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Characterization of polyphenols, lipids and dietary fibre from almond skins (*Amygdalus communis* L.)

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ABSTRACT

Almond skins and blanch water are underutilized by-products of the almond processing industry. Nevertheless, they contain exploitable components that may contribute to the health benefits associated with almond consumption. We have compared natural almond skin powder (NS) prepared by a novel freeze-thawing method with blanched almond skin powder (BS). Microstructural studies were carried out, and we analyzed both types of almond skin for phenolic compounds (by HPLC), lipids (by solvent extraction), proteins (by micro-Kjeldahl), and fibre content (by the enzymatic-gravimetric AOAC method). Antioxidant activity (by measuring the reduction of the 2,2-diphenyl-1-picrylhydrazyl radical) was also monitored. We identified a combination of flavonols, flavan-3-ols, hydroxybenzoic acids and flavanones in NS, BS and in industrially obtained blanch water (BW). As expected, the total phenolic content was higher in NS compared to BW and BS, although the latter showed high antioxidant properties. Almond skins had high fibre content as well as significant amounts of lipid; both of these components may be relevant to fermentation in the large intestine. In addition, the processing of almond skins and blanch water clearly has economic potential for lowering the environmental impact of waste fill and pollution.

1. Introduction

Almonds (*Amygdalus communis* L.) are a rich source of nutrients and phytochemicals such as vitamin E, monounsaturated fatty acids, polyunsaturated fatty acids, arginine and potassium, which are associated with improvement in heart health and obesity-related diseases (Nuts, Almonds, 2007). Other health-promoting compounds in almonds are polyphenols, which have been shown to be protective agents against cancer and cardiovascular disease (Liu, 2004; Knekt et al., 2002; Yang et al., 2009).

Almond skins (also referred to as almond bran) are industrially removed from the nut by hot water blanching, and constitute 4–8% of the total shelled almond weight. Blanching results in a substantial loss of polyphenols to the blanch water, so greater quantities are recovered in the water than in the removed skins (Milbury et al., 2006). Therefore, it is not unexpected that the antioxidant capacity of almond skins, which is largely due to the presence of the phenolic compounds, varies in relation to the process used to isolate the skins. Both blanched skins and blanch water are considered by-products, which, if not processed further, become waste and might potentially cause environmental pollution. The almond-processing industries are interested in the valorization of these by-products, which at present are mainly used in cattle feed (Grasser et al., 1995) and in gasification plants to produce energy (Gonzáles et al., 2006).

An extensive understanding of the overall composition and carbohydrate constituents of the cell walls of blanched skins is required to optimize feed utilization for dairy cattle and for the generation of other potentially valuable fractions. Furthermore, almond skins are rich in fibre, which is recognized as a useful ingredient for the control of oxidative processes in food products and as a functional food ingredient (Monagas et al., 2007). Dietary fibre consists of intrinsic plant cell-wall polysaccharides which are not degraded by endogenous enzymes in the upper gastro-intestinal tract. However, through fermentation in the large bowel, cell-wall polysaccharides exert a major control on colonic

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function, including bowel habit, transit times, metabolism and balance of the commensal flora and large bowel epithelial health (Cummings and Stephen, 2007). Almonds (nut plus skin) have a total fibre content of 12.2% and are therefore considered to have one of the highest fibre contents of all the edible nuts (Holland et al., 1991).

A number of flavonoids and phenolic acids, including flavonols, flavanols, flavanones and simple phenolic acids have been identified in blanched almond skins, and may play a role in reducing risk factors against chronic inflammatory diseases and ageing disorders (Milbury et al., 2006; Garrido et al., 2008). The aim of this study was to evaluate the composition of natural almond skins removed from the seed using liquid nitrogen, and blanched almond skins removed from the seeds using hot water. The dietary fibre was characterized in an attempt to evaluate industrially obtained skins as sources for extraction. Furthermore, the polyphenolic composition of industrial blanch water was analyzed to identify new value-added antioxidant compounds to be used as dietary antioxidant ingredients.

2. Materials and methods

2.1. Source of almond skins

Natural almonds (Maisie Jane's, California) were kindly provided by the Almond Board of California and stored in the dark. Natural almond skins (NS) were isolated by freeze–thaw methods developed at the Institute of Food Research, UK. Almonds were kept in an atmosphere of 100% relative humidity at 4 °C for 4–5 days in order to facilitate loosening of the skins for easier removal. They were then immersed in liquid nitrogen for 20–30 min, taken out of the liquid nitrogen and allowed to thaw at room temperature; the skins could then be removed by hand. An alternative method, which was nearly as effective, was to omit the initial hydration step and carry out the freeze-thawing cycle twice. In this case, the second freezing step was done after the almonds had been thawed but were still cold and had taken up some condensed water from the atmosphere. The skins were milled using an analytical mill (Janke & Kunkel A10). Blanched almond skins (BS) produced by ABCO Laboratories (Almond Skins powder 1912) were supplied by the Almond Board of California. Industrial almond blanching was carried out by soaking brown skin almonds in near boiling water (90–100 °C) for 2–3 min. Industrially obtained almond blanch water (BW) was provided by the Almond Board of California.

2.2. Microstructural analysis of almond skin

Thin transverse sections of the outer layers of whole, shelled almonds, the natural skins (NS) prepared using liquid nitrogen, and the skins from blanched almonds (BS) were cut using a sharp razor blade, either by hand or by Vibratome. Some sections were examined and photographed unstained under bright field illumination to show the topography and pigmented regions of the skin using an Olympus BX60 microscope with a ProgRes® C10 ^{plus} (Jenoptik, Germany) digital camera and software. Further sections were examined in water under UV light (filter cube U-MWU, exciter filter BP330-385, barrier filter BA420) to reveal autofluorescent components in the almond skin. For visualization of lipids, another set of sections was stained with Sudan IV in 96% ethanol and mounted on slides in 30% glycerol.

