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# Antioxidant activity of extracts produced by solvent extraction of almond shells acid hydrolysates

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**Abstract**

The ethyl acetate-soluble fraction generated during acid hydrolysis of almond shells was evaluated for radical-scavenging capacity and protection against fish oil oxidation. The influence of the operational conditions during acid hydrolysis on: (i) the total phenolics produced; (ii) the recovery yield of ethyl acetate solubles; (iii) the phenolic content in the ethyl acetate extracts; (iv) the antioxidant activity of extracts was assessed. A one-at-a-time variation study of the hydrolysis time and sulfuric acid concentration was carried out. For a given temperature and hydrolysis time, the influence of the acid concentration was noticeable; whereas the maximal phenolics production, measured in the hydrolyzate (2.2 g gallic acid equivalents/100 g shells) was achieved with 2% sulfuric acid, the maximal recovery in the organic phase required at least 5% acid. The crude extracts showed DPPH radical-scavenging activities ($EC_{50} < 0.5$ g/l) comparable to those of synthetic antioxidants, and protected labile lipid systems, such as fish oils and fish oil-in water emulsions, from oxidation as efficiently as did propyl gallate.


*Keywords:* Almond shells; Acid hydrolysis; Solvent extraction; DPPH; Fish oils; Emulsions

## 1. Introduction

Almonds (*Prunus amygdalus*) are used worldwide in bakery and confectionery. The processing byproducts, shells and hulls, account for more than 50% by dry weight of the fruit (Fadel, 1999; Martínez, Granado, Montané, Salvadó, & Farriol, 1995). Almond hulls contain triterpenoids (Takeoka et al., 2000), lactones (Sang et al., 2002a), phenolics (Sang, Lapsley, Rosen, & Ho, 2002c), and sterols (Takeoka & Dao, 2003). The isolation and identification of phenolic compounds in almond skins has been reported (Sang et al., 2002b). The water extraction of hulls (Rabinowitz, 2004) and solvent extraction of shells (Pinelo, Rubilar, Sineiro, & Núñez, 2004) to produce food ingredients and antioxidants, respectively, has been studied.

The high xylan content of almond shells makes them a suitable substrate for the production of xylose (Pou-Ilinas, Canellas, Driguez, Excoffier, & Vignon, 1990), furfural (Quesada, Teffo-Bertaud, Croué, & Rubio, 2002) or for fractionation into cellulose, pentosans and lignin (Martínez et al., 1995). This latter utilization consists of an acid-catalysed hydrolysis performed under mild conditions, which causes depolymerization and solubilization of the main components present in hemicelluloses. The liquid phase (hydrolyzate) contains sugars, sugar-dehydration products, acetic acid and compounds derived from the acid-soluble lignin, which can be used for the production of oxyaromatics of interest for the health, cosmetics and food industries (Quesada et al., 2002). Almond shell is highly lignified (30–38% of the dry weight) (Martínez et al., 1995) and the guaiacyl:syringyl phenylpropane units ratio is similar to that of hardwoods (Quesada et al., 2002). Even if most of the lignin is acid-insoluble (Klason lignin), a part of it

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can be solubilized in acidic media. The antioxidant potential of depolymerized lignin fractions produced after mild acid hydrolysis of lignocellulosics has been reported (Cruz, Domínguez, & Parajó, 2004, 2005; Garrote, Cruz, Domínguez, & Parajó, 2003; González, Cruz, Domínguez, & Parajó, 2004).

Deterioration of lipids in food systems, due to autoxidation, is a free-radical chain reaction responsible for off-flavour development and degradation of overall quality (Chan, 1987; Flick & Martin, 1992). The use of natural antioxidant extracts as components of foodstuffs have risen lately as the main methodology for stabilizing food lipids. Consumers and food industries have boosted the substitution of synthetic antioxidants by natural compounds that are often more effective. Among lipids, marine lipids are the main goal of companies, claimed for bioactive n-3 fatty acids, and there have been several attempts at stabilizing them by the use of natural phenolic compounds (Löliger, 1983; Medina et al., 2003). Due to their contents of polyunsaturated fatty acids, fish oils are more susceptible to oxidation than are other oils.

The objective of the present work is to select the operational conditions for the acid hydrolysis of almond shells in order to optimize both the solubilization or production and the recovery of phenolics as well as their DPPH radical-scavenging capacity. The extracts produced under optimal operational conditions were characterized and evaluated for their potential as protector agents for fish oils in relevant model lipid systems.

## 2. Materials and methods

### 2.1. Material

Almond (*P. amygdalus*) shells were kindly provided by Borges S.A., (Tárrega, Lleida, Spain). The shells were ground in a wood mill and the particles smaller than 1 mm were stored in sealed plastic bags and kept in a dark and dry place before use. Results from quantitative acid hydrolysis indicated that glucan, xylan and arabinan accounted for 30.9%, 30.4% and 2.5% of the dry material, respectively, and the Klason lignin for 30.6% of the dry weight.

