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EFFECT OF PHENOLIC COMPOUNDS ON THE OXIDATIVE STABILITY OF GROUND
WALNUTS AND ALMONDS

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ABSTRACT

Walnuts and almonds are mainly constituted by unsaturated fatty acids and, in consequence, are readily prone to oxidation reactions. These nuts are also rich in antioxidant substances as polyphenols, which are mainly concentrated in the brown skins (BS) or seed coats. This study analyzes the effect of BS phenolic compounds on the oxidative stability of walnuts and almonds. For this reason, peeled and unpeeled ground nuts were comparatively evaluated after thermal treatments as well as after room temperature storage. Furthermore, volatile compound emission due to thermally induced oxidation of ground nuts was analyzed by SPME-GC-MS. Several secondary oxidation products were identified in the headspace, volatile products formation was considerably larger in nuts with BS than in peeled ones. Consistently, the first one showed higher malondialdehyde levels after heating which indicates a prooxidant effect of BS presence. In order to verify these findings, a water-in-oil micro emulsion was used as a model system to assess the role of nut phenolic compounds on lipid oxidation promotion. Hydroperoxides and malondialdehyde contents were determined, as a measurement of primary and secondary oxidation advance, respectively. A dose dependent prooxidant effect on linoleic acid thermal oxidation was also observed when BS polyphenolic extracts as well as pure phenolic compounds were added.

KEYWORDS: nuts; TBARS; volatile compounds; lipid oxidation; SPME-GC

1. INTRODUCTION

Walnuts and almonds are worldwide consumed foods and frequently included as ingredients in the diet of numerous ethnical groups. Besides, it has been demonstrated that the regular consumption of such nuts exerts beneficial effects on health.¹ The main constituent of these nuts is the oil, predominantly constituted by unsaturated fatty acids and hence, highly prone to oxidation reactions.²

Lipid oxidation is a multi-stage radical mediated process where the primary products are lipid hydroperoxides.³ Since these are relatively unstable, they can be further oxidized yielding diverse secondary products⁴ as aldehydes, acids, hydrocarbons and oxoacids.⁵ Aldehydes can undergo classic auto-oxidation process forming short-chain hydrocarbons, carboxylic acids and dialdehydes like malondialdehyde (MDA) as main products.⁶ These volatile compounds not only imparts undesirable flavors, but also decreases the nut nutritional quality and results in the formation of potentially toxic species.⁴ There are several factors which can promote lipid peroxidation. Among the endogenous agents, the enzyme lipoxygenase (LOX) is able to catalyze the oxidation of fatty acids containing a *cis,cis*-1,4-pentadiene system as specific substrates like linoleic acid (LA).⁷ LOX occurrence in walnuts and almonds has been studied and the enzyme inactivation by heating has also been described.⁸ Even when LOX is not activated, lipid oxidation may take place by an auto-oxidation process but at a slower rate. Another important external factor capable to promote the oxidation reaction of lipid-containing foods is the thermal treatment in aerobic medium, conditions very frequent during food preparation.

Walnuts and almonds are also rich in other bioactive molecules as phenolic compounds. In a previous study, we have determined that hydrophilic phenolic compounds are concentrated in the nut brown skins (BS) or pellicles that cover the kernels as seed coats, whereas less polar phenolic compounds such as tocopherols are located in the oily matrix of the kernel or nut meat.⁹ BS represents a 4% w/w of the shelled seed. Numerous phenolic compounds have been found in nut BS, flavonoids

and non-flavonoid being identified.¹⁰ Methanolic and aqueous extracts of nut BS are able to effectively scavenge free radicals and inhibit LOX-induced oxidation of LA.⁹ Other antioxidant mechanisms of flavonoids are also well known including metal chelation and antioxidant-enzyme activation.¹¹ On the contrary, the prooxidant effect of polyphenols has also been described in lipid systems in a dose-dependent manner in the presence of active metal ions.^{12,13}

Walnuts and almonds, consumed as shelled nuts, are ingredients of several processed food frequently subjected to cooking. These nuts are worldwide used in cooked food preparation as crushed, ground, or powdered nuts. Their oxidative stability has previously been studied in whole nuts exposed to different treatments.^{14,15} However, as far as we know, the effect of BS phenolic compounds on the oxidative stability of walnuts and almonds submitted to thermal treatments has not previously been reported. The main aim of this work was to compare the oxidative susceptibility of peeled and unpeeled ground nuts after heating and during room temperature storage in aerobic conditions in order to elucidate the role of the phenolic constituents in the oxidation reaction mechanism. Besides, a model system formed by a water-in-oil micro emulsion was designed to mimic the nut matrix in order to isolate the main factors, and to simulate the interactions that occur when nut lipids are heated in the presence of BS phenolic compounds.

2. EXPERIMENTAL SECTION

2.1. MATERIALS

Walnuts (*Junglans regia* var. Franquette) and almonds (*Prunus dulcis* var. Amara) were purchased in a local market. Nuts were cracked and shelled. A portion was peeled by soaking in water in order to remove the BS and afterwards, the material was dried under nitrogen. Peeled and unpeeled

nuts were cooled at -20 °C and ground before analyses into a fine powder (particle size approx. 0.5 mm) using a coffee grinder for 1 min.

Hexanal and acetophenone were purchased from Sigma-Aldrich (Saint Louis, MO), pentanal from Fluka (Steinheim, Germany) and 1,1,3,3-tetraethoxypropane (TEP), thiobarbituric acid (TBA), and trichloroacetic acid (TCA) from Merck (Buenos Aires, Argentina). A total amount of 37 saturated and unsaturated fatty acids used as reference compounds (Supelco 37 FAME Mix) were purchased from Sigma (Newport, MA). Tween 20 (polyoxyethylene-sorbitan monolaurate) and catechin hydrate were purchased from Aldrich; linoleic acid was obtained from Riedel de H en; sodium bis(2-ethylhexyl) sulphosuccinate or docusate sodium salt (AOT) and Folin-Ciocalteu's phenol reagent were purchased from Sigma (Sigma-Aldrich, Buenos Aires, Argentina); GA was obtained from Anedra (Buenos Aires, Argentina) and quercetin were provided by Parafarm, (Buenos Aires, Argentina) .

2.2. METHODS

2.2.1. Fatty acid characterization of nut oils

Walnut and almond oil extraction was carried out by cold extraction method with hexane according to Miraliakbari and Shahidi report¹⁶ with minor modifications. All extractions were performed in triplicate. After extractions, oil samples were stored at -20 °C under nitrogen. Fatty acids were transformed in their corresponding methyl esters before GC analysis. Methyl undecanoate (C:11) was used as internal standard.¹⁷ Results were expressed in mg of fatty acid/100 mg of extracted oil.

2.2.2. Phenolic content in walnut and almond extracts

Phenolic extract preparation: Approximately 2 g of whole nuts or 0.5 g of their BS were mixed with 50 mL ultra-pure water and stirred for 45 min, then, filtered under vacuum and centrifuged at 10000 rpm for 10 min. The supernatant was recovered and total phenolic compound content was

determined by spectrophotometry according to the Folin Ciocalteu method¹⁸ and phenolic extract concentration was expressed as $\mu\text{g GAE/mL}$. This solution was used in model-system experiments.

