EFFECTS OF DIFFERENT PHENOLS EXTRACTION CONDITIONS ON ANTIOXIDANT ACTIVITY OF ALMOND (*PRUNUS DULCIS*) FRUITS

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ABSTRACT

*In order to determine the optimum operational conditions for phenols extraction, a series of assays were performed in the Guara variety of almond (*Prunus dulcis*). Four variable operational conditions factors were considered: solvent type, solvent volume, temperature and extraction time. Phenols extracts antioxidant properties were evaluated, either chemically, by screening the free radical scavenging activity, or biochemically, by measuring the inhibition of thiobarbituric acid reactive substances formation in brain cells used as model for the study of lipid peroxidation damage in biomembranes. The extraction of the totality of phenols, confirmed by their antioxidant properties, allows the calculation of ideal doses for almond intake concerning antioxidant effects. The best outcome, regarding these proposals was obtained in the following conditions: 50 mL of methanol with 60 min of extraction time at 25C.*

PRACTICAL APPLICATIONS

The quantification of total phenols in the extracts allows the definition of the most adequate doses of almond to be included in healthy diets in order to obtain the best antioxidant effects. This is quite important as it is not yet well defined if the over-intake of these compounds can cause deleterious effects.

The correlations obtained between phenols and antioxidant activity allows an estimation of extract concentration providing 50% inhibition values instead of their experimental determination. In future works with the same or other varieties we can achieve antioxidant activity parameters through phenols determination. From an industrial point of view, the obtained results offer a possibility to valorise almond as a better food product obtained from an economical natural resource. Furthermore, almond tree could be cultivated more intensively, namely in areas with little agricultural potential, as it represents a culture with modest needs in terms of practical management.

INTRODUCTION

Almond, *Prunus dulcis* (Miller) D.A. Webb, apparently originated from one or more wild species that evolved in the deserts and lower mountain slopes of central and southwestern Asia (Micke and Kester 1998). Almond tree is an important crop and it produces fruits of high commercial value currently used worldwide in bakery and confectionery (Cordeiro and Monteiro 2001). It is especially spread through and well adapted to the whole Mediterranean region from which about 28% of the world production is obtained. In Portugal, almond is a traditional crop, mainly spread through Algarve and Baixo Alentejo in the south, and "Terra Quente" of Trás-os-Montes in the north (Cordeiro and Monteiro 2001; Martins *et al.* 2003).

Prunus species are reported to have interesting biological properties such as sedative, anti-inflammatory, anti-hyperlipidemic, antitumoral and antioxidant activities (Donovan *et al.* 1998; Wang *et al.* 1999; Sang *et al.* 2002). The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary disease and cancer is raising interest among scientists, food manufacturers and consumers since the future trend is toward functional foods with specific health effects (Löliger 1991). Some synthetic antioxidants (2-*tert*-butyl-4-methoxyphenol) are used nowadays in the food industry with serious concerns about their health effects. Therefore, there is great interest in replacing them by natural antioxidants. The most common plant phenolic antioxidants include flavonoid compounds, cinnamic acid derivatives, coumarins, tocopherols and polyfunctional organic acids (Shahidi and Wanasundara 1992). Even though it is unclear whether active compounds are active against free radicals after being absorbed and metabolized by cells in the body, radical-scavenging assays have gained acceptance for their capacity to rapidly screen materials of interest (Ferreira *et al.* 2007). The antioxidant activity of phenolic compounds is mainly because of their redox properties, which allow them to act as reducing agents or

hydrogen-atom donors. Thus, natural antioxidants may work as free-radical scavengers and chain breakers, complexers of pro-oxidant metal ions and quenchers of singlet oxygen present in the environment (Amarowicz *et al.* 2004).