Crude extracts from *Eucalyptus globulus* wood acid hydrolysates, produced under conditions previously selected: (130 °C, 45 min, 5% sulfuric acid) (González et al., 2004), were used. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), soybean lecithin (40% L-α-phosphatidylcholine), the Folin–Ciocalteu reagent, gallic acid, and propyl gallate were purchased by Sigma (St. Louis, MO).

A high quality fish oil was purchased from Fluka (New-Ulm, Switzerland) and composed of: 5.8% of 14:0; 11.9% of 16:0; 6.7% of 16:1 ω7; 1.8% of 18:0; 2.8% of 18:1 ω7; 11.6% of 18:1 ω9; 1.9% of 18:2 ω6; 3.7% of 18:3 ω3; 7.4% of 20:1 ω9; 1.3% of 20:4 ω3; 0.6% of 20:4 ω6; 14.9% of 20:5 ω3; 8.8% of 22:1 ω11; 2.8% of 22:5 ω3 and 17.9% of 22:6 ω3.

### 2.2. Acid hydrolysis of shells and production of extracts (Fig. 1)

Ground shells were suspended in an acidic solution (0.5–5% sulfuric acid) at a liquid to solid ratio of 10 (g/g), including the moisture content of shells ($8.9 \pm 0.13\%$). Optionally, shells were previously extracted in a Soxhlet extractor with a mixture 2:1 of toluene:ethanol. Hydrolysis was carried out in closed flasks in an autoclave at 110, 120 and 130 °C. Non-isothermal heating and cooling periods were not taken into account. A control experiment, without acid addition was also carried out (autohydrolysis). After hydrolysis, solid–liquid separation was accomplished by vacuum filtration and the liquid phase (hydrolyzate) was extracted three times, either with ethyl acetate or with diethylether. Solvent was added to the aqueous phase at a solvent:hydrolyzate volume ratio of 3 and stirred at room temperature during 15 min. Aqueous and organic phases were separated by decantation and the organic phase was vacuum evaporated to recover the solvent, which was reutilized up to three times to avoid concentration of traces which could interfere with the analytical methodology used. The remaining sludge (extract) was freeze-dried and analysed for total solids, total phenolics and radical-scavenging capacity. Aliquots of the aqueous phase, before and after solvent extraction were analyzed for phenolic content. The phenolics production yield was defined as the phenolics released to the aqueous phase during hydrolysis, expressed as weight percent of the initial raw material. The phenolics recovery yield refers to the phenolics extracted in the ethyl acetate phase, expressed as percent of initial sample. The phenolic content or purity in the extracts was expressed as the weight percent of phenolics in the ethyl acetate extracts.

### 2.3. Analytical methods

Quantitative acid hydrolysis with 72% sulfuric acid according to standard methods (Browning, 1967) was carried out, followed by HPLC analysis of the hydrolyzates to determine polysaccharide composition. Klason lignin was gravimetrically measured as the insoluble fraction after digestion with 72% sulfuric acid. The total phenolic content was determined by Folin–Ciocalteu reagent (Singleton & Rossi, 1965), and expressed as gallic acid and catechin equivalents.

### 2.4. Antioxidant activity

#### 2.4.1. α,α-Diphenyl-β-picrylhydrazyl (DPPH) radical-scavenging activity

Two millilitres of a $3.6 \times 10^{-5}$ M methanolic solution of DPPH (Fluka), were added to 50 μl of a methanolic solution of the antioxidant. The decrease in absorbance at 515 nm was recorded for 16 min. The inhibition percentage (IP) was defined as the percent reduction in absorbance with respect to the initial value, using a selected concentra-