2.2.3. Determination of volatile secondary products by SPME-GC-MS

Amounts of 5 g of ground walnuts and almonds (previously peeled or unpeeled) were placed in round-bottom flasks with hermetic lids. The samples were heated for 24 h at 100 °C under controlled conditions. Appropriate amounts of acetophenone were added to the flasks 20 min before finishing the heating time as internal standard. Volatile compounds were collected from the head-space by SPME using a 50/30 μm divinylbenzene-carboxen-polydimethylsiloxane stable flex fiber. DVB carboxen/PDMS fiber (Supelco, Spain) was exposed in the head-space for the last 10 min of thermal treatment. After the volatile compound extraction, the fiber was inserted directly into the GC injector where it was kept during the run time. A Thermo Scientific gas chromatograph equipped with a mass spectrometer detector, split injector and a 30 m \times 0.25 mm \times 0.25 μm Thermo Scientific TR-5MS capillary column were used in the analysis. The mass range scanned was 50 - 600 m/z. The ionization mode was electron impact at 70 eV. The injector temperature was 200 °C, the injector split mode was used with a rate of 50 mL/min, while total and column flows were 10 and 1 mL/min. The initial temperature was 37 °C and it was held for 5 min; then, it was increased at 10 °C/min to 140 °C and held for 5 min; finally, it was increased at 10 °C/min to 240 °C and held for 5 min. Peak identification was carried out by comparison of its mass spectra with those contained in NIST library Mass Spectral Search Program, version 2.0. In order to quantify the volatile compounds formed, pure authentic samples were used for calibration purposes. Individual response factors were calculated with the internal standard to quantify the oxidation products. Results were expressed as headspace concentration of volatile compounds in $\mu\text{g/g}$ ground nut.

2.2.4. Determination of secondary products by TBARS method

Samples of 0.6 g of ground nuts (previously peeled or unpeeled) were placed into hermetically capped flasks and heated in a water bath for different times. Seven series of samples were kept at 60 °C for 10 and 30 min, at 70 °C for 10, 30 and 60 min, and at 100 °C for 30 and 60 min. Thiobarbituric acid reactive substances (TBARS) contents of were determined in nuts after treatment.¹⁹ Controls were kept at room temperature (25 °C) for 10, 30, 60 and 120 min. An experiment kept at room temperature immediately after nut grinding was considered as a blank for all the determinations. The procedure to monitor the formation of TBARS was carried out mixing an amount of 5 mL of 0.3 M TCA solution with 0.6 g of sample. The mixture was stirred and centrifuged for 15 min at 10000 rpm. A 2 mL supernatant aliquot was mixed with an equal volume of 34 mM TBA solution and heated at 100 °C for 1 h. Finally, the solution was cooled in a cold-water bath containing ice to stop the reaction. After reaching room temperature, the absorbance was measured at 532 nm using 700 nm values to correct baseline. As a malondialdehyde (MDA) precursor, TEP was used for calibration purposes. Results were expressed as MDA equivalents in mg/Kg of sample. In order to verify if TBA is able to react with another carbonyl compounds different to MDA, 6 mM pentanal and hexanal pure solutions were also analyzed.

2.2.5. Thermal induced oxidation of LA in a model system

To mimic a low water content food although rich in lipids, a water-in-oil model system was designed as AOT/isooctane/water reverse micelles containing LA. Stock solutions of AOT and LA were prepared both in isooctane in capped tubes to final concentrations of 25 and 8 mM, respectively. A fixed aliquot of the aqueous media was added with a microsyringe. Total water content in the system was expressed as the mole ratio between water and surfactant: $W = [\text{H}_2\text{O}]/[\text{AOT}]$

W value was fixed at 62 according to our previous report to reach humidity level in nuts.⁹ In order to modify the phenolic concentration from 0 to 6 μg GAE/mL for walnuts, and for almonds from 0 to 0.3 μg GAE/mL, water- aqueous extract ratio was varied in the aqueous pool of the reverse micelle system. The mixtures were incubated in sealed vials at 60 and 100 $^{\circ}\text{C}$ for time lengths of 30 min to 15 h. Subsequently, they were cooled in a cold-water bath to stop the reaction in order to analyze the oxidation advance. The same procedure was carried out adding to the system aqueous solutions of GA, catechin and quercetin in order to compare the nut aqueous extracts behavior with those of pure compounds. Phenolic concentrations varied between 0 and 6 $\mu\text{g}/\text{mL}$. No precedents have been found about using temperatures close to 100 $^{\circ}\text{C}$ in reverse micelles systems formed by AOT/ isooctane/ water; thus, the boiling point of the water in oil system and its stability were previously determined. The determination was carried out putting the glass tubes containing the micelle system in a vaseline bath. Porous plate was previously added to the tubes to prevent overheating, and the instrument used in this determination was a thermocouple. The boiling point was $103.0 \pm 0.1^{\circ}\text{C}$, which was higher than the working temperature.

2.2.5.1. Determination of primary oxidation products in the model system

Hydroperoxide formation was evaluated spectrophotometrically at 234 nm using a reduced optical-path cuvette, with 0.2 cm of path length. Hydroperoxide concentrations were calculated by Lambert-Beer Law and a mole extinction coefficient of LA hydroperoxides at the same wavelength, in reverse micelles of $\epsilon = 23005 \text{ M}^{-1}\text{cm}^{-1}$. To calculate this value, a given oxidized LA sample was prepared in Tween 20 aqueous micelles and measured at 234 nm. These values were converted to hydroperoxide concentrations using Lambert-Beer Law, taking ϵ as $25000 \text{ M}^{-1}\text{cm}^{-1}$. This value is the mole extinction coefficient of LA hydroperoxides reported in direct micelles.²⁰ The same LA sample

was used to prepare the AOT/isooctane reverse-micelle system and the mole extinction coefficient in this system was calculated by measuring the absorbance at 234 nm.

2.2.5.2. Determination of secondary products in the model system

After hydroperoxide measurement, secondary oxidation products were determined by TBARS method as previously described. Amounts of 2 mL of 0.3 M TCA solution and 34 mM TBA were added to the model system. The mixture was homogenized and heated at 100 °C in a water bath for 1 h. Then, the reaction was stopped by cooling in a cool-water bath to reach room temperature. Subsequently, the mixtures were centrifuged at 10000 rpm for 10 min at 20 °C in order to separate the aqueous and organic phases. The first one was used to measure the absorbance at 532 and 700 nm. Results were expressed as $\mu\text{g MDA/mg LA}$.

2.2.6. Statistical analysis

All analyses were performed in triplicate. Results were expressed as the mean of the values obtained for each sample ($n=3$). Analysis of variance models (one-way ANOVA) was performed using Infostat computing software. Multiple comparison procedure using Duncan's test was applied to determine which means were significantly different at $P<0.05$ confidence level.