2.3. Cell-wall material

Cell-wall material (CWM) was prepared from NS and BS following the modified method previously reported (Mandalari et al., 2008a). Briefly, milled NS and BS were weighed (5 g) and defatted by Soxhlet automatic Soxtec 2050 extraction using *n*-hexane as a solvent for 3 h. The defatted skins were blended in 1.5% (w/v) sodium dodecyl sulphate to solubilize proteins in an Ystral homogenizer (Ystral GmbH, Dottingen, Germany) for 2 min. A few drops of octanol were added to reduce foaming. The homogenate was filtered through a 70 μm nylon filter mesh, and the residue washed with 0.5% sodium dodecyl sulphate and sufficient deionized water to reduce bubbles on the filter. The iodine test and light microscopy were performed to ensure that the CWM was free of starch and any intracellular material. After several washes with water, the residue was filtered through a Whatman filter grade 1 (Whatman PLC, Brentford, Middlesex, UK) overlaid with a nylon mesh (70 μm). After washes with acetone and ethanol, the CWM was allowed to dry under the fume hood overnight. The yields of CWM were 47.7% (w/w) from NS and 45.4% (w/w) from BS. This method removed unbound phenolic compounds from the CWM.

2.4. Cell-wall bound phenolic analysis

The cell-wall bound, ester-linked phenolics of NS and BS were analyzed by hydrolysis of CWM (5 mg) in 4 M NaOH (1 mL) overnight at room temperature. Samples were centrifuged, neutralized with 11.6 M HCl (0.5 mL), extracted with ethyl acetate before being reduced to dryness, resuspended in MeOH:H_2O (50:50, v/v) and injected onto a 5 μm LUNA C18 HPLC column (250 by 4.6 mm inner diameter, Phenonomex, Macclesfield, UK) after filtration using a 0.2 μm PVDF membrane filter. Solvents for the mobile phase were: solvent A (acetone, 40%, methanol, 40%, water, ~20% made up with 1 mM TFA), solvent B (acetonitrile, 10%, water, ~90% made up with 1 mM TFA). The elution program was the following: (I) solvent A (10%), solvent B (90%) for 0.5 min, gradient = 0; (II) solvent A (75%), solvent B (25%) for 25 min, gradient = 1; (III) solvent A (100%), solvent B (0%) for 5 min, gradient = 1; (IV) solvent A (10%), solvent B (90%) for 10 min, gradient = –3; (V) solvent A (10%), solvent B (90%) for 2 min, gradient = 0. The injection volume was 40 μL, flow rate 1 mL/min. The detector was a diode array (PerkinElmer); *trans*-cinnamic acid (200 μL, 1.67 mg/50 mL methanol) was added as an internal standard. Ferulic acid (FA) dehydrodimers were quantified according to the method of Waldron et al. (1996).

2.5. Neutral sugar composition

Analysis of the sugar composition of the CWM from NS and BS and BW was carried out by acid hydrolysis of polysaccharides followed by reduction, acetylation and quantification of alditol acetates by gas–liquid chromatography (GLC), using methods adapted from Blakeney et al. (1983). Sugars were released from the samples after hydrolysis with 72% H_2SO_4 for 3 h, followed by dilution to 1 M (Saeman hydrolysis). Hydrolyzed monosaccharides were analyzed as their alditol acetates by GLC as previously reported (Mandalari et al., 2006). A second set of samples was hydrolyzed with 1 M sulphuric acid only (100 °C) and then treated the same as the first set; this second analysis excluded cellulose. Sugar analysis was performed in triplicate.

2.6. Uronic acid content

Total uronic acids were determined colorimetrically at 580 nm from a standard curve of galacturonic acid by the method of Blumenkranz and Asboe-Hansen (1973). Galacturonic acid and glucuronic acid are not differentiated by this method.

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2.7. Dietary fibre determination

Total dietary fibre (TDF), insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) were measured by the enzymatic-gravimetric AOAC method (Lee et al., 1992; Prosky et al., 1992). Triplicate defatted samples of NS and BS were homogenized in MES/Tris buffer (0.05 M, pH 8.2) and incubated at 100 °C with a heat-stable α-amylase for 15 min under constant agitation, then at 60 °C in a water bath for 30 min with protease in MES/Tris buffer (0.05 M, pH 8.2) in order to solubilize proteins. Finally, after adjusting the reaction mixture to pH 4.7, an amyloglucosidase solution was added and samples were incubated for 30 min in a water bath at 60 °C with continuous agitation. To determine the TDF content, samples were treated with 95% (v/v) ethanol (ethanol/sample ratio, 4:1, v/v) at room temperature for 1 h in order to precipitate soluble fibre and remove depolymerized protein and glucose (from starch). The residues were filtered on G4-sintered glass and washed sequentially with 78% (v/v) ethanol, 95% (v/v) ethanol and absolute acetone, and dried at 40 °C to a constant weight. To determine IDF and SDF content, the enzyme-digested sample was filtered and the insoluble material washed twice with preheated water at 60 °C. The final insoluble material was treated as above to give IDF. Four volumes of 95% (v/v) ethanol were then added to the filtrate and placed at room temperature for 1 h. After centrifugation and filtration, the recovered precipitate, SDF, was dried to a constant weight. TDF, IDF and SDF were corrected for residual protein and ash. Experiments were carried out in duplicate.

2.8. Klason lignin analysis

Klason lignin was quantified gravimetrically in NS and BS by a modification of the method of Theander and Westerlund (1986). Accurately weighed skin samples of approximately 50 mg were dispersed into 0.75 mL of 72% H_2SO_4 and incubated at room temperature for 3 h with frequent shaking. The samples were further incubated in a temperature-controlled oven set at 100 °C for 2.5 h after having been diluted with 9.0 mL water. After filtration through pre-weighed sintered glass funnels (10 mm diameter, Fisher Scientific), residues were recovered and the insoluble material was washed with warm water until the residue was free of acid. The glass filters were dried at 50 °C overnight or until a constant weight was reached, and the Klason lignin content was calculated gravimetrically. Experiments were carried out in duplicate.

2.9. Total protein assay

The total protein content of both NS and BS was determined by a micro-Kjeldahl according to AOAC (2000). Values were expressed as $N \times 6.25$. Protein concentration of BW was determined by the Bradford method (1976) using the Coomassie Protein Assay Reagent (Pierce, USA).