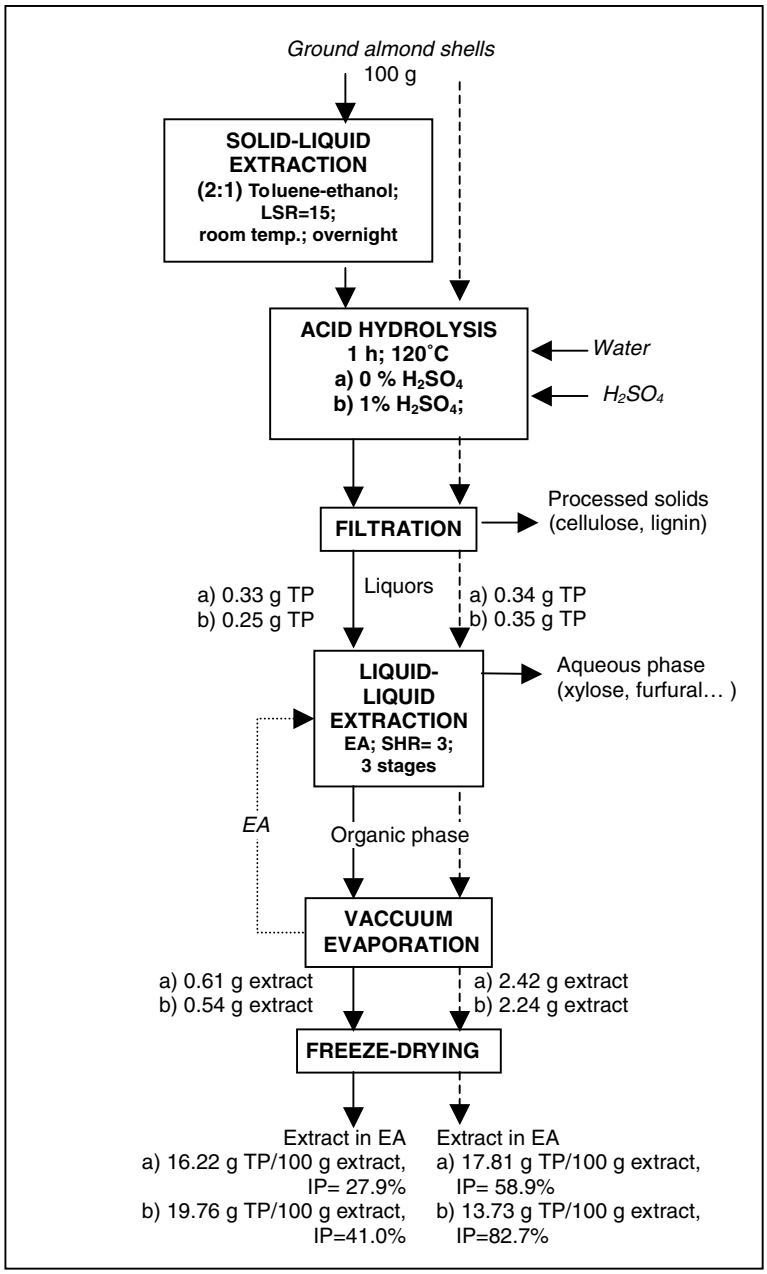


Fig. 1. Flow diagram of the acid hydrolysis (with and without added acid) of almond shells comparing the effect of a previous extraction with (2:1) toluene:ethanol on the phenolics recovery yield, phenolic content in the ethyl acetate extracts and IP of DPPH.

tion of extract. $EC_{50}$ was calculated as the amount of extracts (redissolved in methanol) causing a 50% inhibition of the DPPH radical.

### 2.4.2. Antioxidant activity in fish oil and in fish oil-in-water emulsions

Samples of fish oils (5 g) were introduced into 50-ml Erlenmeyer flasks and oxidized with shaking at 40 °C. For each antioxidant and control, triplicate samples were prepared and subjected to oxidation induced by temperature. Oxidative stability was determined by measuring, in duplicate, conjugated dienes and triene hydroperoxides. The total experiment was repeated at two completely separated times. Oil-in-water emulsions containing 1% lecithin and 10% fish oil were prepared in water as previously described (Huang, Frankel, Schwarz, Aeschbach, & German, 1996a; Huang et al., 1996b). Samples of fish oil-in-water emulsions were oxidized with shaking at 30 °C. For each antioxidant and control, triplicate samples were prepared and subjected to oxidation induced by temperature. Oxidative stability was evaluated by measuring, in duplicate, conjugated dienes and triene hydroperoxides and

fluorescence compounds. The total experiment was repeated at two complete and separated times.

Inhibition of oxidation was calculated during the propagation period of controls. The induction period was calculated as the time (in days) required for a sudden change in the rate of oxidation by the method of tangents to the two parts of the kinetic curve (Alaiz, Hidalgo, & Zamora, 1997).

### 2.5. Measurement of conjugated diene hydroperoxides

One hundred milligrams of fish oil samples and emulsion samples were dissolved in hexane and in ethanol, respectively, and absorbance was measured at 234 and 268 nm (UV–Vis Spectrophotometer Perkin–Elmer,) and calculated as mmol hydroperoxydes/kg of oil as described previously (Huang et al., 1996a). Duplicate analyses were performed for each sample.

### 2.6. Measurement of fluorescence compounds

One hundred milligrams of emulsion samples were dissolved in ethanol. Fluorescence was measured at 345/416 nm and 393/463 nm (Perkin–Elmer LS 3B) and was standardized with a quinine sulphate solution (1 μg/ml in 0.05 M $H_2SO_4$) at the corresponding wavelengths (Nielsen, Finto, & Hurrell, 1985). Duplicate analyses were performed for each sample.

### 2.7. HPLC determination of phenols

Ethyl acetate extracts (obtained from 25 ml hydrolysates) were dissolved in methanol (10 ml) and analysed by HPLC in a Hewlett–Packard 1050 instrument operating at conditions reported by Cruz et al. (2004).

### 2.8. GC-MS analysis

Samples were derivatized as reported by Quesada, Rubio, and Gómez (1997) and analyzed in a Hewlett–Packard 5989 chromatograph fitted with a Hewlett–Packard 5972 mass spectrometer, as described by (Cruz et al., 2004).