3. RESULTS AND DISCUSSION

3.1. Fatty acid composition of nut oils

Walnut and almond oils were obtained through hexane extraction in order to determine their fatty acid profiles by gas chromatography (GC). Table 1 shows the fatty acid composition of walnut and almond oils. In the case of walnuts, LA was the major component, followed by oleic, linolenic, palmitic, and stearic acids. In contrast, oleic acid was the major component of almonds followed by LA, palmitic, stearic, and palmitoleic acids. No significant differences in the oil composition were

observed between peeled and unpeeled nuts (data not shown). According to these results, walnut oil is richer in poly-unsaturated fatty acids (PUFA) than that of almonds, while the latter is richer in mono-unsaturated fatty acids, being in agreement with previous reports.^{2, 14} Consistently, a lower oxidative stability has been found for walnut oil than those of other nut oils measured by Rancimat method, being walnut and almond induction times of 4.7 and 21.8 h, respectively.²¹

3.2. Thermally induced oxidation of nuts ground with and without BS

Concerning to the oxidative stability of walnuts and almonds, different experiments were carried out by heating some portions of ground peeled and unpeeled nuts in aerobic conditions to evaluate the BS effect. The oxidative status after each treatment was evaluated by analyzing the volatile compound emission by SPME-GC-MS and the TBARS formation. Control experiment was carried out keeping the material at room temperature for the same time period.

3.2.1. Analysis of volatile oxidation products

The volatile compound emission produced as a consequence of the secondary oxidation was taken as one of the parameters to evaluate the changes in oxidation advance according to BS presence. Figure 1 shows the typical chromatograms corresponding to the volatile fraction of the oxidation products formed after the thermal treatment of ground walnuts and its BS for 24 h at 100 °C. A complex mixture of volatile products was observed. Several compounds were found and identified in the headspace of peeled and unpeeled nuts after heating. Significantly different behaviors were observed between peeled and unpeeled nuts. Experiments performed by heating exclusively the BS taken from walnuts and almonds, indicate that this material did not produce the emission of these volatile compounds in absence of lipid material.

Table 2 clearly indicates that BS induces the oxidation product formation in walnuts as well as in almonds, volatile compound levels in the headspace being higher in walnuts than in almonds. In the

case of ground walnuts with skin, several compounds were found, the major one being 2,4-decadienal, followed by 3-decen-1-ol, 2-octenal, 2-heptenal, 2-undecenal, 2,4-heptadienal, hexanal, nonanal, 2-nonenal and pentanal. By contrast, the major product in walnuts without skin was hexanal, followed by 2,4-decadienal and 3-decen-1-ol. Total volatile contents in the headspace were 183 and 5762 $\mu\text{g/g}$ for ground walnuts without and with BS, respectively after 24 h of heating.

In ground almonds with BS, the volatile compounds found in the headspace were hexanal, 2-octenal, 2-heptenal, nonanal, 2-undecenal, 3-decen-1-ol, pentanal, 2,4-decadienal and 2-undecenal. In ground peeled almonds, the compounds detected were hexanal, 3-decen-1-ol, 2,4-decadienal and 2-undecenal. Total volatile contents in the headspace were 87 and 1014 $\mu\text{g/g}$ for ground almonds without and with BS, respectively after 24 h heating.

The formation of 2,4-decadienal, the major volatile aldehyde found in oxidized walnut headspace, was markedly induced by the BS presence; being 34 times higher in walnuts ground with peel than in the one previously peeled.

Hexanal levels in the headspace of walnuts with skin showed values 4.5 times higher than in walnuts without skin. Furthermore, almonds with skin showed emissions 10 times higher than in almonds without skin. Pentanal emission was exclusively detected in nuts with skin, being in walnuts more than 2 times higher than in almonds. The presence of 2-heptenal, 2-octenal, 2-nonenal and nonanal was detected only after heating nuts with skin, being the values between 3 and 4,5 times higher in walnuts than in almonds. In the case of nonanal, the values were similar in both nuts. On the other hand, 3-decen-1-ol and 2,4-heptadienal was detected mainly in walnuts with skin.

Development of low-molecular weight aldehydes during storage of fat-rich food can be produced as a consequence of hydroperoxides decomposition from lipid oxidation reactions.^{5,22} The possible scissions of primary oxidation compounds give place to different volatile products as hexanal,

2,4-decadienal, 3-nonenal, 2-heptenal.⁶ Hexanal has been reported as the main oxidation product from LA oxidation which has the highest correlation with the sensorial perception of specific rancidity in walnuts.²³ Pentanal has been detected as one of the major volatile constituents of walnut aroma and its presence has been attributed to LA breakdown. Pentanal is then considered an important contributor to the typical walnut aroma.²⁴ Hexanal formation in walnuts stored for a period of 12 months packaged in different conditions have been reported,²⁵ hexanal values in dark storage and under light exposure varied between 0.0285 and 36 mg/Kg, respectively. In a similar study, the effect of packaging on hexanal contents among other parameters of almonds stored under different conditions for 12 months have been investigated, ranging values between 0.0285 and 4.88 mg/Kg.¹⁵ As previously mentioned, hexanal and pentanal as well as 1-pentanol and 1-hexanol have been identified as the major volatile compounds in the aroma headspace for walnuts from different provenances, especially in those which contain high PUFA levels.²⁴ On the other hand, these volatile carbonylic substances formed as secondary oxidation products from unsaturated fatty acid breakdown can be toxic at high concentrations and may be associated to the development of off-flavors, making the products undesirable to consumers.^{26,27}

3.2.2. Secondary oxidation products by TBARS method

TBARS levels were analyzed in ground peeled and unpeeled nuts treated at different temperatures and time lengths. Nut samples kept at room temperature for the same time periods were taken as control. Results corresponding to the analysis of TBARS contents in these nut samples are shown in Table 3. Significant differences were observed in the oxidation degrees of the treated samples depending on BS presence. It is important to emphasize that this is the first study where the BS effect on the oxidative stability of ground nuts is taken into account.

After storage at 25 °C, oxidative levels of ground peeled nuts were higher than those nuts ground with their peels even up to 120 min. This indicates a protector effect of the BS in lipid auto-

oxidation or enzymatically induced lipoxidation. In contrast to this antioxidant effect, walnuts with BS showed higher TBARS levels than the peeled ones after heating at 60, 70 and 100 °C, whereas almonds showed behavior similar to walnuts only at 70 and 100 °C. In the case of almonds, the protector effect remained after heating at 60 °C for 10 and 30 min although at 70 °C only for 10 min. However, BS produced a pro-oxidant effect in harder conditions, indicating that nut skins exerted a pro-oxidant effect when the thermally induced lipoxidation was the main mechanism.

After thermal treatment, MDA equivalents determined in walnuts were larger, in all cases, than that of almonds. In both cases, nuts ground with peels presented larger amounts than nuts after thermal treatment. The increases in TBARS levels were between 25 and 38 % in walnuts, whilst they were between 34 and 57 % in almonds, for the different temperatures and time lengths assayed being the highest for 70 °C and 30 min.