2.10. Ash determination

The ash content of NS and BS was determined gravimetrically following the method of AOAC International (2005). Duplicate samples of NS and BS were incinerated in a muffle furnace (Vulcan 3-550 Jencons, UK) at 525 °C until constant weight was reached.

2.11. Lipid content

Total lipid content and fatty acids were determined using the method described by Mondello et al. (2000). Vitamin E recovered in almond skin oil was determined by HPLC and quantified using authentic α-, β-, γ- and δ-tocopherol standards (Sigma–Aldrich) as previously described (Mandalari et al., 2008a).

2.12. Phenolic profile and radical scavenging activity

All solvents were HPLC grade, and water was ultra-pure grade. All flavonoid and other phytochemical standards (flavanone glycosides and aglycones, flavone glycosides, phenolic acids) were obtained either from Sigma–Aldrich (Poole, UK) or Extrasynthese (Genay, France). Other chemicals were of analytical grade.

2.12.1. Sample preparation

NS and BS (5 g) were extracted three times with *n*-hexane (10 mL) for 6 h under constant agitation in order to remove the lipids. After filtration, the residue was mixed with 50 mL of methanol/HCl 0.1% (v/v) and sonicated for 15 min. The sample was centrifuged (5000 × *g*, 10 min, 4 °C), and the pellet extracted two more times. The methanolic fractions were combined and evaporated by a rotary evaporator; the residue was dissolved in 20 mL of distilled water and extracted four times with 20 mL of ethyl acetate. The organic phases were combined and dried with Na_2SO_4 for 20 min. The yields of the residues from NS and BS were 7.6 and 3.6%, respectively.

An aliquot (10 mL) of BW was dried on a rotary evaporator, and the residue extracted with methanol/HCl 0.1% (v/v) by sonication for 10 min. The mixture was centrifuged (5000 × *g*, 10 min, 4 °C) and the sediment extracted as above. The methanolic fractions were combined and dried on a rotary evaporator, and the yield was 0.39%.

The dried extracts of NS, BS and BW were dissolved in methanol and used for total phenol determination, HPLC analysis and radical scavenging activity.

2.12.2. Total phenols

Total phenol content of NS, BS and BW was determined colorimetrically by the Folin-Ciocalteu method as modified by Singleton et al. (1999). Gallic acid was used as reference compound. Total phenol content was expressed as mg of gallic acid equivalents (GAE)/100 g of NS, BS and BW. Each determination was performed in triplicate and repeated at least three times.

2.12.3. Radical scavenging activity

The anti-radical activity of NS, BS and BW was determined using the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^•$) and the procedure previously described (Bonina et al., 1998). In its free radical form, DPPH$^•$ has an absorption band at 517 nm which disappears upon reduction by an anti-radical compound. Absorbance at 517 nm was measured on a Shimadzu UV-1601 UV–vis spectrophotometer 20 min after starting the reaction. The DPPH$^•$ concentration in the reaction medium was calculated from a calibration curve analyzed by linear regression. Each determination was carried out in triplicate and repeated at least three times. Results were expressed as μg of GAE or as mg of extract needed to scavenge 50 μmoles of initial DPPH$^•$ concentration (SE 50).

2.12.4. HPLC analysis of phenolics and flavonoids

The qualitative/quantitative determination of phenolics and flavonoids in NS, BS and BW was carried out using a Shimadzu high performance liquid chromatography system equipped with a UV–vis photodiode-array detector (DAD) and a fluorescence detector (Hewlett Packard 1046A). The apparatus was controlled by a control system (SCL-10A VP) equipped with an LC pump (LC-10 AD VP) and an auto-injector (SIL-10AD VP). The chromatographic separation was obtained by a 5 μm ODS3 reversed-phase Prodigy column (250 mm × 4.6 mm; Phenomenex) with solvent A (water/acetic acid, 98/2, v/v), solvent B (water/acetonitrile/acetic acid, 73/

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25/2, v/v/v) and solvent C (acetonitrile) as mobile phase (Garrido et al., 2008). The gradient program started with 100% A to reach 20% A and 80% B at 55 min, 10% A and 90% B at 57 min, 100% B at 90 min and 100% C at 105 min. The flow rate was 1 mL/min, and the column was thermostatically controlled at 25 °C. Extracts were filtered through a 0.22 μm filter and the injection volume was 75 μL. All analyses were carried out in triplicate. Identification of compounds was carried out by comparing their spectra and retention times with those of external standards. Detection was performed at 270 nm for hydroxybenzoic acids and flavanones and at 370 nm for flavonols. The UV spectra of the different compounds were recorded from 240 to 400 nm. The wavelengths used for fluorescence detection of flavan-3-ols were: λ_{ex}: 276 nm, λ_{em}: 316 nm. Data acquisition was performed using Class-VP5 Chemstation software (Shimadzu, Japan).

3. Results and discussion

3.1. Microstructure of almond skins

Unstained transverse sections of the outer surface of whole shelled almonds (Fig. 1A) showed the characteristic tissue arrangement of almond skin (s) with loose outer pigmented sclerenchyma cells (sc), pigmented flattened parenchyma (p), xylem tissue (x), a thin cuticle (c) and compressed nucellar remnants (n) overlying the aleurone cells (a). In whole almonds, the aleurone is firmly attached to the outer surface of the almond cotyledon (ac). Transverse sections of NS (Fig. 1C) and BS (Fig. 1E) showed a similar arrangement of tissue types in the skin, and in both treatments the skins separated at the junction of the aleurone and the cotyledons. After staining with Sudan IV, red coloured lipid droplets (l) were visible in the skin, particularly in the cells of the aleurone layers, and in the cotyledons of whole shelled almonds (Fig. 1B). Lipid droplets were also seen in NS (Fig. 1D) and BS (Fig. 1F) particularly in the aleurone cells. The droplets tended to be larger in BS compared to either whole shelled almonds or NS. This suggests that some droplet coalescence may occur in the high temperatures experienced during blanching.