### 2.9. Statistical analysis

The data were compared by one-way analysis of variance (ANOVA) (Sokal & Rohlf, 1981), and the means were compared by a least squares difference method (Statsoft, 1994).

## 3. Results and discussion

### 3.1. Effect of the operational conditions on the production of antioxidants

Fig. 1 shows the flow diagram of the process with the mass balance of solids and total phenolic content in the ethyl acetate extracts, expressed as gallic acid equivalents. The influence of previous removal of toluene:ethanol solubles on the yield and antioxidant capacity of the extracts produced by solvent extraction of acid hydrolyzates was examined. For this purpose, acid hydrolysis was performed under operational conditions corresponding to intermediate severity in the experimental equipment used (Cruz et al., 2004; González et al., 2004). Previous extraction with (2:1) toluene:ethanol did not influence the total phenolics production yield during autohydrolysis (0% added acid) and only slightly during acid hydrolysis (1% $H_2SO_4$). The recovery yield of total ethyl acetate-soluble solids (g extract/100 g shells, determined in the stream after vacuum evaporation) from non-extracted shells was four times higher than that from the toluene:ethanol extracted ones, regardless of they were processed by acid hydrolysis or by autohydrolysis. Ethylacetate and diethylether were used as solvents in the liquid–liquid extraction, the former being selected for further experiments due to the higher recovery yields. Phenolic content in the ethyl acetate extracts and antioxidant activity, determined after freeze-drying and expressed as the IP of the DPPH radical, are influenced by the processing technology. The highest phenolic content (19.8 g phenolics/100 g extract) was recorded in the ethyl acetate solubles from extracted shells processed by acid hydrolysis, followed by the ethyl acetate solubles from autohydrolysis liquors of non-extracted (17.8 g phenolics/100 g extract) and extracted (16.2 g phenolics/100 g extract) shells, and by the ethyl acetate solubles from acid hydrolysis of non-extracted shells (13.7 g phenolics/100 g extract). When assayed at 1 g/l, the ethyl acetate solubles produced by acid hydrolysis of non-extracted shells showed an IP value of 82.7%. Those obtained by acid hydrolysis were less effective (IP = 58.9%), both being superior to those of hydrolyzates from toluene:ethanol extracted shells. In further experiments, almond shells were directly processed by acid hydrolysis and the hydrolyzates were extracted with ethyl acetate.

The effects of hydrolysis time of non-extracted shells with 1% sulfuric acid at 120 °C on the production yield, determined in the hydrolysate, phenolic recovery in ethyl acetate, and phenolic content in the ethyl acetate extract were assessed (Fig. 2a). A steady increase over 6 h (and a decrease from that time onwards) was observed in the production and recovery yields. The phenolic content in the extract increased with increasing hydrolysis time up to 6 h. The reduction in the production and recovery yields did not cause a concomitant reduction in phenolic content in the extract, suggesting that the thermal degradation of both phenolic and non-phenolic compounds occurred simultaneously with increasing severity. Fig. 2b shows $EC_{50}$ values, referred both to the ethyl acetate solubles and to the phenolics in the extracts. The $EC_{50}$ values of the extracts were minimal (1.17–1.28 g total EA solubles/l) for extracts produced in 2–8 h.

The influence of the acid concentration during 6 h of hydrolysis on the total phenolics production, recovery