On the other hand, walnut control samples presented also higher MDA values than almond ones. However, differences were found between peeled and unpeeled nuts in control samples. MDA values of ground peeled nuts were higher than those of nuts ground with their peels; the BS protective effect being of 37 and 41 % for walnuts and almonds, respectively.

The BS effect on TBARS levels is consistent with the observed variations in volatile compound formation ascribed to BS presence. Control experiments were also performed with pure pentanal and hexanal using TBARS method conditions. It was verified that these carbonylic compounds do not interfere with MDA in this analysis. This indicates that both parameters have independently a good correlation with lipid oxidation.

TBARS values in raw unpeeled almonds varied in a range between 0.65 and 1.8 mg MDA/Kg.⁴ In the case of raw unpeeled walnuts, TBARS values measured varied between 0.2 and 11 mg MDA/Kg

of walnuts stored for a period of 12 months packaged in different conditions, when they were stored in the dark and exposed to light, respectively.²⁵

Nut heating increases the rate of all single reactions involved in the oxidation process. Furthermore, the concentration of lipid oxidation markers in nuts increases dramatically from room temperature to storage temperatures above 40 °C.²⁸ High temperatures and light exposure during storage dramatically increase lipid oxidation in almonds; besides, the oxygen presence has been recognized as one of the most significant extrinsic agents affecting lipid oxidation of nuts.¹⁵ Moreover, roasting of walnut kernels was found to increase the peroxide value and conjugated dienoic acid value of the oil, parameters which were used as indicators of its oxidative stability.²⁹ Walnuts and almonds are foods rich in polar and nonpolar phenolic compounds with recognized antioxidant ability as free radical scavengers, but also as LOX inhibitors. In both nuts, the major fraction corresponds to polar compounds concentrated in BS, and non-polar ones located in the oil as minor constituents, including tocopherols and tocotrienols.

We have previously reported the phenolic contents of whole nuts and of their BS.⁹ In both nuts, walnuts and almonds, BS and whole walnut extracts presented approximately 10 times higher phenolic contents than that of almond ones. Besides, BS extracts presented noticeably higher contents than whole nut extracts, indicating that phenolic compounds are concentrated in the nut BS.

Numerous phenolic compounds have been found in nut BS. Flavonoids and non-flavonoid phenolic compounds have been identified in almond skins.¹⁰ Among the non-flavonoid compounds, protocatechuic, vanillic and *p*-hydroxybenzoic acids have been informed, while among flavonoids, several flavonols, flavanols, dihydroflavonols and flavanons have been reported. Besides, the presence of different kinds of proanthocyanidins has also been found.³⁰ Several flavonoids and phenolic acids have been described exclusively in almond BS;³¹ among them, quercetin, quercetin-3-*O*-galactoside,

quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, dihydroxykaempferol, eriodictyol, kaempferol and *p*-hydroxy-benzoic acid can be mentioned. Many other compounds as catechin and epicatechin have found in the kernel, although they are mainly located in the almond BS. Potential uses of almond skins as a source of phytochemicals have driven several of studies of their composition.³² In walnut BS, compounds as ellagic acid, gallic acid and methyl gallate have been identified.^{33,34} These compounds can be present as polymers and bound to sugars and constitute a major fraction of nut phenolic compounds. Moreover, sixteen phenolic constituents have been found in walnut extracts, three of them were hydrolysable ellagitannins named glansrins and thirteen ones are well known polyphenolic compounds.³⁵ Besides, walnuts presented noticeably higher antioxidant activity and larger phenolic contents than almonds.⁹ Walnut oil has demonstrated to present higher levels in both, non-polar phenolic compounds and polyunsaturated fatty acids than the almond oil. However, BS protective effect towards thermally induced lipid oxidation was not observed on the contrary to our initial expectations. Moreover, thermal treatment of nuts ground with peels indeed induced the volatile carbonyl compound formation. By contrast, the differences found in control samples between peeled and unpeeled nuts can be attributed to a protective effect of phenolic compounds present in their BS towards enzymatic oxidations. Taking into account that phenolic compounds are good LOX inhibitors, these results are new evidences that BS phenolic compounds prevent LOX-induced lipoperoxidation which is the major reaction pathway taking place at room temperature.

3.3. Effect of BS phenolic extract on thermally induced lipid oxidation in a model system

In order to isolate the main factors controlling the processes and simulating the interactions that occur when peeled and unpeeled nuts are heated, a model system formed by a water-in-oil micro emulsion was proposed. Nuts as dried foods have 2-4 % of moisture content but are rich in lipids. Since the lipids are located in a hydrophobic region called lipid droplets or oily bodies and water is located in

pools or “pockets”, a liquid model was chosen to mimic the nut microstructure. Reverse micelles of AOT/ isooctane/ water at $W= 62$ allow to mimic such low water content dispersed in a lipophilic medium enriched in LA. In the aqueous core, phenolic extracts of nut BS at different levels were assayed. Samples were heated for 15 h to monitor primary and secondary oxidation products.

3.3.1. Primary oxidation products

Oxidation advance after 15 h of thermal treatment at 60 and 100 °C was analyzed in terms of hydroperoxide formation. In systems containing walnut and almond BS extracts, heating at 60 °C did not form hydroperoxides in absence of BS phenolic compounds, or in their presence. LA thermally induced oxidation at 100 °C yielded a peroxide level of $6 \pm 0.5 \mu\text{g}/\text{mg}$ LA after 15 h in absence of phenolic compounds. Nut phenolic extract presence markedly increased the formation of oxidation products giving as the highest peroxides levels 64.5 ± 4.1 and $21.3 \pm 1.2 \mu\text{g}/\text{mg}$ LA, at 4.4 and 0.05 μg GAE/mL for walnuts and almonds extracts, respectively. After reaching the maximum level of hydroperoxides, a decrease was observed in samples with both kinds of extracts, as a function of phenolic concentration. The highest values of hydroperoxides in systems containing walnuts extracts are consistent with their higher LA content. The fact that non detectable levels of hydroperoxides were found at higher phenolic concentrations, suggests that the oxidation-promoting effect of BS extract shifted the reaction to more advanced stages leading to secondary oxidation products. Room temperature experiments were also carried out to simulate those conditions where the enzymatically induced oxidation is the major pathway taking place. An aliquot of LOX aqueous solution was added to the reverse micelle system containing LA to induced oxidation. The hydroperoxide formation was evidenced as the absorbance increase at 234 nm due to the conjugated diene formation. When the same experiment was performed with BS extract addition, an abundant precipitate was formed and impeded the spectrophotometric measurement. This fact, far from being negative, was very positive since it

demonstrated that BS phenolic compounds have the ability to interact with LOX. The association between tannins and proteins has been widely studied and reported. However, this studies were carried out in solution but results presented in this work evidence that this interaction can take place in a food matrix and this explains the protective effect of BS phenolic compounds towards enzyme-induced oxidation observed in ground nuts stored at room temperature.

