresis analysis (NDND-PAGE) of WF protein stored in different package from 0 to 8 months.

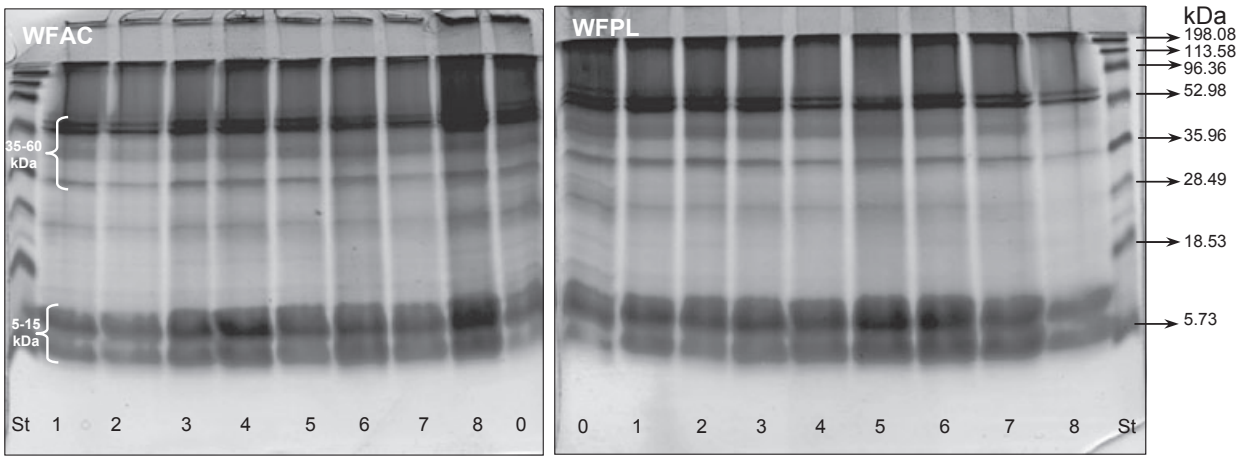

Figure 3 Electrophoresis analysis (SDS-PAGE) of WF protein stored in different package from 0 to 8 months. Except for the standard marker proteins, the protein loaded in each lane was 40 μg.

amino acids may be buried into the non-polar environment of the proteins, causing lower absorbance values.

Electrophoretic analysis

Electrophoretic analysis of the soluble fraction in phosphate buffer at pH 8 (and precipitated in acetone) allows analysing protein species responsible for the protein solubility at this pH. Under non-denaturing, non-dissociating conditions (NDND-PAGE) (Fig. 2), WF stored in both types of packages showed a predominant band at the top of the separating and stacking gels (indicated by arrows in the figure), implying a large molecular weight protein stabilised by disulphide linkage. No variation at molecular level was observed during the 8-month storage period.

Under SDS-PAGE in the absence of reducing agent (Fig. 3), two major groups of complex polypeptide bandings were evident in both WFAC and WFPL along all storage time. The size ranges of these groups were between 5–15 and 35–60 kDa (shown by brackets in the figure) and part of the protein remained at the top of the separating gel, indicating protein stabilised by disulphide linkages. Similar results were informed by Sze-Tao & Sathe (2000) for the walnut glutelin fraction run on 8–25% acrylamide gradient gel.

Results from SDS-PAGE-2ME are shown in Fig. 4. The total walnut proteins are composed of a large