When sections of NS (Fig. 2A) and BS (Fig. 2B) were illuminated by UV light, blue autofluorescence was emitted from the phenolics within the walls of the sclerenchyma cells (sc), the lignified cells of the xylem (x), and from the cuticle (c) overlying the aleurone cells (a). A similar pattern was found in skins of whole shelled almonds (not shown) indicating that the method of skin removal did not

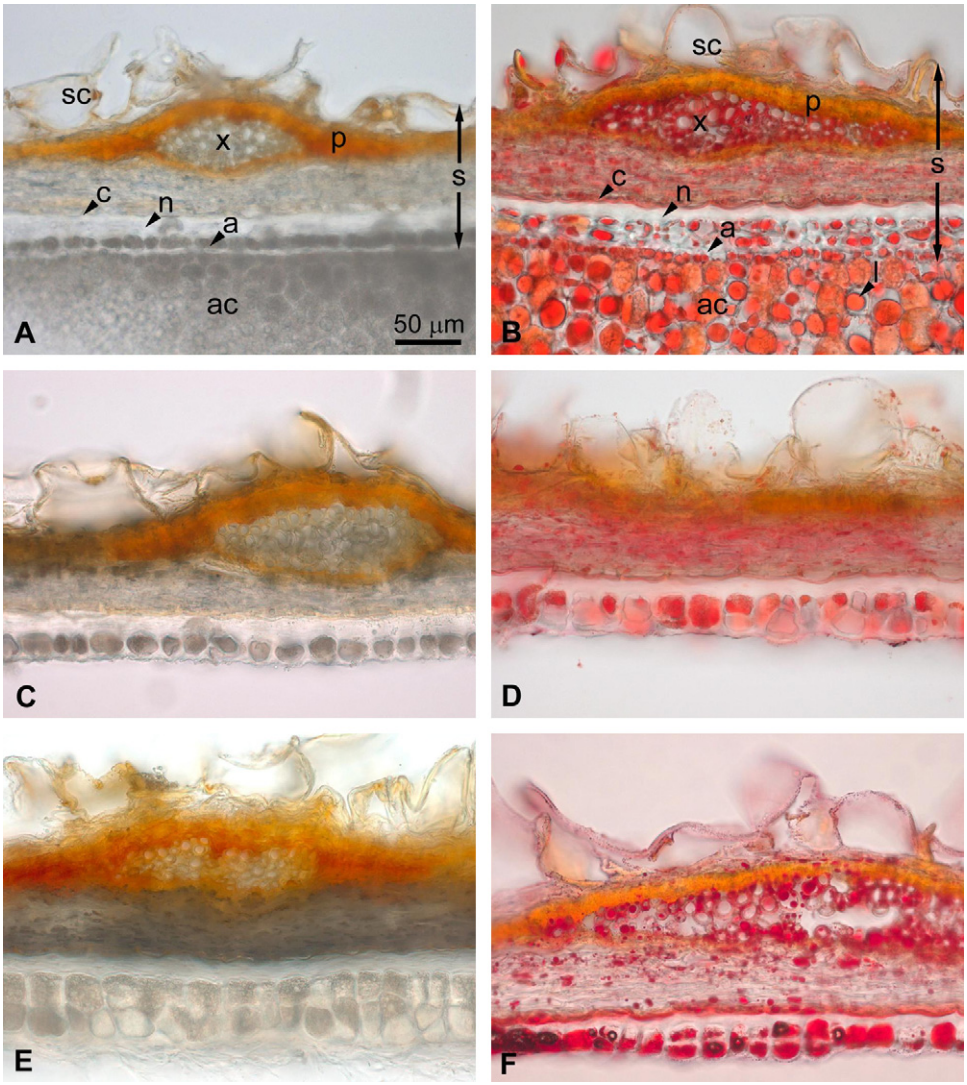

Fig. 1. Transverse sections of almond skins unstained to show natural pigmentation, or stained with Sudan IV to show distribution of lipids. (A) Outer layers of whole almond unstained, and (B) stained with Sudan IV, (C) natural skin prepared using liquid nitrogen unstained, and (D) stained with Sudan IV, (E) blanched skin prepared using hot water unstained, and (F) stained with Sudan IV. Scale bar in (A) applies to all images. *Abbreviations*: s = skin, sc = sclerenchyma cells, p = pigmented flattened parenchyma, x = xylem, c = cuticle, n = nucellar remnants, a = aleurone, ac = almond cells, l = lipid drops stained red with Sudan IV.