3.3.2. Secondary oxidation products

After hydroperoxides determination in the model system, TBARS levels were also analyzed as a function of phenolic concentration. This experiment was carried out in order to verify if the decrease in hydroperoxides values previously observed was related to the secondary oxidation products formation at the experimental conditions. Samples containing BS extracts, showed higher MDA levels than samples without BS extracts after thermal treatments at 100 °C for 15 h. Significant differences were observed in oxidation levels of treated samples, depending on their phenolic compound contents. The maximum yield of MDA was found in samples with walnut extract, this value being 31% higher than that of samples with almond extract. The increment in MDA level was of 53% in samples with walnuts extract, and of 33% in samples with almond extract. This increase in MDA level was coincident with the decrease in hydroperoxide values previously observed.

Figure 2 A) and B); and Figure 3 A) and B) show the effect of phenolic concentration on LA oxidation as the variation of primary and secondary oxidation products after thermal treatment in the model system. Results clearly indicate a prooxidant action of phenolic compounds at 100 °C treatment of the reverse micelle system. This observation is in good agreement previous report where the prooxidant effect of polyphenols over an oxidisable substrate has been reported promoted by metal ions.³⁶ Besides, Zhou and Elias¹³ have recently reported the turning from antioxidant to prooxidant activity of phenolics in direct micelles in the presence of active metal ions.

To the best of our knowledge, this is the first report about the promotion of lipid oxidation by phenolic compounds in a model system as well as its correlation with the natural food behavior in absence of active metal ions.

3.4. Effect of pure phenolic compounds on thermally induced lipid oxidation in a model system

Three different pure phenolic compounds were used to study their antioxidant/prooxidant behavior, and compare it with that than of nut aqueous extracts. They were chosen since as was previously cited, GA is an important phenolic acid present in walnut BS,^{33,34} and both flavonoids catechin and quercetin are main constituents of almond BS.³¹

3.4.1. Primary oxidation products

LA thermally induced oxidation yielded an initial peroxide level approximately of 10 $\mu\text{g}/\text{mg}$ LA in the absence of phenolic compounds. The three pure substances employed exerted a prooxidant action at the working conditions. After 15 h at 100 °C, an increase in peroxides levels was observed, followed by a decrease in all cases. The highest peroxides levels were 60.8 $\mu\text{g}/\text{mg}$ in the presence of 4.4 $\mu\text{g}/\text{mL}$ of GA, 51.2 $\mu\text{g}/\text{mg}$ LA with 4.4 $\mu\text{g}/\text{mL}$ of catechin and 80.4 $\mu\text{g}/\text{mg}$ LA with 2.2 $\mu\text{g}/\text{mL}$ of quercetin. The behavior observed to the three compounds studied was consistent with that observed in systems containing aqueous extracts. Figure 4 shows the variation of LA primary oxidation products in a dose dependent manner, after thermal treatment in the model system. Results clearly indicate a prooxidant action of phenolic compounds at 100 °C treatment of the reverse micelle system.

3.4.2. Secondary oxidation products

The systems containing phenolic substances showed that TBARS levels increased as a function of its concentration. In the same way that aqueous extracts, the decrease in hydroperoxides values was related to the secondary oxidation products increase. Maximum oxidation levels were 0.32, 0.3 and 0.41 μg MDA/mg AL in the presence of 6 $\mu\text{g}/\text{mL}$ of GA, catechin and quercetin, respectively. Figure 5

show the effect of phenolic concentration on LA oxidation as the variation of secondary oxidation products after thermal treatment in the model system. Results clearly indicate a prooxidant action of phenolic compounds at 100 °C treatment of the reverse micelle system. Summarizing, the model system designed allowed corroborate the prooxidant effect on LA of phenolic compounds after thermal treatment, in the same way to that observed in the food matrix.

Main factors influencing the pro-oxidant activity of polyphenols in oil-in-water emulsions have been well described in a system containing iron ions.¹³

In our present study, polyphenol prooxidant action takes place in reverse micelles being the micro- heterogeneous systems which describes well what take place in the food matrix as nuts. As far as we know, this is the first report evidencing the prooxidant action of phenolic compounds in systems free of active metal ion addition.

The interaction of phenolic compounds toward free radicals (like ROS) produces phenoxyl radicals as intermediates stabilized by resonance; further oxidation of these species, leads to final degradation products of the phenolics. In the case of low free radical levels (few initiation events for a radical reaction), phenolics produced the breaking of the reaction chain, and due to stability of the phenoxyl radical intermediates formed, the results leads to antioxidant behavior. However, prooxidant behavior can take place in a lipid rich system containing peroxides and hydroperoxides thermally unstable precursors of free radicals. When the production of these radicals is excessive due to heat induction, this leads to a redox imbalance (oxidative stress) and these phenoxyl radical species are able to promote the propagation of the radical chain reaction and thus, to induce the advance of the oxidation reaction in a dose dependent manner.

4. CONCLUSION

Walnuts are more susceptible to oxidation than almonds as a consequence of their lipid composition. Thermal treatment of nuts ground with their BS produced higher TBARS levels and higher volatile emission than those of ground peeled nuts; hence, the BS presence promotes lipid oxidation in ground walnuts and almonds subjected to short-term heating.

On the contrary, during room temperature storage of ground nuts, antioxidant behavior was found exerted by the presence of BS. This action is ascribed to the inhibition of LOX induced oxidation by BS phenolic constituents. This protective ability was no longer effective when the nuts were heated, being BS prooxidant action more important than their antioxidant effect. The proposed water-in-oil model system validates these results; a dose-dependent prooxidant effect on LA thermal oxidation was observed after heating for phenolic extract additions; this behavior being reverted in LOX induced process at room temperature. Nut skin induces specific lipid oxidation reactions yielding volatile compounds.

This work shows the importance of peeling the nuts before the grinding for culinary preparations that require cooking to preserve not only their sensorial and nutritional characteristics but also their safety. On the other hand, for those recipes without cooking including ground nuts, keeping their skins is important to prevent oxidation.

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Abbreviations

BS: brown skin, GA: gallic acid, GAE: gallic acid equivalents, GC: Gas chromatography, LA, linoleic acid, LOX: Lipoxygenase, MDA: Malondialdehyde, MS: mass spectrometry, PUFA: polyunsaturated fatty acids, ROS: reactive oxygen species, SPME: Solid phase micro-extraction, TBARS: thiobarbituric acid reactive substances, TCA: Trichloroacetic acid, TEP, 1,1,3,3-tetraethoxypropane.