Several studies were performed on almond phenolics determination (Donovan *et al.* 1998; Sang *et al.* 2002; Takeoka and Dao 2003; Moure *et al.* 2007). Yet, they were only carried out on by-products of almond such as shells, skins and green bark, and curiously, not on the fruit itself, which is precisely the edible part. Also, none of those studies reported optimization procedures for phenols extraction. Extraction efficiency is commonly a function of process conditions. Previous findings have reported the influence of some variables (e.g., temperature, time contact, solvent-to-solid ratio, etc.) on the phenolic yields extracted from different natural products such as almond hulls, pine sawdust or apple by-products. The role of each factor in the mass transfer of the process is not always obvious; the chemical characteristics of the solvent and the diverse structure and composition of the natural products ensure that each material-solvent system shows different behavior (Pinelo *et al.* 2005).

The aim of the present work is the establishment of the best phenols extraction conditions for almond in order to obtain the higher antioxidant activity, measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity and thiobarbituric acid reactive substances (TBARS) test. It is necessary to ensure that the totality of phenols (well-known antioxidants) is extracted in order to advice consumers regarding the ideal doses for almond intake. For the optimization of the extraction procedure, we chose mainly solvents with high polarity (water [W] and methanol [M]), taking into account that the high lipid fraction of the almond seed, which ranges from 50 to 60% (Sánchez-Bel *et al.* 2007), interfere significantly in the results when nonpolar solvents were tested.

MATERIALS AND METHODS

Samples

Almond fruits, Guara variety, were collected in August 2006 in an orchard located in Trás-os-Montes, Northeast Portugal. The trees were twelve years old, were not irrigated and no phytosanitary treatments were applied. The fruits were dried at room temperature and exposed to sun, as common practice in the region. The samples (~300 g; whole fruit, after removing the outer shell, but keeping the inner skin) were kept at –20C and protected from light until use.

Standards and Reagents

Gallic acid was purchase from Sigma (St. Louis, MO). DPPH was obtained from Alfa Aesar (Ward Hill, MA). All other chemicals were obtained from Sigma Chemical Co.. M was obtained from Pronalab (Lisboa, Portugal). W was treated in a Milli-Q water purification system (TGI Pure Water Systems, Brea, CA).

Phenols Extraction

For phenols extraction optimization, a fine powder (20 mesh) of sample (2 g) was extracted using different conditions (solvent, temperature, solvent-to-solid ratio, extraction time). The first stage was the selection of the most appropriate solvent among hexane (H), diethyl ether (E), ethyl acetate (A), chloroform (C), M, W and a hydromethanolic mixture (1:1, M/W), fixing temperature at 25C (T1), as well as the solvent volume (50 mL) and the extraction time (30 min) (Fig. 1a). The extraction was performed using a 250 mL beaker and heating magnetic stirrer (VELP Scientific, Europe). In the second stage we carried out new assays with the better solvents but using different temperatures T1 (25C), T2 (50C) and bT (64.7C for M and ~100C for W), solvent volumes (50 and 100 mL) and times (30 min and 60 min) (Fig. 1b). The extractions at 50C and boiling temperature were performed using a refluxing system (250 mL round-bottom flask, heating mantle and condensator).

Extracts obtained with W were filtered through Whatman n° 4 paper (Dassel, Germany) under reduced pressure, frozen and then lyophilized (Ly-8-FM-ULE, Snijders, Tilburg, Holland). Extracts obtained with the other solvents were evaporated under reducing pressure to dryness. HM was obtained extracting the sample in H (50 mL) at 25C for 30 min, and re-extracting the residue in M (50 mL) at 25C for 30 min. All the extracts were re-dissolved in the corresponding solvent at a concentration of 10 mg/mL and analyzed for their content in phenols, DPPH radical scavenging activity (RSA) and formation of TBARS.