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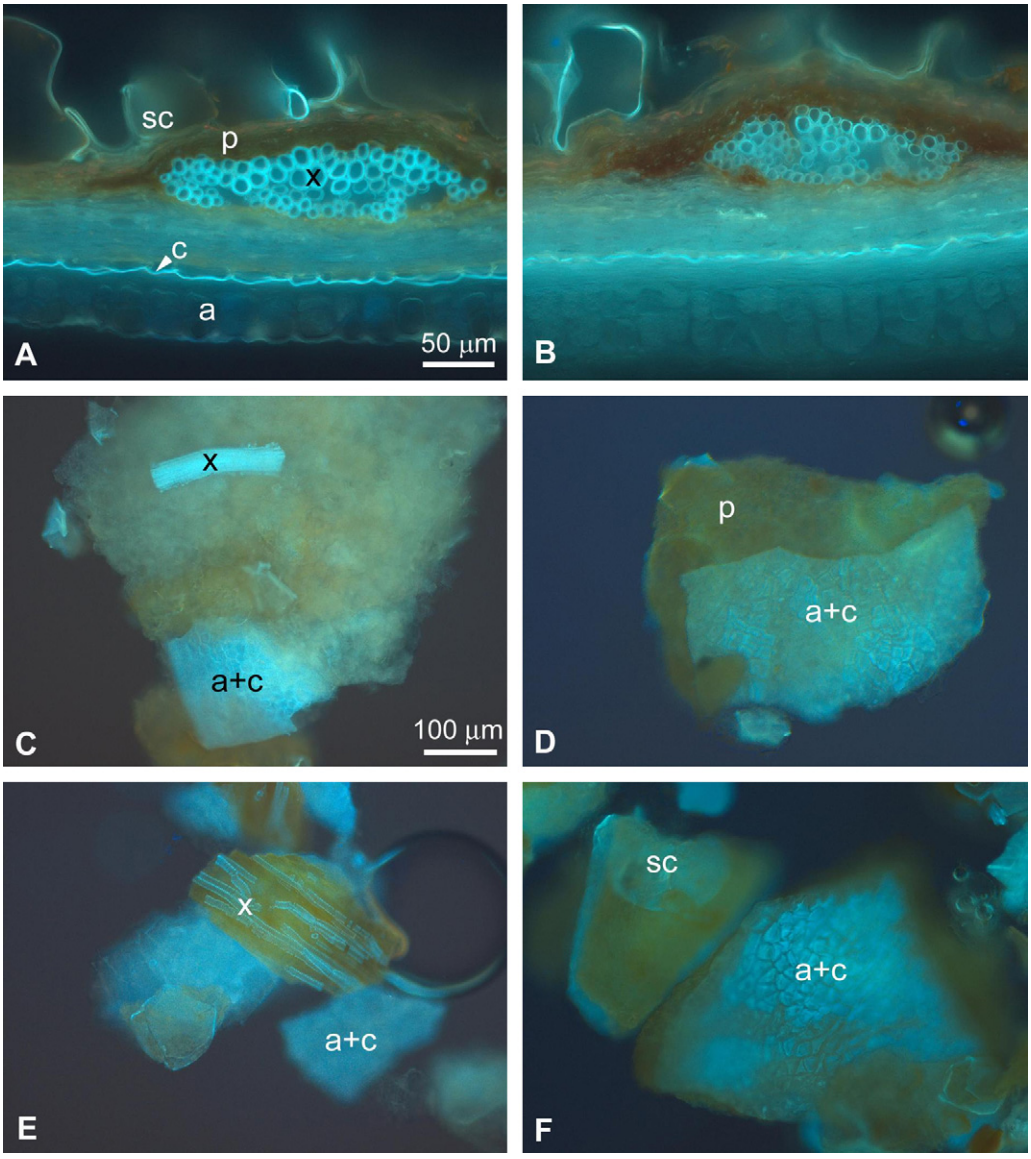

Fig. 2. UV-induced autofluorescence (in distilled water) in transverse sections of (A) natural almond skin prepared using liquid nitrogen, and (B) blanched almond skin prepared using hot water, both at same magnification. UV-induced autofluorescence of milled cell-wall material after fat and protein extraction (C and E) from natural skins, and (D and F) from blanched skins, all at the magnification indicated in (C). Abbreviations as in Fig. 1, additionally a + c = superimposed cuticle and aleurone cells.

affect the phenolic components of the tissue. Cell-wall material derived from NS and BS consists of fragments each comprising many cells (Fig. 2C–F), and the fragments tended to lie flat with tissues superimposed on each other, particularly in the case of the aleurone cells and cuticle (a + c). Examination of the UV autofluorescence of fragments of cell-wall material derived from NS (Fig. 2C and E) and BS (Fig. 2D and F) showed that there were no differences in the distribution of cell-wall bound phenolics between these samples, confirming the analytical data reported in Table 1.

3.2. Cell-wall bound phenolics

Several cell-wall bound phenolics, mainly *p*-hydroxybenzoic acid, vanillic acid and *t*-ferulic acid, were identified in NS and BS and their mean concentrations are reported in Table 1. With the exception of *p*-hydroxy-benzoic acid and 8,5′-diferulate, no significant differences ($p > 0.05$) were observed between NS and BS. The 5,5′-diferulate and the 8,5′-diferulate benzofuran form

Table 1
Cell-wall bound phenolics of natural (NS) and blanched (BS) almond skin cell-wall material.

Compound	NS	BS
Protocatechuic acid	–	–
Chlorogenic acid	–	–
Protocatechuic aldehyde	–	–
p-Hydroxy-benzoic acid	0.62 ± 0.03	0.45 ± 0.02
p-Hydroxy-phenyl acetic acid	–	–
Vanillic acid	0.09 ± 0.00	0.09 ± 0.00
p-Hydroxy-benzaldehyde	0.07 ± 0.00	0.08 ± 0.00
Vanillin	0.03 ± 0.00	0.07 ± 0.00
trans-*p*-Coumaric acid	0.08 ± 0.00	0.07 ± 0.00
trans-Ferulic acid	0.13 ± 0.01	0.13 ± 0.01
5,5′-Diferulate	0.60 ± 0.04	0.58 ± 0.03
8-*O*-4′-Diferulate	–	–
8,5′-Diferulate (benzofuran form)	0.42 ± 0.01	0.20 ± 0.01
Total	2.04	1.67

Values are expressed as μg/mg dry weight and data are the means ± SD ($n = 3$). (–) Not detected.

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were the most abundant ferulic acid dimers detected in both NS and BS.

3.3. Phenolic acids and flavonoids in almond skins and BW

Although almond skins have been previously shown to be rich sources of water-soluble bioavailable flavonoids and other polyphenols, the industrial blanching process is known to remove most of these antioxidant compounds. Therefore blanched skins are generally considered to be by-products with little economic value (Chen et al., 2005; Saura-Calixto et al., 2007). The total phenolic content, expressed as mg gallic acid equivalents (GAE) per 100 g of fresh skin was 3474.1 ± 239.8 for NS and 278.9 ± 12.0 for BS. Although the two samples are not directly comparable due to the different sources of almond skins, these results showed a significant loss of phenolic content resulting from blanching. In peanut skin, blanching was responsible for a significant loss (89%) in the total polyphenol content of skins (Yu et al., 2006). The high phenolic content in natural skins may contribute to the antioxidant and nutritive value associated with almond consumption. As in previous work (Milbury et al., 2006), lipids were removed from the skins in order to prevent any interference in subsequent phenol analysis and chromatographic methods. Our results for total phenols in almond skins are higher than those previously reported by Milbury et al. (2006), but are similar to those obtained by other researchers (Garrido et al., 2008; Sang et al., 2002). A number of different factors, including variety, cultivation practices and environmental conditions, as well as extraction methods, cause difficulties in making meaningful comparisons. Furthermore, ripeness, processing and storage are known to influence the flavonoid content of food plants (Herman, 1976). The total phenolic content of BW (33.3 ± 2.8) was significantly lower than the values reported (between 50.3 and 153.9 expressed as mg GAE released from blanching 100 g of fresh natural almonds) by Milbury et al. (2006), who designed experimental blanching conditions mimicking commercial processing. However, industrial BW was used in this present study and it was not possible to relate volume of water to weight of processed almonds.