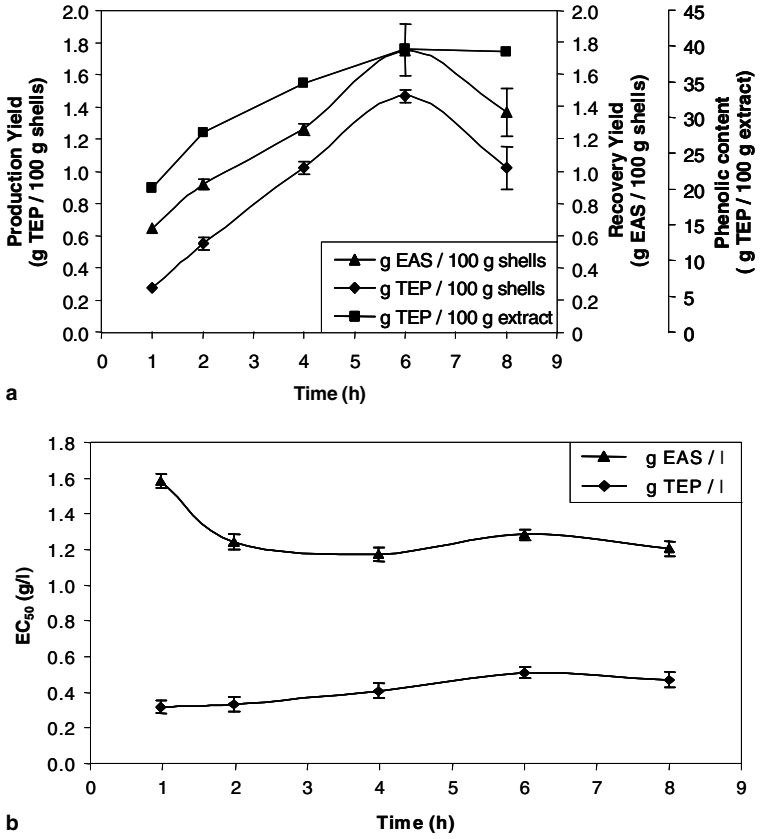


Fig. 2. Effect of the hydrolysis time of almond shells with 1% sulphuric acid at 120 °C on (a) the production yield (aqueous phase) (♦), recovery yield in ethyl acetate (▲) and phenolic content in the extract (■); (b) $EC_{50}$ values measured as the concentration of total solubles in the ethyl acetate (EAS) (▲), and as phenolics (TEP) in the extracts (♦) required to inhibit by 50% DPPH radical.

and content in the ethyl acetate extract is shown in Fig. 3a. Increased phenolics yield in the aqueous phase with increasing acid concentration, up to 2%, was observed. The increased yield when the acid concentration was increased in the range 1–2% did not result in a significantly enhanced recovery in ethyl acetate. Further increase of the acid concentration in the range studied did not improve production yields but doubled the phenolic recovery in ethyl acetate. Providing that no mass transfer limitations occurred during liquid–liquid extraction, the lower recovery from hydrolyzates produced with less than 2% acid could be due to the nature of the compounds released. Breakage into smaller compounds, occurring at higher acid concentrations, could be in concordance with the increased recovery yields under these conditions. The production of monomeric compounds is favoured by increasing severity, but beyond a certain level, decomposition of these monomers to volatile compounds could occur, either in the presence of externally added acid or under autohydrolysis conditions (Heitz, Wu, Lapointe, & Rubio, 1995; Lora & Wayman, 1978). The concentration of phenolic acids and the corresponding aldehydes, formed by the acid-catalyzed degradation of the β-aryl-ether linkages of lignin and their oxidative degradation, increase as the operational conditions become more severe. However, at higher severity, condensation and repolymerization reactions lead to formation of pseudo-lignin from hemicellulose degradation products and low-molecular-weight lignin fractions, as reported by Martínez et al. (1995) during acid hydrolysis of almond shells harvested in the same area as those used in the present study.

The extraction and recovery yield of total phenolics attained in the proposed process are in the range of those reported for conventional aqueous or organic solvent extraction from different vegetal materials and agricultural residues. The advantage of the present process lies on the integral utilization of the lignocellulosic residue in a fractionation technology useful for yielding: (i) almost intact cellulose; (ii) hemicellulose monomers and their decomposition products; (iii) a phenolic fraction with antioxidant activity. This scheme is valid for other lignocellulosics with similar composition, once the operational conditions are optimized.

The most active extracts (Fig. 3b) were produced with 2.5% sulfuric acid, under conditions leading to maximal phenolics concentration in the hydrolyzates and in the extracts, and intermediate solids recovery in ethyl acetate. Increased severity caused by higher acid concentrations led to higher recovery of ethyl acetate solubles in the extract, which were of non-phenolic nature (lower phenolic content) and/or less active (higher $EC_{50}$), requiring more grams of ethyl acetate solubles to achieve the same radical-scavenging capacity. The extracts produced