Determination of Phenols Content

Phenolic concentration in the extracts was estimated by a colorimetric assay based on procedures described by Singleton and Rossi (Singleton and Rossi 1965) with some modifications. Basically, 1 mL of extract solution was mixed with 1 mL of Folin and Ciocalteu's phenol reagent. After 3 min, 1 mL of saturated sodium carbonate solution was added to the mixture and adjusted to 10 mL with distilled W. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm (Analytik Jena 200-2004 spectrophotometer, Jena, Germany). Gallic acid was used for constructing the

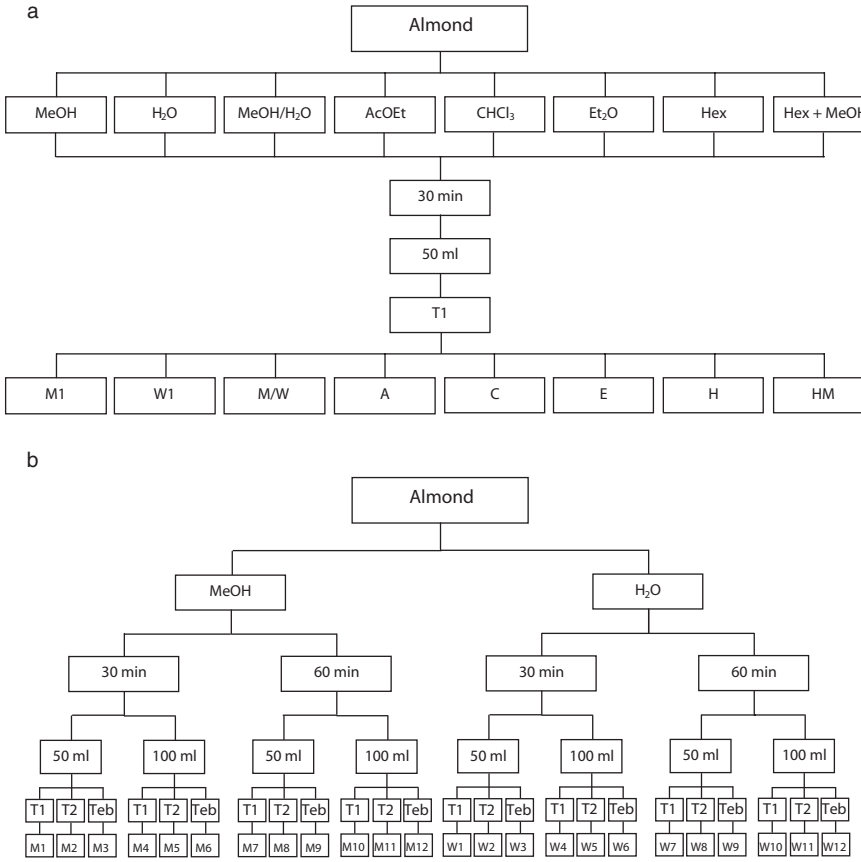

FIG. 1. EXPERIMENTAL DESIGN FOR OPERATIONAL CONDITIONS USED IN ALMOND PHENOLS EXTRACTION

(a) Solvent selection; (b) temperature, extraction time and solvent-to-solid ratio selection. T1: 25C; T2: 50C; bT: solvent boiling temperature.

standard curve (0.01–0.4 mM; $y = 2.94848x - 0.09211$; $R^2 = 0.99914$, for W; $y = 2.08926x - 0.10207$, $R^2 = 0.99842$, for M) and the results were expressed as milligram of gallic acid equivalents/gram of extract (GAEs).

RSA

The capacity to scavenge the "stable" free radical DPPH was monitored according to the method of Hatano (Hatano *et al.* 1988). Several concentrations of extracts solutions (0.3 mL) were mixed with 2.7 mL of methanolic

solution containing DPPH radicals (6×10^{-5} mol/L). The mixture was shaken vigorously and left to stand for 60 min in the dark (until stable absorbance values were obtained). The reduction of the DPPH-radical was determined by continuously monitoring the decrease of absorption at 517 nm. DPPH scavenging effect was calculated as a percentage of DPPH discoloration using the equation: % scavenging effect = $([A_{DPPH} - A_S]/A_{DPPH}) \times 100$, where A_S is the absorbance of the solution when the extract solution has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution. The extract concentration providing 50% inhibition (EC_{50}) was calculated from the graph of scavenging effect percentage against extract concentration. BHA and α-tocopherol were used as reference compounds.