5. REFERENCES

- 1- Simopoulos, A. P. Health Effects of Eating Walnuts. *Food Rev. Int.*, 2004, 20, 91-98.
- 2-Venkatachalam, M., Sathe, S. K. Chemical composition of selected edible nut seeds. *J. Agric. Food Chem.*, 2006, 54, 4705-4714.
- 3- Halliwell, B., Gutteridge, J. M. C. Free Radicals in Biology and Medicine. 2nd Edition. 1989. London, Oxford University Press.
- 4-Yaacoub, R., Saliba, R., Nsouli, B., Khalaf, G., Birlouez-Aragon, I. Formation of lipid oxidation and isomerization products during processing of nuts and sesame seeds. *J. Agric. Food Chem.*, 2008, 56, 7082-7090.
- 5-Spiteller, P., Kern, W., Reiner, J., Spiteller, G. Aldehydic lipid peroxidation products derived from linoleic acid. *Biochim. Biophys. Acta*, 2001, 1531, 188-208.
- 6-Frankel, E. Volatile lipid oxidation products. *Prog. Lipid Res.* 1982, 22, 1-33.
- 7-López-Nicolás, J. M., Pérez-Gilabert, M., García Carmona, F. Eggplant lipoxygenase (*Solanum melongena*): product characterization and effect of physicochemical properties of linoleic acid on the enzymatic activity. *J. Agric. Food Chem.*, 2001, 49, 433-438.

- 8-Buranasompob, A., Tang, J., Powers, J. R., Reyes, J., Clark, S., Swanson, B. G. Lipoxygenase activity in walnuts and almonds. *LWT- Food Sci. Technol.*, 2007, 40, 893-899.
- 9-Salcedo, C. L., López de Mishima, B. A., Nazareno, M. A. Walnuts and almonds as model systems of foods constituted by oxidisable, pro-oxidant and antioxidant factors. *Food Res. Int.*, 2010, 43, 1187-1197.
- 10-Monogas, M., Garrido, I., Lebrón-Aguilar, R., Bartolomé, B., Gómez-Cordovés, C. Almond (*Prunus dulcis* (Mill.) D.A. Webb) skins as a potential source of bioactive polyphenols. *J. Agric. Food Chem.*, 2007, 55, 8498-8507.
- 11-Heim, K. E., Tagliaferro, A. R., Bobilya, D. J. Flavonoid antioxidants: Chemistry, metabolism and structure–activity relationships. *J. Biochem.*, 2002, 13, 572-584.
- 12-Fukumoto, L. R., Mazza, G. Assessing antioxidant and prooxidant activities of phenolic compounds. *J. Agric. Food Chem.*, 2000, 48, 3597-3604.
- 13-Zhou, L., Elias, R. J. Factors influencing the antioxidant and pro-oxidant activity of polyphenols in oil-in-water emulsions. *J. Agric. Food Chem.*, 2012, 60, 2906-2915.
- 14-Amaral, J. S., Casal, S., Pereira, J. A., Seabra, R. M. Oliveira, B. P.P. determination of sterol and fatty acid compositions, oxidative stability, and nutritional value of six walnut (*Juglans regia* L.) Cultivars Grown in Portugal. *J. Agric. Food Chem.*, 2003, 51, 7698-7702.
- 15-Mexis, S. F., Badeka, A. V., Kontominas, M. G. Quality evaluation of raw ground almond kernels (*Prunus dulcis*): Effect of active and modified atmosphere packaging, container oxygen barrier and storage conditions. *Innovat. Food Sci. Emerg. Tech.*, 2009, 10, 580-589.
- 16-Miraliakbari, H., Shahidi, F. Antioxidant activity of minor components of tree nut oils. *Food Chem.*, 2008, 111, 421-427.

- 17-Sancho, R. A. S., Lima, F. A., Costa, G. G., Mariutti, L. R. B., Bragagnolo, N. Effect of annatto seed and coriander leaves as natural antioxidants in fish meatballs during frozen storage. *J. Food. Sci.*, 2011, 76, 838-845.
- 18-Singleton, V., Rossi, J. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vit.*, 1965, 16, 144-158.
- 19-Zacheo, G., Capello, A. R., Perrone, L. M., Gnani, G. V. Analysis of factors influencing lipid oxidation of almond seeds during accelerated ageing. *LWT- Food Sci. Technol.*, 1998, 31, 6-9.
- 20-López-Nicolás, J. M., Bru, R., García-Carmona, F. Kinetic characteristics of the enzymatic conversion in presence of cyclodextrins: study of the oxidation of polyunsaturated fatty acids by lipoxygenase. *Biochim. Biophys. Acta*, 1997, 1347, 140-150.
- 21-Arranz, S., Cert, R., Pérez-Jiménez, J., Cert, A., Saura-Calixto, F. Comparison between free radical scavenging capacity and oxidative stability of nut oils. *Food Chem.*, 2008, 110, 985-990.
- 22-Pastorelli, S., Valzacchi, S., Rodriguez, A., Simoneau, C. Solid-phase microextraction method for the determination of hexanal in hazelnuts as an indicator of the interaction of active packaging materials with food aroma compounds. *Food Additives and Contaminants: A*, 2006, 23, 1236-1241.
- 23-Crowe, T. D., Crowe, T. W., Johnson L. A., White P. J. Impact of extraction method on yield of lipid oxidation products from oxidized and unoxidized walnuts. *J. Am. Oil Chem. Soc.*, 2002, 79, 453-456.
- 24-Elmore, J. S., Nisyrios, I., Mottram, D. S. Analysis of the headspace aroma compounds of walnuts (*Juglans regia* L.). *Flav. Frag. J.*, 2005, 20, 501-506.
- 25-Mexis, S. F., Badeka, A. V., Riganakos, K. A., Karakostas, K. X., Kontominas, M. G. Effect of packaging and storage conditions on quality of shelled walnuts. *Food Control*, 2009, 20, 743-751.

- 26-Guillén, M. D., Cabo, N., Ibargoitia, M. L., Ruiz, A. Study of both sunflower oil and its headspace throughout the oxidation process. Occurrence in the headspace of toxic oxygenated aldehydes. *J. Agric. Food Chem.*, 2005, 53, 1093-101.
- 27-Goicoechea, E., Brandon, E. F. A. Blokland, M. H., Guillén, M. D. Fate in digestion in vitro of several food components, including some toxic compounds coming from omega-3 and omega-6 lipids. *Food Chem. Toxicol.*, 2011, 49, 115-124
- 28-Tazi, S., Plantevin, F., Di Falco, C., Puigserver, A., Ajandouz, E. H. Effects of light, temperature and water activity on the kinetics of lipoxidation in almond-based products. *Food Chem.*, 2009, 115, 958-964.
- 29-Vaidya, B., Eun, J. B. Effect of roasting on oxidative and tocopherol stability of walnut oil during storage in the dark. *Eur. J. Lipid Sci. Technol.*, 2013, 115, 348-355.
- 30-Brieskon, C. H., Betz, R. Procyanidin polymers crucial to the structure of the almond seed coat. *Z. Lebensm. Unters. Forsch.*, 1998, 187, 347-353.
- 31-Milbury, P. E., Chen, C. Y., Dolnikowski, G. G., Blumberg, J. B. Determination of Flavonoids and Phenolics and Their Distribution in Almonds. *J. Agric. Food Chem.*, 2006, 54, 5027-5033.
- 32-Bolling, B. W., Dolnikowski, G., Blumberg, J. B., Chen, C. Y. O. Quantification of Almond Skin Polyphenols by Liquid Chromatography-Mass Spectrometry. *J. Food Sci.* 2009, 74, 326-332.
- 33-Anderson, K. J., Teuber, S. S., Gobeille, A., Cremin, P., Waterhouse, A. L., Steinberg, F. M. Walnut polyphenolics inhibit in vitro human plasma and LDL oxidation. *Biochem. Mol. Action Nutr.*, 2001, 131, 2837-2842.
- 34-Papoutsis, Z., Kassi, E., Chinou, I., Halabalaki, M., Skaltsounis, L. A., Moutsatsou, P. Walnut extract (*Juglans regia* L.) and its component ellagic acid exhibit anti-inflammatory activity in