In agreement with total phenolic content, NS showed the highest radical scavenging activity, followed by BS and BW (Table 2). This data reflected both the amount of phenols (as μg GAE) present in the almond skins and water and the different phenolic profile of the samples, also in relation to their reactivity to the radical scavenging test. BS showed an excellent radical scavenging activity in the DPPH• assay, and compared well with trolox and quercetin used as reference standards (Table 2). Its antioxidant capacity may be affected by the biodegradation of bioactive compounds at high temperatures, generating other products with antioxidant properties by the Maillard reaction (Durmaz and Alpaslan, 2007). Garrido et al. (2008) have recently demonstrated an increase in antioxidant capacity of dried blanched skins and roasted samples.

Table 2
Radical scavenging activity measured by DPPH• in natural (NS) and blanched (BS) almond skins, and in blanch water (BW).

Sample	μg GAE	mg of skin (or water)
NS	8.33 ± 0.51	0.24 ± 0.01
BS	14.57 ± 0.83	5.25 ± 0.42
BW	12.12 ± 0.46	36.34 ± 15.29
Quercetin[a]	1.49 ± 0.09	
Trolox[a]	4.25 ± 0.34	

Activity is expressed as SE50 (amount needed to scavenge 50 μmoles of the initial DPPH• solution).
Data are expressed as mean ± SD ($n = 3$).
[a] Quercetin and trolox were used as reference compounds.

Table 3
Phenolic compounds identified in natural (NS) and blanched (BS) almond skins and in blanch water (BW).

Peak no.	Compound	r.t. (min)	λ_{max} (nm)
1	Protocatechuic acid	18.46	259.4; 293.5
2	*p*-Hydroxybenzoic acid	26.07	255.3
3	Catechin	31.76	279.1
4	Chlorogenic acid	33.61	291.0; 319.4
5	Vanillic acid	35.19	262.5; 291.0
6	Epicatechin	41.21	279.1
7	*trans*-*p*-Coumaric acid	45.95	310.2
8	Eryodictiol-7-*O*-glucoside	55.10	284.0
9	Quercetin-3-*O*-rutinoside	56.04	255.3; 354.2
10	Quercetin-3-*O*-galactoside	56.61	255.6; 354.1
11	Quercetin-3-*O*-glucoside	57.67	255.7; 354.0
12	Kaempferol-3-*O*-rutinoside	62.84	265.1; 346.9
13	Naringenin-7-*O*-glucoside	64.09	284.0
14	Isorhamnetin-3-*O*-rutinoside	64.58	254.5; 355.2
15	Kaempferol-3-*O*-glucoside	65.26	265.1; 346.9
16	Isorhamnetin-3-*O*-glucoside	66.36	254.5; 354.0
17	Eryodictiol	80.01	287.5
18	Quercetin	85.78	255.2; 369.0
19	Naringenin	99.82	289.4
20	Kaempferol	101.23	265.1; 364.5
21	Isorhamnetin	102.96	254.5; 368

Peak identification was performed by matching retention time and UV spectra against standards.

A typical chromatogram of almond skin flavonoids and phenolics is shown in Fig. 3 A. A total of 21 flavonoids (flavanols, flavonols and flavanones) and phenolic acids have been identified (Table 3). As reported by others (Milbury et al., 2006; Garrido et al., 2008), the most abundant phenolic compounds were identified by matching the retention time and their spectral characteristics against standards. During the optimization of HPLC conditions, peak overlapping was avoided by using the HPLC Workstation software to monitor peak spectra. Pure standards (needed to increase around 40% the area of peak of interest) were added at sample solution where necessary and the new analytical parameters (r.t., absorption spectra, peak shape) were compared with those obtained from pure standards and original samples. The UV spectra of the different compounds were recorded from 240 to 400 nm. Detection was performed at 270 nm for hydroxybenzoic acids and flavanones (Fig. 3A) and at 370 nm for flavonols (Fig. 3B). The wavelengths used for fluorescence detection of flavan-3-ols were: λ_{ex}: 276 nm, λ_{em}: 316 nm (Fig. 3C).

As previously reported (Milbury et al., 2006), the major almond flavonoids identified were (+)-catechin, (−)-epicatechin, kaempferol and isorhamnetin, the latter (as the 3-*O*-rutinoside or 3-*O*-glucoside) being predominant in both NS and BS (Table 4). The main conjugated sugars were rhamnose (Rha) and glucose (Glc); mono-glycosides (mono-Glc), or rutinosides (–Glc [6 → 1]Rha). Neither quercetin nor kaempferol were found in BW, probably because of their low water solubility.

Hydroxybenzoic acids (as protocatechuic, *p*-hydroxybenzoic, chlorogenic, vanillic and *trans*-*p*-coumaric acids), flavan-3-ols (as (+)-catechin and (−)-epicatechin), flavonols (as aglycones; isorhamnetin, quercetin, kaempferol and glycosides; quercetin-3-*O*-rutinoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside, kaempferol-3-*O*-glucoside, isorhamnetin-3-*O*-glucoside) and flavanones (as aglycones; naringenin and eriodictyol and glycosides; eryodictiol-7-*O*-glucoside and naringenin-7-*O*-glucoside) were identified in the almond skin samples. The most abundant forms of flavonol glycosides were rutinosides, mainly isorhamnetin-3-*O*-rutinoside (narcissin). The antioxidant capacity of the above mentioned phenolic compounds is largely reported in the literature (Balasundram et al., 2006; Harrison and Were, 2007), suggesting that BS could be further utilized to produce high value