Inhibition of Lipid Peroxidation (LPO) Using TBARS

Brains were obtained from pig (*Sus scrofa*) of body weight ~150 Kg, dissected and homogenized with a PT-2100 Polytron (Kinematica AG, Lucerne, Switzerland) in ice-cold Tris–HCl buffer (20 mM, pH 7.4) to produce a 1:2 (w/v) brain tissue homogenate that was centrifuged at 3,000× g for 10 min. An aliquot (0.1 mL) of the supernatant was incubated with the almond extracts (0.2 mL) in the presence of $FeSO_4$ (10 μM; 0.1 mL) and ascorbic acid (0.1 mM; 0.1 mL) at 37C for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28% w/v, 0.5 mL), followed by thiobarbituric acid (TBA; 2%, w/v, 0.38 mL), and the mixture was then heated at 80C for 20 min. After centrifugation at 3,000× g for 10 min to remove the precipitated protein, the color intensity of the malondialdehyde-TBA complex in the supernatant was measured by its absorbance at 532 nm. The inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%) = $[(A - B)/A] \times 100\%$, where A and B were the absorbance of the control and the compound solution, respectively. The extract concentration providing 50% LPO inhibition (EC_{50}) was calculated from the graph of antioxidant activity percentage against extract concentration. BHA was used as reference compound.

Statistical Analysis

For each one of the assayed conditions, three portions of the sample were simultaneously extracted, and the obtained extracts were analyzed in triplicate. The results are expressed as mean values and standard error or standard deviation (SD). The differences between extraction conditions (solvent, temperature, volume and time) were analyzed using one-way analysis of variance followed by Tukey's honestly significant difference test with $\alpha = 0.05$. This treatment was carried out using SAS v. 9.1.3 (Cary, NC) program. The regression analysis between phenol contents and EC_{50} values for scavenging activity used the same statistical package.

RESULTS AND DISCUSSION

In order to optimize phenols extraction procedure in almonds, a well-known and widespread variety (*Guara*) of *P. dulcis* was assayed under different experimental conditions. The first step was the selection of the most appropriate solvent among solvents with different polarities: H, E, A, C, M, W and a hydromethanolic mixture (1:1). To compare the extraction efficiencies among different solvents, the temperature was fixed at T1 (25C), as well as the volume (50 mL) and the extraction time (30 min) (Fig. 1a).

As it was expected, the most polar solvents (M and W) were more efficient (Table 1). To evaluate the influence of lipids present in methanolic extracts, a previous assay was conducted (HM extract) using a sequential extraction, first with H to remove lipids and then re-submitting the residue to an extraction with M. As we did not observe significant differences between M1 (similar assay but without removing fat) and HM (Table 1), we decided to carry out the experiments without the H preliminary extraction.

As M and W prove to be the most efficient solvents, we carried out new assays with these solvents at different temperatures, T1 (25C), T2 (50C) and bT (64.7C for M and ~100C for W), different solvent volumes (50 and 100 mL) and times (30 min and 60 min) (Fig. 1b). The higher amount of phenolic compounds was obtained at 25C, with 50 mL of solvent, and for 60 min of extraction time, both for M (M7, 14.01 mg/g) and W (W7, 14.46 mg/g) (Table 1).

Temperature is the variable with greater influence. The assay conducted at boiling temperature was carried out only to conclude if the heating could influence the phenols extraction for each specific solvent. Some authors reported that phenolic compounds are unstable and easily became non-antioxidative under heating (Leffer 1993; Yen and Hung 2000), whereas the other authors (Choi *et al.* 2006) explained that heat treatment might produce changes in phenols extractability because of the disruption of the plant cell wall; thus bound polyphenolics may be released more easily relative to the assays carried out at lower temperatures. The acquired results in the present study indicate that some of the phenolics could have been destroyed at high temperatures.