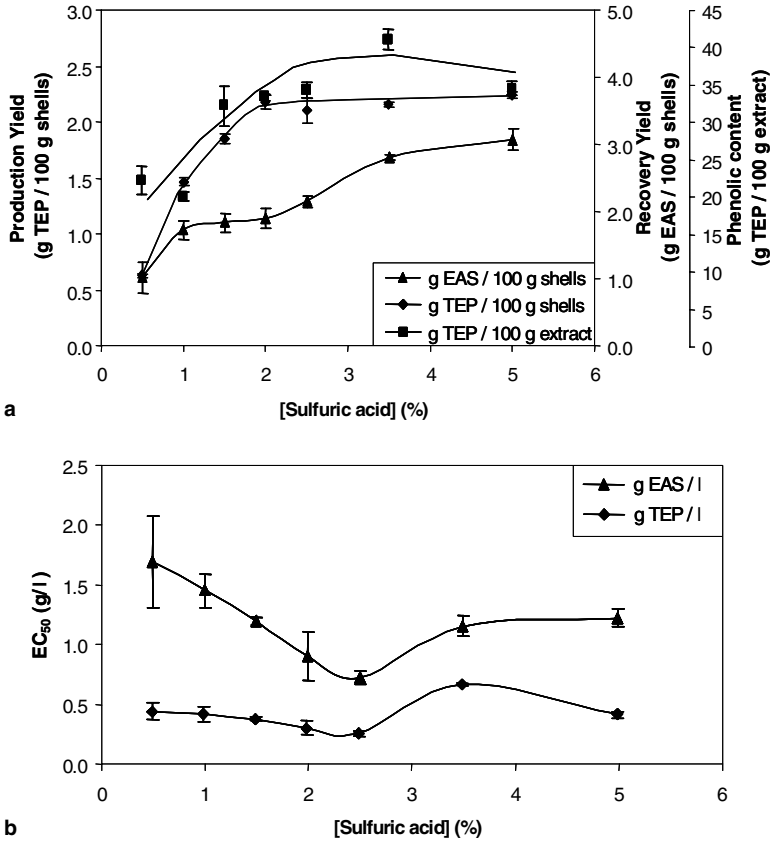


Fig. 3. Effect of the sulphuric acid concentration during hydrolysis of almond shells during 6 h at 120 °C on (a) the production yield (♦) recovery yield in ethyl acetate (▲) and phenolic content in the extract (■), (b) $EC_{50}$ values measured as the concentration of total solubles in the ethyl acetate extract (▲), and as phenolics (TEP) in the extracts (♦) required to inhibit (by 50%) DPPH radical.

showed antiradical activity comparable to that of BHA ($EC_{50,\,BHA} = 0.24$ g/l) and were higher than the value of BHT ($EC_{50,\,BHT} = 2.79$ g/l). The activity was similar to that of ethyl acetate-soluble extracts from hydrolysates of red grape pomace, corn cob or eucalyptus wood (0.18–0.47 g/l) (Cruz et al., 2004; Garrote et al., 2003; González et al., 2004), although lower than that from purified fractions from eucalyptus wood (0.15 g/l) (Cruz, Domínguez, & Parajó, 2005) and red grape pomace (0.07 g/l) (Cruz et al., 2004) hydrolysates.

Since lignin–lignin and lignin–carbohydrate bonds are very sensitive to temperature, greater lignin depolymerization is expected with increasing temperature. The effect of increasing the hydrolysis temperature to 130 °C on the phenolics production and recovery yields is shown in Table 1 for different hydrolysis times and two acid concentrations. Acid concentration exerted more influence on both production and recovery yields than did hydrolysis time. In spite of the higher recovery yield and content in the extracts produced at 130 °C, the extracts from hydrolysates produced at 120 °C showed higher radical-scavenging capacities. The extracts from ethyl acetate-soluble compounds present in hydrolysates produced with 2.5% (EASAS-2.5) and with 5% (EASAS-5) sulfuric acid at 120 °C were further selected and evaluated for their potential in protecting lipids from oxidation.

### 3.2. GC–MS and HPLC analyses

The ethyl acetate extracts from almond shell hydrolysates were composed of phenolic acids, aliphatic acids, aliphatic esters and other minor compounds. The aliphatic fatty acids were butanedioic, octadecanoic and *trans*-9 octadecenoic. They are usually formed during removal of hemicelluloses caused by mild-acid based processing of lignocellulosics (Klinke, Schmidt, & Thomsen, 1998). The phenolic fraction of the extract EASAS-2.5 was composed mainly of vanillic, syringic and *p*-coumaric acids in higher proportions than that corresponding to the extract EASAS-5, which showed other hydroxycinnamic derivatives, not identified. Vanillin was also found in both extracts. These compounds are also present in hydrolysates from mild acid hydrolysis of lignocellulosics (Garrote, Cruz, Moure, Domínguez, & Parajó, 2004).

### 3.3. Assessment of antioxidant activity of extracts on fish oils and fish oil-in-water emulsions

The antioxidant activity of the crude almond shell extracts, EASAS-2.5 and with EASAS-5, was assessed in bulk fish oil and in oil-and-water emulsions. Propyl gallate was used as a reference of synthetic antioxidants in each system. The activity was compared with that of an ethyl

Table 1
Effect of the operational conditions during acid hydrolysis of almond shells on the phenolics production yield and recovery in the ethyl acetate extracts, on the phenolic content in the extracts and on the DPPH radical-scavenging capacity