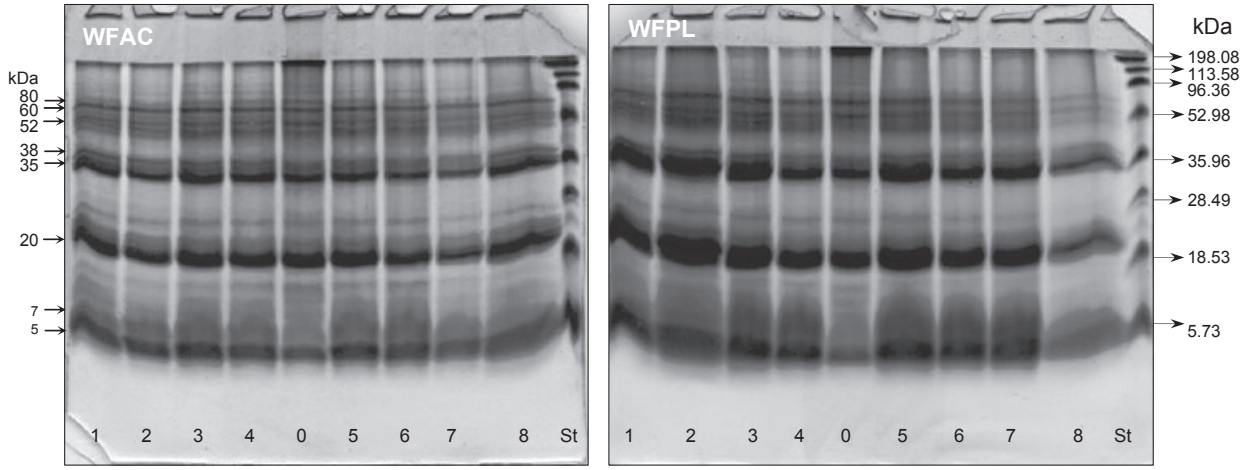

Figure 4 Electrophoresis analysis (SDS-PAGE with 2-ME) of WF protein stored in different package from 0 to 8 months. Except for the standard marker proteins, the protein loaded in each lane was 40 μg.

number of polypeptides with molecular weights in the range 5000–20 000. The darkest bands were at ~80, 60, 52, 38, 35, 20, 7 and 5 kDa (indicated by the left-hand arrows). These band patterns agreed with previously reported data for walnut-reduced protein (Sathe *et al.*, 2009). Comparing the SDS-PAGE patterns in the absence (Fig. 3) and the presence (Fig. 4) of the reducing agent (2-ME), the action of the 2-ME produced a disruption of the S–S linkages and consequently a reduction in intensity or disappearance of the bands corresponding to high-molecular weight peptides (> 50 kDa) present in SDS-PAGE without 2-ME. On the other hand, an increase in the number and intensity of bands at molecular mass under 50 kDa was observed in SDS-PAGE-2ME, compared with the electrophoretic profiles without reducing conditions. The molecular mass range of the SDS-PAGE polypeptides bands was within the reported range from several seed protein polypeptides (Abugoch *et al.*, 2009; Sathe *et al.*, 2009; Sharma *et al.*, 2010).

Considering the electrophoretic profiles from both WFAC and WFPL for each storage time, the banding patterns were almost identical, suggesting that the protein constituents of WF be unaffected by storage time.

Protein solubility

Protein solubility in water at natural pH is a practical indicator of denaturation and also gauges the potential or limitation of proteins as functional ingredients. The natural pH for WF was 6.7. At time zero, the protein solubility was around 5.45%, a similar value to that obtained by Sze-Tao & Sathe (2000) for water-extracted walnut proteins. At any time, total protein solubility had no significant differences between WFAC and WFPL, and no clear behaviour was observed during storage

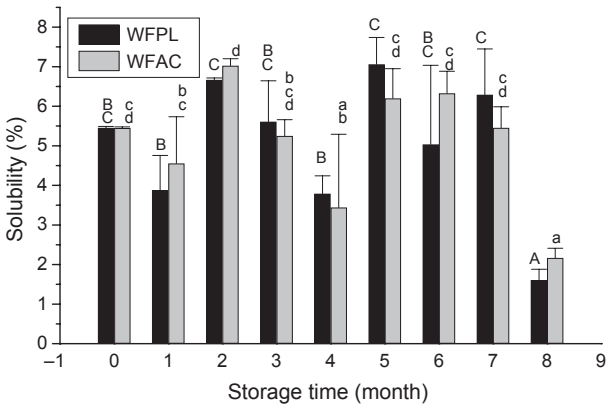

Figure 5 Effect of package and storage time on protein solubility of walnut flour packaged in plastic-laminated (WFPL) and in plastic-laminated, aluminium-coated (WFAC) (% of total protein) containers. Mean value of three independent determinations. Standard error is indicated by bars.

(Fig. 5). A significant decrease in solubility was found for both WFAC and WFPL at the end of the storage period (month 8). This fact may be related to an increase in surface hydrophobicity of proteins and the formation of aggregates through hydrophobic interactions. In control samples (−18 °C), no differences in protein solubility were found during the 8 months of storage (5.71% initial time, 5.73% after 8 months of storage).