The extraction yields were calculated in order to find a relation between yield values and phenols content. Nevertheless, that relation was not observed. For example, nonpolar or weakly polar solvents (A, C, E and H) allow higher extraction yields than highly polar solvents such as M or W, but the extracted mass did not correspond to phenolic compounds (Table 1). This fact is probably related to the low solubility of those compounds in nonpolar solvents, which extract mainly the lipids (Sánchez-Bel *et al.* 2007). Concerning the

TABLE 1.
EXTRACTION YIELDS (η), TOTAL PHENOLS CONTENT, SCAVENGING EFFECT (EC_{50}) AND CORRESPONDING COEFFICIENTS OF VARIATION FOR ALMOND EXTRACTS OBTAINED USING DIFFERENT CONDITIONS

Extracts	η (%)	CV (%)	Phenols (mg/g extract)	CV (%)	DPPH EC_{50} (mg/mL)	CV (%)	LPO EC_{50} (mg/mL)	CV (%)
M1	9.36 ± 0.73 hij	7.81	11.60 ± 2.70 def	23.28	2.13 ± 0.04 hij	3.84	2.02 ± 0.07 klm	3.52
M2	9.29 ± 3.21 hij	34.52	11.00 ± 1.34 efgh	12.22	2.73 ± 0.53 efg	19.44	2.56 ± 0.49 ij	19.18
M3	15.60 ± 7.33 efghi	46.98	6.00 ± 1.68 m	27.96	4.54 ± 0.30 ab	6.48	4.38 ± 0.39 bcd	8.89
M4	13.51 ± 0.61 fghij	4.48	8.53 ± 0.72 kl	8.48	2.64 ± 0.52 efgh	19.83	2.48 ± 0.50 ij	20.16
M5	16.54 ± 2.03 defgh	12.27	7.38 ± 0.71 l	9.58	4.18 ± 0.40 bc	11.29	4.11 ± 0.46 bcde	11.23
M6	10.62 ± 2.94 hij	27.65	9.37 ± 1.42 ijk	15.17	4.11 ± 0.21 bc	10.65	4.24 ± 0.46 bcde	10.73
M7	8.97 ± 0.54 hij	5.97	14.01 ± 1.13 ab	8.12	1.93 ± 0.11 j	5.55	1.80 ± 0.10 m	5.42
M8	7.45 ± 1.35 ij	18.00	11.31 ± 1.24 ef	10.96	3.05 ± 0.59 e	19.47	2.86 ± 0.55 i	19.31
M9	7.38 ± 1.21 ij	16.34	7.87 ± 0.74 l	9.40	4.32 ± 0.21 bc	4.96	4.06 ± 0.22 cdef	5.29
M10	11.80 ± 0.84 hij	7.11	12.03 ± 1.36 de	11.34	2.76 ± 0.20 ef	7.12	2.59 ± 0.19 ij	7.34
M11	15.63 ± 1.76 efghi	11.27	5.66 ± 0.65 m	11.48	4.11 ± 0.29 cd	7.03	3.87 ± 0.28 efgh	7.20
M12	7.36 ± 0.19 ij	2.62	9.98 ± 1.82 ghij	18.25	4.33 ± 0.21 bc	19.62	4.08 ± 0.80 bcde	19.66
W1	25.88 ± 3.50 c	13.53	13.27 ± 1.19 bc	8.97	2.88 ± 0.53 ef	18.41	2.71 ± 0.51 ij	18.96
W2	25.20 ± 5.63 cd	22.34	12.74 ± 1.38 cd	10.86	3.72 ± 0.37 d	9.88	3.51 ± 0.35 h	10.09
W3	8.59 ± 0.45 hij	5.22	9.85 ± 0.98 hij	9.92	3.73 ± 0.37 d	9.90	3.51 ± 0.35 h	9.96
W4	20.86 ± 1.41 cdefg	6.70	13.45 ± 0.99 abc	7.33	3.70 ± 0.63 d	17.15	3.47 ± 0.61 h	17.67
W5	21.98 ± 1.61 cdef	7.33	13.99 ± 0.93 ab	6.62	2.45 ± 0.23 fghi	9.26	2.29 ± 0.22 jkl	9.58
W6	12.74 ± 1.20 ghij	9.42	11.10 ± 0.45 efg	4.05	2.04 ± 0.28 ij	13.70	1.91 ± 0.27 lm	14.29
W7	21.19 ± 3.59 cdefg	16.93	14.46 ± 0.53 a	3.67	4.46 ± 0.37 abc	8.69	4.31 ± 0.39 bcd	9.11
W8	21.51 ± 1.08 cdefg	5.00	9.08 ± 0.22 jk	2.42	>5 a	na	4.46 ± 0.35 bc	7.86
W9	10.53 ± 0.94 hij	8.94	11.87 ± 1.15 de	9.71	3.10 ± 1.25 e	40.43	3.60 ± 0.77 gh	21.42
W10	23.77 ± 3.09 cde	12.98	11.56 ± 1.23 def	10.67	4.61 ± 0.56 ab	12.15	3.96 ± 0.47 defg	11.75
W11	29.01 ± 3.75 c	12.94	11.54 ± 0.91 ef	7.89	>5 a	na	4.52 ± 0.22 b	5.02
W12	10.67 ± 0.49 hij	4.58	12.05 ± 0.55 de	4.57	2.25 ± 0.11 ghij	4.98	2.39 ± 0.45 jk	18.70
MW	10.96 ± 0.04 hij	37.60	11.76 ± 1.07 de	9.14	3.71 ± 1.18 d	31.74	3.62 ± 0.96 fgh	26.56
A	54.28 ± 4.30 a	5.60	ND	NA	>5 a	NA	>5 a	NA
C	40.29 ± 1.80 b	4.48	ND	NA	>5 a	NA	>5 a	NA
E	38.78 ± 3.44 b	8.88	ND	NA	>5 a	NA	>5 a	NA
H	39.73 ± 4.07 b	10.25	ND	NA	>5 a	NA	>5 a	NA
HM	6.14 ± 1.23 j	20.10	10.16 ± 0.64 fghi	4.49	2.25 ± 0.02 ghij	0.91	2.39 ± 0.37 jk	15.47