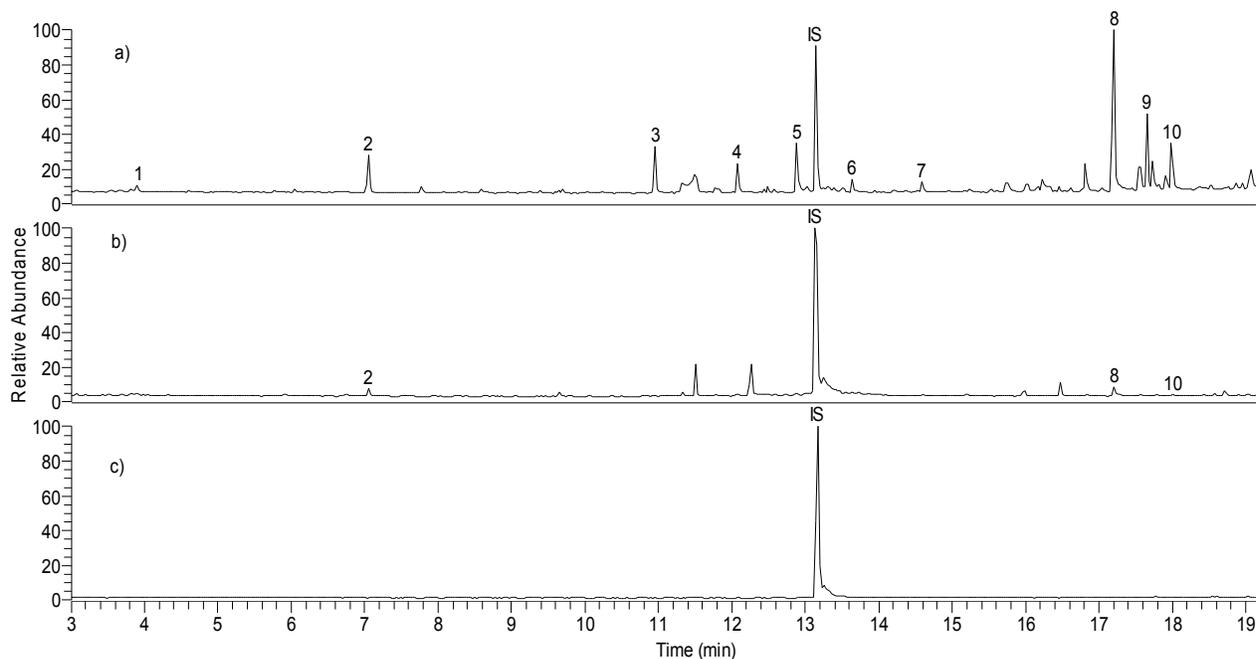
human aorta endothelial cells and osteoblastic activity in the cell line KS483. *Br. J. Nutr.*, 2007, 99, 715-722.

35-Fukuda, T., Ito, H., Yoshida, T. Antioxidative polyphenols from walnuts (*Juglans regia* L.). *Phytochem.*, 2003, 63, 795-801.

36-Sakihama, Y., Cohen, M. F., Grace, S. C., Yamasaki, H. Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals in plants. *Toxicology*, 2002, 177, 67-80.

Figure 1. Chromatographic profiles of volatile oxidation products of ground walnuts after thermal treatment at 100 °C for 24 h analyzed by SPME-GC-MS.

a) Walnuts with brown skin. b) Walnuts without brown skin. c) Walnut brown skin.



Peak identification: 1) pentanal, 2) hexanal, 3) 2-heptenal, 4) 2,4-heptadienal, 5) 2-octenal, 6) acetophenone, 7) nonanal, 8) 2-nonenal, 9) 2,4-decadienal, 10) 2-undecenal, 11) 3-decen-1-ol, IS: Internal standard.

Figure 2. Effect of walnut brown skin phenolic extracts on the LA oxidation in water-in-oil micelle systems after 15 h of thermal treatment. A) primary oxidation products; B) secondary oxidation products

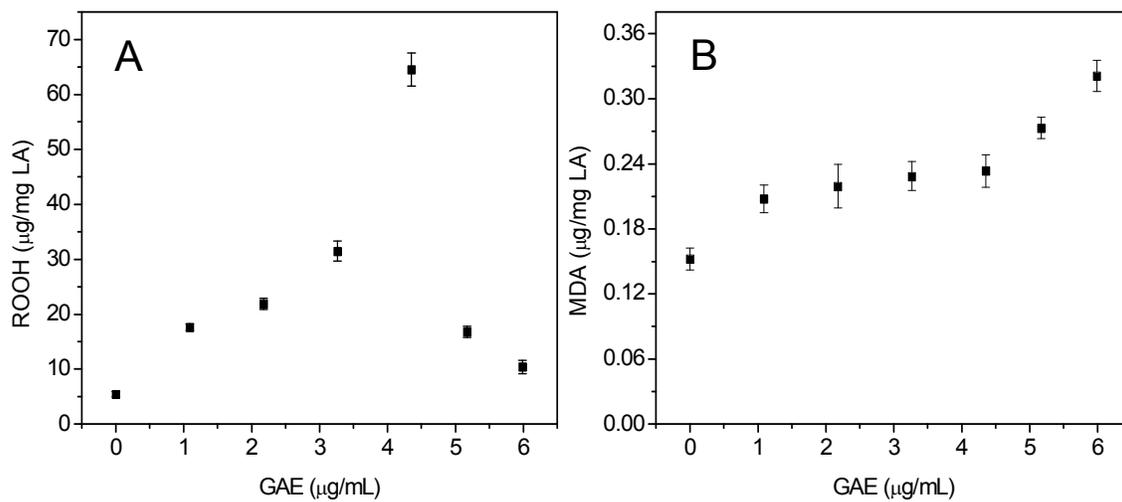


Figure 3. Effect of almond brown skin phenolic extracts on the LA oxidation in water-in-oil micelle systems after 15 h of thermal treatment. A) primary oxidation products; B) secondary oxidation products