Water-holding capacity

Water-holding capacity of the flour represents the ability of a matrix to absorb and retain water, therefore, is an important physical characteristic affecting the manufacture of foods. The major components of WF that

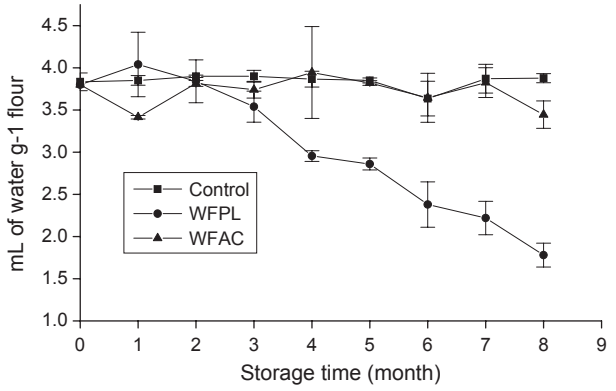

Figure 6 Effect of storage time and package on water-holding capacity of walnut flour packaged in plastic-laminated (WFPL) and in plastic-laminated, aluminium-coated (WFAC) (mL of water g^{-1} of flour) containers.

enhance this property are proteins and carbohydrates, because these constituents contain polar or charged side chains. The NaOH-soluble glutelins are the major protein fraction in WF (≅70%) (Sze-Tao & Sathe, 2000; Labuckas *et al.*, 2008), which in terms are insoluble in water, so this fraction largely contribute with the hydration of the flour. The WHC of WF at time zero was 3.8 mL of water g^{-1} of flour (Fig. 6). This value is higher than the values reported by Yu *et al.* (2007) for fermented and unfermented raw and roasted peanut flour. Based on data reported by Tang (2007), the WHC of WF has higher value than buckwheat protein products and slightly lower than soy protein isolates. When compared with other nuts, WF was found to possess higher WHC than cashew nut protein isolate, concentrate and nut powder (2.2, 1.74 and 0.81 mL of water g^{-1} of samples, respectively) (Ogunwolu *et al.*, 2009).

Table 2 Fatty acid composition (per cent) of oil extracted from analysed WF as a function of storage time. Mean values ($n = 3$) ± standard deviations (SD)

	WF	**Control (−18 °C)**		**WFAC**								**WFPL**					
	Time	**Time**		**Storage time (month)**								**Storage time (month)**					
	0	**1**	**8**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**1**	**2**	**3**	**4**	**5**	**6**
16:0	7.08	6.88	6.91	7.22	6.78	6.85	7.16	6.82	6.59	7.03	6.96	7.07	7.1	7.32	8.27	8.8	10.07
SD	0.2	0.3	0.02	0.2	0.1	0.1	0.1	0.3	0.02	0.1	0.1	0.2	0.1	0.4	0.1	0.2	0.8
*	ab	ab	ab	b	ab	ab	b	ab	a	ab	ab	ab	ab	b	c	c	d
18:0	2.22	2.22	2.21	2.16	2.04	2.1	2.3	2.19	2.16	2.29	2.07	2.13	1.59	2.24	2.47	2.36	3.69
SD	0.04	0.01	0.01	0.04	0.1	0.1	0.02	0.01	0.1	0.1	0.1	0.02	0.1	0.1	0.5	0.3	0.3
*	bc	bc	bc	bc	b	b	bc	bc	bc	bc	b	b	a	bc	c	bc	d
18:1	23.84	24.97	24.65	25.02	24.99	24.96	25.15	24.2	25.46	24.31	25.52	23.26	24.42	25.95	28.23	27.73	33.6
SD	1.6	0.1	0.3	0.2	0.1	0.2	0.03	2.1	0.04	1.2	0.1	1.8	0.2	1.2	0.6	0.8	0.03
*	ab	abc	abc	abc	abc	abc	abc	abc	bc	abc	bc	a	abc	cd	e	de	f
18:2	53.24	52.34	51.95	53.33	52.85	52.96	52.53	53.87	52.9	53.52	52.96	53.94	54.33	53.48	54.27	54.13	46.56
SD	1.6	0.2	0.2	0.2	0.4	0.4	0.1	1.5	0.1	1.0	0.1	1.2	0.4	1.1	1.8	1.9	1.2
*	bcd	bc	b	bcd	bcd	bcd	bcd	bcd	bcd	bcd	bcd	bcd	d	bcd	cd	cd	a
18:3	13.61	13.57	13.83	12.18	13.33	13.13	12.84	12.92	12.88	12.86	12.50	13.58	12.55	11.0	6.76	6.96	6.06
SD	0.3	0.03	0.4	0.3	0.0	0.0	0.2	0.4	0.04	0.2	0.01	0.4	0.4	3.0	0.6	0.6	0.1
*	cd	cd	d	bc	cd	cd	cd	cd	cd	cd	bcd	cd	bcd	b	a	a	a
SFA	9.30	9.10	9.12	9.38	8.82	8.95	9.47	9.01	8.76	9.32	9.03	9.2	8.69	9.57	10.75	11.16	13.76
SD	0.3	0.3	0.01	0.3	0.2	0.1	0.1	0.2	0.1	0.04	0.02	0.2	0.2	0.7	0.6	0.5	1.1
*	ab	ab	ab	ab	ab	ab	ab	ab	ab	ab	ab	ab	a	b	c	c	d
MUFA	24.97	23.27	24.65	25.02	24.99	24.96	25.15	24.2	25.46	24.31	25.52	23.26	24.42	25.95	28.23	27.73	33.6
SD	1.6	0.1	0.3	0.2	0.1	0.2	0.03	2.15	0.04	1.2	0.1	1.8	0.2	1.2	0.6	0.8	0.03
*	ab	a	ab	ab	ab	ab	ab	ab	b	ab	b	a	ab	bc	d	cd	e
PUFA	66.85	65.92	65.78	65.51	66.18	66.09	65.37	66.8	65.78	66.38	65.46	67.53	66.88	64.48	61.03	61.1	52.62
SD	1.9	0.3	0.1	0.02	0.4	0.4	0.1	1.9	0.03	1.2	0.1	1.6	0.01	1.9	1.2	1.3	1.0
*	cd	cd	cd	cd	cd	cd	cd	cd	cd	cd	cd	d	cd	c	b	b	a