In each row different letters mean significant differences ($P < 0.05$).
EC_{50}, extract concentration providing 50% inhibition; NA, not applicable; ND, not detected.

yield, the coefficients of variation (CV) values (calculated by the ratio between standard deviation and mean, presented in percentage) revealed a great heterogeneity of the results varying from 2.62, in M12, to 46.98, in M3. Nevertheless, the extraction procedures M4, M7, M12, W3, W8, W12, A and C presented high reproducibility, with CV less than 6%.

As the plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical quenchers (Miliauskas *et al.* 2004), the total phenol contents of the selected fruit extracts and assays of the corresponding antioxidant activity, namely DPPH RSA and LPO inhibition were performed. These assays were performed on the whole phenolic extracts, as that could be more beneficial than isolated constituents because of the additive and synergistic effects, and considering that a bioactive individual component can change its properties in the presence of other compounds present in the extracts. In fact, bioactive compounds are best acquired through whole-food consumption ("cocktail" of bioactive compounds), than from expensive dietary supplements or individual antioxidant compounds (Liu 2003).

The EC_{50} values for RSA and LPO inhibition obtained for each almond extract, are presented in Table 1, except for H, A, ether and C, as these extracts were considerably less effective radical scavengers, presenting percentages lower than 20% at the maximal concentration, 5 mg/mL (percentages obtained through the formula presented in the experimental section). Accordingly, with these values it was not possible to achieve 50% of inhibition and, therefore, the EC_{50} values were unavailable.

M and aqueous extracts were quite effective DPPH radical scavengers as well as LPO inhibitors. Some of the evaluated extracts for these two solvents (e.g., M7 and W6) revealed percentages of antioxidant activity higher than 90%, at 5 mg/mL. In the case of RSA assay, those percentages can be considered as full absorption inhibition of DPPH, because after completing the reaction, the final solution always possesses some yellowish color, and therefore its absorption inhibition compared with colorless M solution cannot reach 100% (Miliauskas *et al.* 2004).