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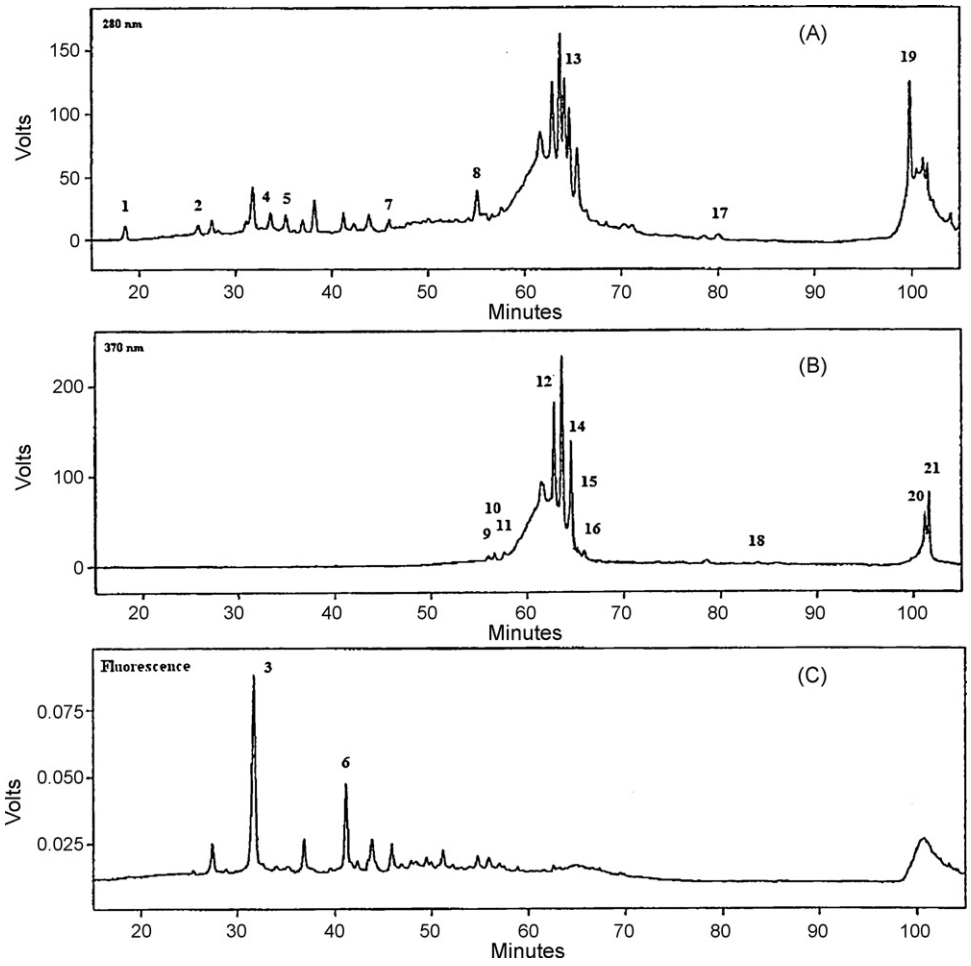

Fig. 3. Typical HPLC chromatograms of phenolic compounds in natural almond skin (NS): (A) absorbance at 280 nm, (B) absorbance at 370 nm and (C) fluorescence (λ_{ex}: 276 nm, λ_{em}: 316 nm). Peaks are marked with the same numbers reported in Tables 2 and 3. Peak identification was performed by matching retention time and UV spectra against standards.

Table 4
Flavonoids and phenolic acids in natural (NS) and blanched (BS) almond skins and in blanch water (BW).

Peak no.	Compound	NS	BS	BW
1	Protocatechuic acid	1541.67 ± 123.12	426.90 ± 36.08	36.55 ± 2.57
2	5-Hydroxybenzoic acid	6401.20 ± 427.28	887.85 ± 74.16	223.26 ± 16.41
3	Catechin	15,569.00 ± 997.84	5057.07 ± 382.21	231.38 ± 12.45
4	Chlorogenic acid	9571.26 ± 211.58	1190.84 ± 90.25	42.49 ± 3.16
5	Vanillic acid	5805.23 ± 612.25	861.44 ± 58.41	18.78 ± 1.24
6	Epicatechin	10,955.64 ± 654.52	1589.58 ± 86.35	127.31 ± 10.63
7	*trans-p*-Coumaric acid	367.71 ± 24.19	67.30 ± 5.52	3.99 ± 0.25
8	Eryodictiol-7-*O*-glucoside	3382.47± 254.11	77.88 ± 5.18	10.96 ± 0.87
9	Quercetin-3-*O*-rutinoside	3197.65 ± 147.55	550.70 ± 32.73	25.18 ± 1.98
10	Quercetin-3-*O*-galactoside	1339.65 ± 55.74	140.19 ± 11.37	0
11	Quercetin-3-*O*-glucoside	896.45± 95.16	60.51 ± 5.02	2.42 ± 0.17
12	Kaempferol-3-*O*-rutinoside	22,949.27 ± 1050.21	4076.25 ± 281.23	276.09 ± 21.55
13	Naringenin-7-*O*-glucoside	14288.10 ± 547.81	981.61 ± 66.12	286.92 ± 23.15
14	Isorhamnetin-3-*O*-rutinoside	54,901.07 ± 309.94	5379.92 ± 356.27	20.82 ± 1.73
15	Kaempferol-3-*O*-glucoside	39,012.26 ± 1343.08	1124.97 ± 81.53	8.78 ± 0.54
16	Isorhamnetin-3-*O*-glucoside	16,940.91 ± 638.22	895.46 ± 78.82	54.28 ± 3.95
17	Eryodictiol	470.09 ± 52.29	41.74 ± 5.01	4.69 ± 0.33
18	Quercetin	214.32 ± 26.17	147.43 ± 11.38	0
19	Naringenin	20,634.38 ± 987.54	1381.77 ± 101.44	14.70 ± 0.94
20	Kaempferol	1249.97 ± 87.39	291.00 ± 17.52	0
21	Isorhamnetin	4551.85 ± 205.84	492.29 ± 32.95	0
	Total amount	234,240.15	25,722.70	1388.60

Values are expressed as μg/100 g and represent average (±SD) of triplicate measurements.