| Operational conditions | Phenolics production yield (g TEP/100 g shells) | Phenolic recovery yield (g EAS/100 g shells) | Phenolic content (g TEP/100 g extract) | EAS extraction yield (%) | $EC_{50}$ (g EAS/l) | $EC_{50}$ (g TEP/l) |
|---|---|---|---|---|---|---|
| 110 °C, 2.5%, 6 h | 1.28 ± 0.2 | 1.74 ± 0.08 | 55.0 ± 4.6 | 69.9 ± 0.7 | 1.41 | 0.85 |
| 120 °C, 2.5%, 6 h | 2.10 ± 0.11 | 2.10 ± 0.08 | 34.3 ± 1.0 | 66.2 ± 1.2 | 0.71 | 0.23 |
| 120 °C, 5%, 6 h | 2.24 ± 0.34 | 2.93 ± 0.98 | 34.6 ± 1.2 | 64.2 ± 3.3 | 1.18 | 0.47 |
| 130 °C, 2.5%, 0.5 h | 1.97 ± 0.18 | 2.68 ± 0.002 | n.d. | 87.9 ± 4.3 | n.d. | n.d. |
| 130 °C, 2.5%, 1 h | 2.10 ± 0.15 | 2.36 ± 0.03 | n.d. | 75.3 ± 1.0 | n.d. | n.d. |
| 130 °C, 2.5%, 5 h | 2.27 ± 0.09 | 2.84 ± 0.18 | 98.7 ± 9.5 | 66.4 ± 5.3 | 1.10 | 1.07 |
| 130 °C, 2.5%, 6 h | 2.36 ± 0.04 | 2.68 ± 0.12 | 43.2 ± 1.4 | 63.9 ± 1.2 | 1.72 | 0.75 |
| 130 °C, 5%, 0.5 h | 3.26 ± 0.04 | 4.29 ± 0.15 | n.d. | 82.7 ± 0.6 | n.d. | n.d. |
| 130 °C, 5%, 1 h | 2.36 ± 0.11 | 4.69 ± 0.09 | n.d. | 78.7 ± 3.4 | n.d. | n.d. |
| 130 °C, 5%, 6 h | – | 5.48 ± 0.11 | 9.78 ± 0.14 | n.d. | – | – |

n.d., not determined.

acetate extract from acid hydrolysates of *Eucalyptus globulus* wood (EASEW). This last extract contained vanillin, syringaldehyde, vanillic acid, protocatechuic acid, siryngic acid, *p*-coumaric acid and resorcylic acid as the main phenolics (16), and showed significant antioxidant activity in vitro ($EC_{50}$ = 0.39–0.55 g/l) (González et al., 2004).

Both, almond and eucalyptus extracts supplemented at concentrations of 100 ppm, inhibited oxidation in lipid systems (Fig. 4a and b). The rate and the amount of oxidation products were significantly lower in supplemented samples than in controls. In fish oils, oxidation of controls occurred by the fourth day and, in samples with antioxidants oxidation, arose by the third day. In emulsions, oxidation of controls was evident by the second day. However, antioxidants preserved emulsions from oxidation during the whole experiment. In fish oils, almond and eucalyptus extracts were similarly effective for inhibiting the formation of hydroperoxides and showed less inhibition than did propyl gallate (Table 2). Conjugated diene value measures primary breakdown products and the formation of peroxides in the early stages of oxidation (Frankel, 1998). The absorption at 268 nm measures conjugated trienes arising as secondary oxidation products and fluorescent compounds measure tertiary oxidation products (Frankel, 1998). Therefore, propyl gallate was a more effective antioxidant by avoiding the formation of volatiles related to flavour deterioration in oils.

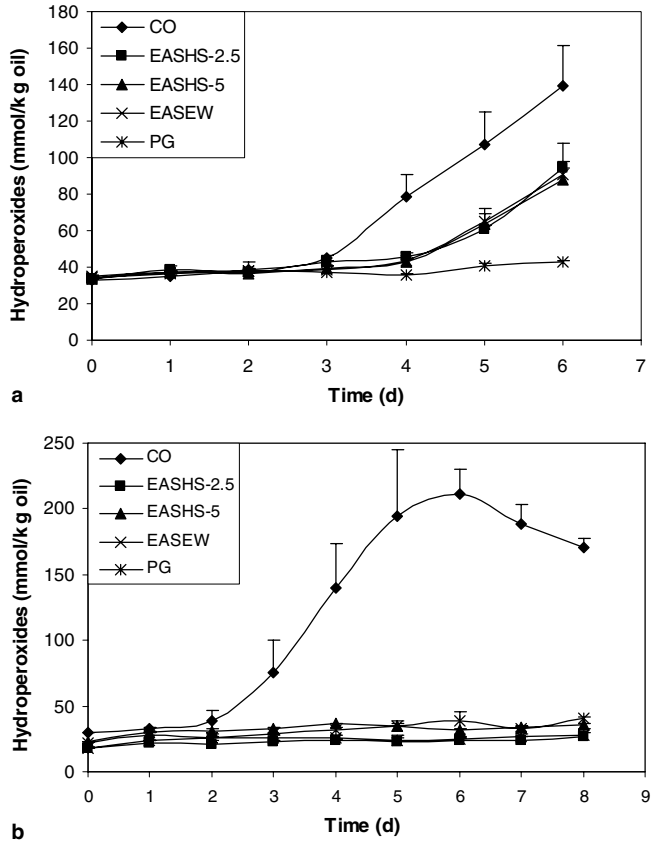


Fig. 4. Formation of hydroperoxides (a) in bulk fish oil during oxidation at 40 °C (b) in fish oil-in-water emulsion during oxidation at 30 °C.