Figure 2 shows the free radical scavenger capacity of almond extracts obtained as a function of their concentration. From the analysis of Fig. 2 we can observe that the extracts scavenging effects on DPPH radicals improved as the concentration increases. Methanolic extracts were generally better, with maximum values obtained for M7 (94.6% $\pm$ 2.1, at 5 mg/mL). These values were similar to the scavenging effect obtained for the standards BHA (96% at 3.6 mg/mL) and α-tocopherol (95% at 8.6 mg/mL).

Figure 3 shows the LPO inhibition of almond extracts obtained as a function of their concentration. From the analysis of Fig. 3 we can also observe that the extracts LPO inhibition capacity increased as the concentra-

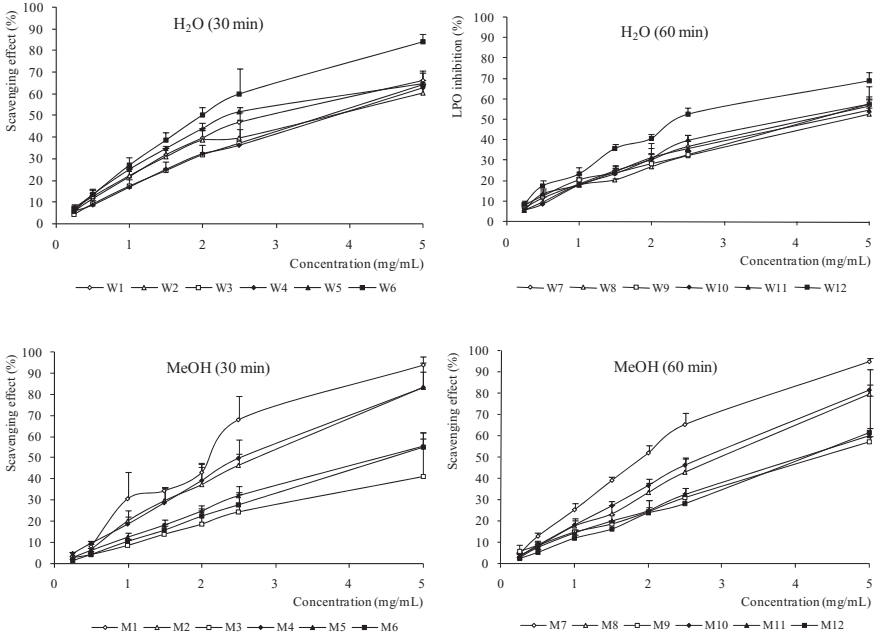

FIG. 2. RADICAL SCAVENGING ACTIVITY OF ALMOND EXTRACTS OBTAINED USING WATER AND METHANOL AS SOLVENTS UNDER DIFFERENT EXTRACTION TIMES, TEMPERATURES AND SOLVENT VOLUMES
Each value is expressed as mean ± standard error.

tion rise. Methanolic extracts were once more generally better, with maximum values obtained for the same extract (M7; 95.3% ± 3.5, at 5 mg/mL).

In summary, the lower RSA EC_{50} values (higher antioxidant activity) were obtained at 25C for M, with 50 mL of solvent, and for 60 min of extraction time (M7, 1.93 ± 0.11 mg/mL), and at boiling temperature for W, with 100 mL of solvent, and for 30 min of extraction time (W6, 2.04 ± 0.28 mg/mL). The lower LPO inhibition EC_{50} values were obtained for the same operational conditions (M7, 1.80 ± 0.10 mg/mL; W6, 1.91 ± 0.27 mg/mL) (Table 1).