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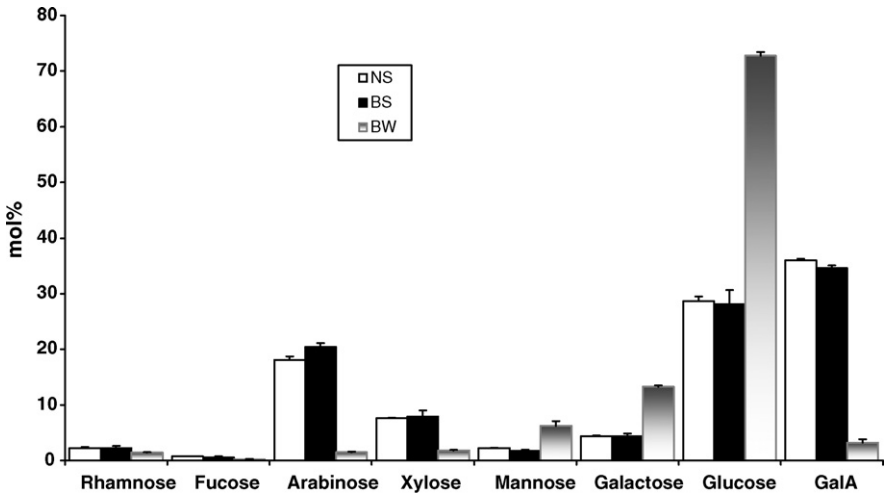

Fig. 4. Monosaccharide composition (mol%) of natural almond skins (white bars), blanched almond skins (black bars), and blanch water (gray bars). Gal A: galacturonic acid. Values for the total and each sugar concentration are the means of three replicates; the SD of the replicates was always <3%.

compounds for cosmetic applications and in the functional food industries.

3.4. Sugar composition

Sugar composition of NS, BS and BW is reported in Fig. 4. Carbohydrate comprised 45% of the dry weight of BS, suggesting this by-product has added value and could be potentially used as a source of dietary fibre. Glucose and galacturonic acid were the major sugars, followed by arabinose, xylose and galactose. Mannose and rhamnose were present in small amounts, whereas only traces of fucose were detected. BW contained mostly glucose (72.28% of total sugar content), followed by galactose (13.27% of total sugar content) and mannose (6.26% of total sugar content). Small amounts of galacturonic acid, xylose and rhamnose were detected. The amounts of different monomeric sugars in NS (as a percentage of total sugar content) were galacturonic acid (35.98%), glucose (28.68%), arabinose (18.07%) and xylose (7.63%).

Approximately 6% of the glucose of NS and BS was released by hydrolysis with 1 M sulphuric acid alone (results not shown), indicating that the bulk of the glucose was cellulosic in origin. The sum of the percentages of arabinose, rhamnose, galactose and galacturonic acid in the 1 M sulphuric acid hydrolysis fraction accounted for 86% of the total sugar content. These results indicated that almond skin cell-wall material was mainly composed of pectic substances, encasing the cellulose microfibrils; this is in agreement with earlier results obtained from Ellis et al. (2004), where the cell walls of raw and roasted almond skins were higher in glucose and uronic acids. The small amounts of noncellulose glucose and xylose indicated that hemicelluloses such as xyloglucan and α-glucans were minor components.

The Klason lignin content was $21.5 \pm 1.05\%$ (w/w) and $20.6 \pm 0.99\%$ (w/w) for NS and BS, respectively. However, this value may be overestimated as the residue representing the Klason lignin is a mixture of lignin with residual protein, suberin and ash.

Total dietary fibre (TDF) accounted for $45.1 \pm 1.9\%$ (w/w) in BS. Most of TDF was insoluble (IDF), whereas only 3–4% was soluble (SDF). A good agreement was found between the measurement of TDF with the AOAC method and the yield of non-starch polysaccharides (NSP) obtained with the CWM preparation. Hence, the determination of total NSP can also be used to quantify accurately the DF in fibre-rich materials, as previously demonstrated for other plant materials (Englyst et al., 1994). The high IDF content of BS could be related to the high cellulose content, supporting the evidence that almond cell walls have been found in the large bowel at various stages of fermentation and may act to enhance colonic health (Mandalari et al., 2008b). No significant differences in terms of dietary fibre content were observed with NS.

3.5. Lipids and other components

Values of 10.3 and 12.8% of the dry weight of NS and BS, respectively, were protein, while in the BW the soluble protein accounted for 0.15 mg/mL. The ash content was 4.8 and 5.2 g/100 g for NS and BS, respectively. The total fat (including wax) content was 22.2 and 24.2% for NS and BS, respectively. Monounsaturated fatty acids (mainly oleic acid) represented the principal component (56.0%) of BS, followed by polyunsaturated (33.6%, mainly linoleic acid) and saturated (10.3%, mainly palmitic acid) fatty acids. The fatty acid profile of BS was shown to be as follows (expressed as percentage of the total fatty acids): myristic (0.05), palmitic (8.01), palmitoleic (0.63), heptadodecanoic (0.11), heptadodecenoic (0.10), stearic (1.81), oleic (55.19), linoleic (33.35), linolenic (0.29), arachidonic (0.20), eicosenoic (0.09), behenic (0.05), lignoceric (0.12). The total vitamin E of NS and BS was 14 and 13 mg/100 g, respectively, of which 99% was represented by α-tocopherol; only traces of β- and γ-tocopherol were detected. Only traces of lipid (0.05%) were found in the BW. Although the two tested samples have different origins, these data show consistent nutrient composition across almond cultivars and varieties.

4. Conclusion

The present study has demonstrated that almond skins contain significant amounts of phenolics and flavonoids potentially bioavailable for absorption in the gastro-intestinal tract. There is clearly scope to process blanched almond skins and blanch water in order to generate not only useful animal feed and bioreactor substrates, but also potential sources of antioxidants for phyto-pharmaceutical applications and functional food ingredients. A further advantage of using almond skin by-products is their high dietary fibre content in relation to the potential health benefits of non-glycaemic carbohydrate functional food preparations, mainly for gut health. Both the dietary fibre-enriched and flavonoid-enriched fractions are quickly and easily generated from the original almond skin by-product, and they use processes and solvents with a negligible impact on the environment compared with the current methods of waste disposal (e.g. as land-fill or being burning).

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