WFAL, walnut flour packaged in plastic-laminated aluminium-coated containers; WFPL, walnut flour packaged in plastic-laminated containers; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
*Mean values from all treatments with different letters in the same row are statistically different ($p < 0.05$).

Water-holding capacity from WFAL did not change significantly along the storage period. On the contrary, a progressive and significant reduction in WHC (47% from time 0 to month 8) was observed for WFPL. This may be the result of partial protein denaturation, leading to the exposure of hydrophobic groups and greater interaction with the non-polar parts of lipids. These interactions could lead to subsequent modifications of the protein structure and progressive reduction in the WHC.

Fatty acid composition

Oil FA composition from WFAC and WFPL samples is shown in Table 2. Initial FA composition was in general agreement with those from Franquette variety from different origins (Martínez *et al.*, 2010). The FA composition from a WF control sample stored at −18 °C in the dark did not change significantly after the 8-month storage period. Similarly, the FA composition from WF stored in plastic-laminated, aluminium-coated containers at 25 °C remained without changes along storage test. On the contrary, PUFA percentages from WFPL samples showed significant decreases in a time-dependent manner.

The double-bond index (DBI) may be used as a useful indicator of PUFA degradation (Senanayake & Shahidi, 2002). When changes in DBI were plotted against storage period (Fig. 7), samples from WFPL showed significant reduction in DBI from month 2 of storage, whereas DBI from WFAC samples did not change significantly along the storage period.

Peroxide value and ultraviolet indices

Peroxide value and conjugated dienes and trienes (CD and CT, respectively) are primary oxidation products and persist during the early stages of lipid oxidation.

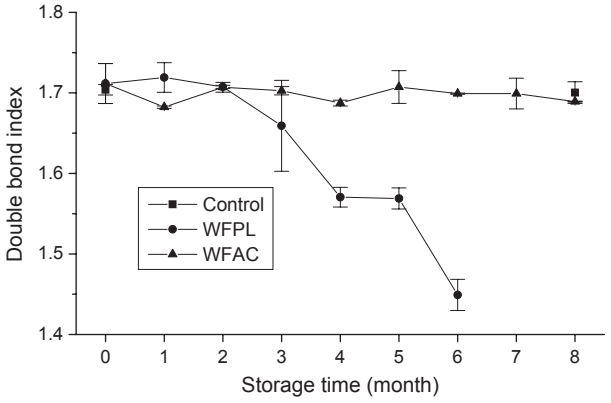

Figure 7 Changes in double-bond index (DBI) of WF oil from walnut flour packaged in plastic-laminated (WFPL) and in plastic-laminated, aluminium-coated (WFAC) containers during storage.

The changes in these chemical parameters from WFAC and WFPL samples are shown in Fig. 8. Oils from WFPL did not change significantly in their PV until 4 months of storage. A value of 25 meq O_2 kg^{-1} oil, considered as a maximum limit of acceptability for nuts (Özgül, 1993), was reached approximately at four and a half months of storage. From this moment, the oil from WFPL increased abruptly and reached a PV of 167 meq O_2 kg^{-1} oil at 6 months of storage. Owing to the high