In fish oil-in-water emulsions, the order of antioxidant efficiency was EASAS-2.5 = EASEW > EASAS-5 = propyl gallate (Table 3). The minor effectiveness of EASAS-5 is probably related to its similar proportion of phenolic acids, such as vanillic, syringic and *p*-coumaric acids. Almond and eucalyptus extracts were much more active in emulsions than in oils. This observation agrees with the order of effectiveness of hydrophobic compounds predicted by Frankel (1998). The phenolic compounds initially present in hydrolysates are water-soluble, but ethyl acetate selectively extracts the less polar ones, resulting in a concentration of hydrophobic compounds in the extract

Table 2
Inhibition by 0.01% phenolics of the formation of hydroperoxides in fish oils at 40 °C (mean ± s.d.)[a,b]

| Antioxidant | Inhibition percentage (%) in the formation of | |
|---|---|---|
| | Conjugated dienes (day 4) | Conjugated trienes (day 4) |
| EASAS-2.5 | 42.2 ± 2.5a | 20.3 ± 8.1a |
| EASAS-5 | 46.0 ± 2.0a | 25.2 ± 5.1a |
| EASEW | 40.7 ± 6.5a | 29.0 ± 6.2a |
| BHT | 55.0 ± 1.0b | 433 ± 6.1b |

[a] % Inhibition = $[(C - S)/C] \times 100$, where $C$ = oxidation product formed in control and $S$ = oxidation product formed in sample.
[b] Values in each column with the same superscript were not significantly different ($p < 0.01$).

Table 3
Inhibition by 0.01% *almonds shells*, of the formation of hydroperoxides and fluorescent compounds in fish oils-in water emulsions at 30 °C (mean ± s.d.)[a,b]

| Antioxidant | Conjugated dienes (day 3) | Conjugated dienes (day 4) | Fluorescence (day 5) |
|---|---|---|---|
| EASAS-2.5 | 69.8 ± 2.4c | 74.4 ± 0.5c | 77.1 ± 2.4b |
| EASAS-5 | 56.1 ± 1.4a | 63.8 ± 0.2a | 74.9 ± 1.7a |
| EASEW | 65.4 ± 2.1bc | 72.9 ± 1.3c | 81.7 ± 2.1b |
| Propyl gallate | 62.1 ± 0.5b | 68.1 ± 2.7b | 75.1 ± 1.4a |

[a] % Inhibition = $[(C - S)/C] \times 100$, where $C$ = oxidation product formed in control and $S$ = oxidation product formed in sample.
[b] Values in each column with the same superscript were not significantly different ($p < 0.01$).

produced. Apolar compounds, tend to locate at the oil–water interfaces, thus protecting more efficiently against oxidation in emulsions than in bulk oils.

Both, almond and eucalyptus extracts contain compounds active against oxidation in bulk oils and oil-in water emulsions. Vanillic, syringic and *p*-coumaric acids significantly preserved linoleic acid and vegetable oils from oxidation (Bratt et al., 2003; Marinova & Yanishlieva, 1996; Papadopoulus & Boskou, 1991). Oxidation of LDL, rat liver liposomes, rat liver microsomes and emulsions could also be protected by vanillic acid (Natella, Nardini, Di Felice, & Scaccini, 1999; Osawa, Ide, Su, & Namiki, 1987), syringic acid (Andreasen, Landbo, Christensen, Hansen, & Meyer, 2001; Natella et al., 1999; Osawa et al., 1987) and *p*-coumaric acid (Natella et al., 1999; Stupans, Kirlich, Tuck, & Hayball, 2002). Vanillin protected efficiently against oxidation of lipids in extruded corn (Camire & Dougherty, 1998) and in cod liver oil (Fujioka & Shibamoto, 2005). Some natural extracts from rosemary leaves, olive oil and grape pomace were also active in fish oil systems and their activity in bulk fish oils and fish oil-in-water emulsions is reported to be related to their polarity (Medina et al., 2003; Pazos, Gallardo, Torres, & Medina, 2004).

## 4. Conclusions

The ethyl acetate solubles, arising from the extractives fraction and from the lignin degradation, caused during acid hydrolysis of almond shells, were characterized for antioxidant activity. The processing scheme suggests the utilization of the less polar fraction present in the hydrolysates generated during acid treatment of almond shells. These liquors contain hemicellulosic sugars, sugar-dehydration products, organic acids, extractives and phenolic compounds. Their removal from hydrolysates is desirable when sugar solutions are intended as carbon source for bioconversions, due to their antimicrobial activity. This general scheme is valid for different lignocellulosic wastes, with the aim of fractionating the cellulose, the hemicellulosic sugars and/or their degradation products and compounds derived from extractives and the lignin fraction, present at lower concentrations and showing antioxidant properties. Up to 4% of the initial raw material could be recovered in the ethyl acetate extract, containing 40% of total phenols solubles, showing radical-scavenging capacity, and could be used for protection against fish oil oxidation both in bulk oil and in emulsions.

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