Antioxidant activity results were correlated to the phenols content found in methanolic extracts (RSA: $y = -0.2995x - 6.2224$; $r^2 = 0.6992$; $F = 23.2477$; df = 11; $p < 0.001$; LPO inhibition: $y = -0.2918x - 6.0441$; $r^2 = 0.6128$; $F = 15.8301$; df = 11; $p < 0.005$). The results obtained for aqueous extracts were very poorly correlated. For example, boiling W leads to a decrease in phenols content that is not reflected in the antioxidant properties, indicating that eventually, other antioxidants besides phenols are present in the

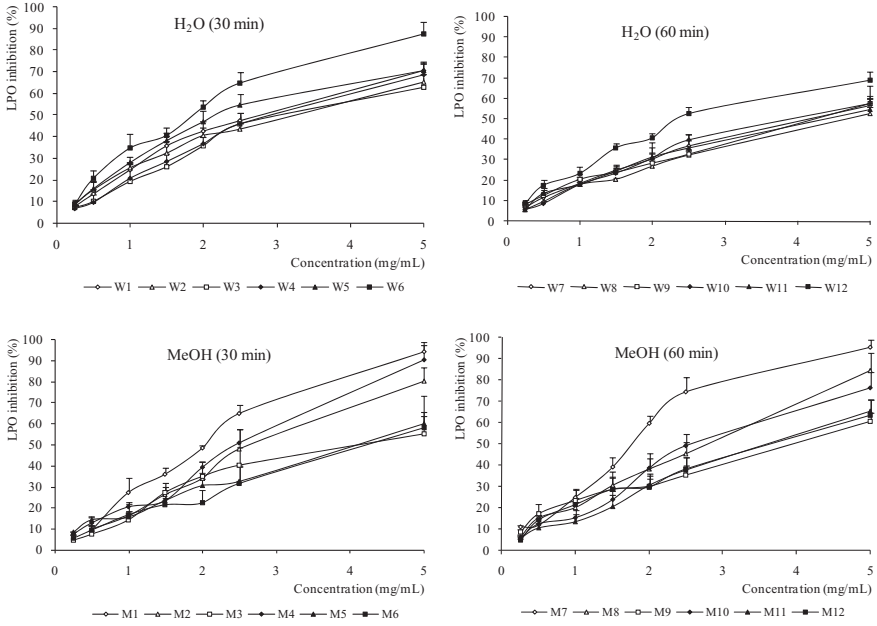

FIG. 3. LIPID PEROXIDATION (LPO) INHIBITION OF ALMOND EXTRACTS OBTAINED USING WATER AND METHANOL AS SOLVENTS UNDER DIFFERENT EXTRACTION TIMES, TEMPERATURES AND SOLVENT VOLUMES
Each value is expressed as mean ± standard error.

extracts and contributing to their antioxidant activity. This is well observed for W6 extract in which the good antioxidant activity EC_{50} values are not proportional to the phenols content.

In the case of the M almond extracts, a significantly negative linear correlation was established between the phenols content and EC_{50} values. This correlation proves that the extracts with highest phenols content show lower EC_{50} values, confirming that phenolics are likely to contribute to the RSA of these fruit extracts, as it has been reported in other species (Velioglu *et al.* 1998). Particularly, the high content of total phenols in M7 (14.01 mg/g) might account for the good results found in its antioxidant activity.

The obtained correlations are particularly useful when the total phenol content is known because it allows the estimation of EC_{50} values instead of their experimental determination. In future works with the same or other varieties we can achieve antioxidant activity parameters through phenols determination.

Previous studies on the influence of different extracting solvents on the antioxidant activity and the yield of phenolics content have already been

reported but with different matrixes (Cheung *et al.* 2003; Sun and Ho 2005). Our results are in accordance with those authors that observed a better scavenging activity in methanolic extracts.

The results obtained in the present work denote that almond may constitute a good source of healthy compounds. So the intake in the diet of almond fruits suggests that it could be useful in the prevention of diseases in which free radicals are implicated. Almond seems to be a natural provider of antioxidants, which are increasing its value as food or food component, as they offer effective protection against oxidative damage.

CONCLUSION

The optimization of operational conditions to extract the maximum phenols content in almond (whole fruit) has made it possible to determine the following conditions as adequate: 50 mL of M at 25C for 60 min. The quantification of total phenols in the extracts allows the definition of the most adequate doses of almond to be included in healthy diets in order to obtain the best antioxidant effects. This is quite important as it is not well defined if the over-intake of these compounds can cause deleterious effects.

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