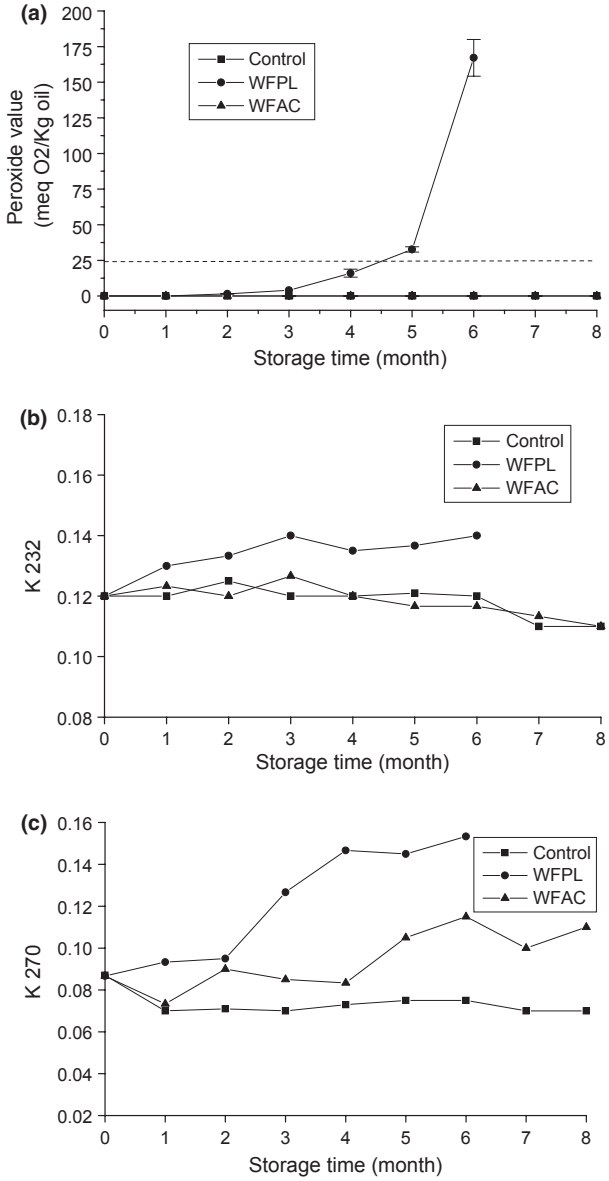

Figure 8 Kinetic curves of (a) peroxide accumulation, (b) conjugated dienes, K_{232}, and (c) conjugated trienes, K_{270}, of WF oil from WFPL and WFAC during storage. Kinetic curves were the average result of three independent experiments.

PV obtained in WFPL in the 6th month, no further determination of PV as well as ultraviolet indices (K_{232} and K_{270}) and FA composition were made in subsequent months. In contrast, PV from WFAC did not vary significantly along storage period and were similar to those obtained from the control sample stored at −18 °C in the dark. A similar trend was observed for CD values. Regarding CT values, samples from WFPL increased significantly from 0.09 (time 0) to 0.15 (month 6); samples from WFAC showed significantly lower increments. Furthermore, in the WFPL treatment, DBI correlated negatively with both PV ($r = -0.86$, $P < 0.01$) and CT ($r = -0.85$, $P < 0.01$). No significant correlations among these oxidative parameters were observed for WFAC treatment. The results described previously are in general agreement with those reported by Jensen *et al*. (2001) who showed that shelled walnuts stored in light develop pronounced oxidative changes, whereas dark storage results in longer shelf life.

Using storage conditions equal to those employed in this study (25 °C, 800 lux), Martínez (2010) found that WO obtained directly from screw-press and stored in aluminium-coated bottles reaches the induction period (time needed to reach a PV of 20 meq O_2 kg^{-1} oil) at 105 days of storage. This implies that the extracted WO is less stable against oxidation than oil contained in WF. Under a flour matrix, the remaining WO could be protected by an array of phenolic compounds, mostly present in the seed coat, which persist in the flour after processing. These phenolic compounds have shown strong antioxidant and free radical-scavenging activities (Arranz *et al*., 2008; Labuckas *et al*., 2008).

Conclusions

Results from this work showed a strong effect of packaging material on lipid-quality parameters from WF stored during the 8-month period. Packaging materials' barrier to light like plastic-laminated, aluminium-coated containers effectively protect WF against PUFA degradation and oil oxidation. Lipids from WF stored in plastic-laminated package showed decreasing DBI values and increasing oxidation rates along storage test.

Regarding protein electrophoretic patterns, minor changes were observed between WFAC and WFPL. However, a progressive reduction in WHC was registered from WFPL.

In summary, the present data indicated that: (i) lipid oxidation is the most important factor affecting WF quality during storage, (ii) protection against light by using aluminium-coated packages results in increased lipid and protein stability and (iii) this packaging material can be used to keep WF quality at room temperature at least for the 8-month storage period.

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