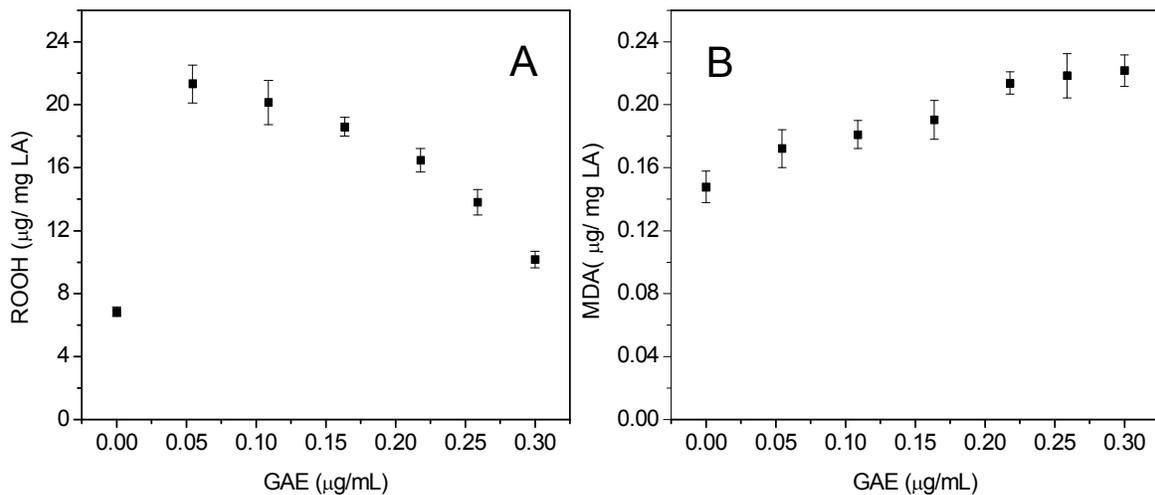


Figure 4. Effect of pure phenolic compounds on the primary oxidation products of LA oxidation in water-in-oil micelle systems after 15 h of thermal treatment.

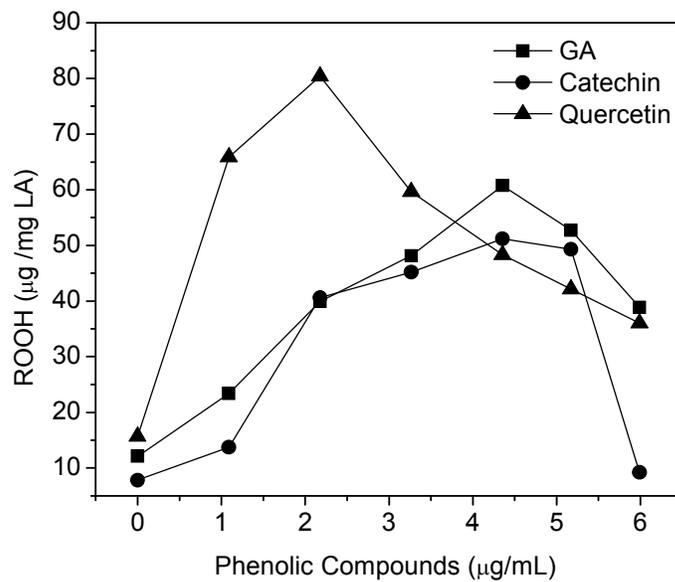


Figure 5. Effect of pure phenolic compounds on the secondary oxidation products of LA oxidation in water-in-oil micelle systems after 15 h of thermal treatment.

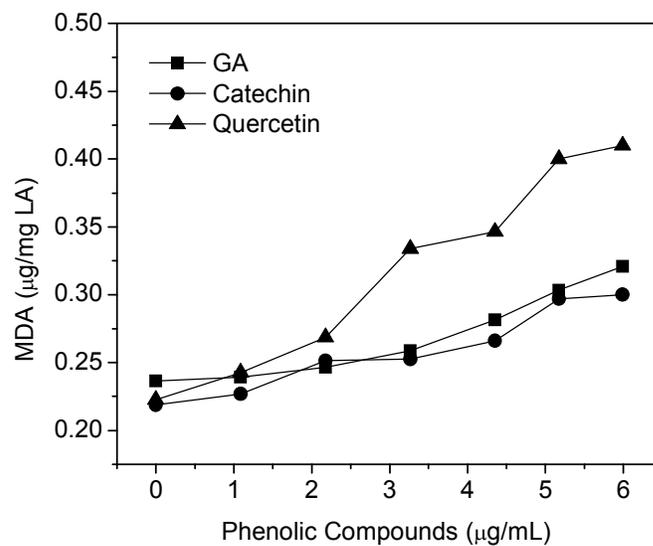


Table 1. Fatty acid composition of walnut and almond oils (mg/100 mg oil)

	Palmitic acid (16:0)	Palmitoleic acid (16:1n-7)	Stearic acid (18:0)	Oleic acid (18:1n-9)	Linoleic acid (18:2n-6)	Linolenic acid (18:3n-3)
Walnuts	6.7 ± 0.5	0.23 ± 0.01	2.3 ± 0.1	21 ± 2	57 ± 7	12 ± 2
Almonds	6.7 ± 0.3	0.49 ± 0.03	1.4 ± 0.1	68 ± 9	24 ± 3	nd

Values indicate the mean ± standard deviation of 3 replicates; n.d.: non detected.

Table 2. Brown skin effect on the emission of volatile oxidation products of ground walnuts and almonds after thermal treatment.

Peak number	Volatile oxidation products	Headspace concentration ($\mu\text{g/g}$ ground nut)			
		Walnuts with skin	Walnuts without skin	Almonds with skin	Almonds without skin
1	Pentanal	88 ± 1	n.d.	36 ± 2	n.d.
2	Hexanal	381 ± 8	84 ± 3	261 ± 15	26 ± 2
3	2-heptenal	452 ± 35	n.d.	143 ± 9	n.d.
4	2,4-heptadienal	354 ± 42	n.d.	n.d.	n.d.
5	2-octenal	576 ± 47	n.d.	176 ± 2	n.d.
6	Nonanal	142 ± 17	n.d.	142 ± 2	n.d.
7	2-nonenal	105 ± 5	n.d.	23.2 ± 0.2	n.d.
8	2,4-decadienal	2478 ± 379	73 ± 10	24 ± 1	22 ± 3
9	2-undecenal	385 ± 15	n.d.	124 ± 7	16 ± 14
10	3-decen-1-ol	799 ± 12	26 ± 2	85 ± 5	23 ± 4

Values indicate the mean \pm standard deviation of 3 replicates; n.d.: non detected. Detection limit = $3 \mu\text{g}$ in the headspace.

Table 3. Brown skin effect on the oxidation levels of ground walnuts and almonds after thermal treatment and room temperature storage

Treatment		TBARS (mg MDA/Kg nut)				
Temperature (°C)	Time (min)	Walnuts		Almonds		
		Without skin	With skin	Without skin	With skin	
25	10	3.5 ± 0.1 ^a	2.2 ± 0.1 ^b	2.5 ± 0.1 ^a	1.7 ± 0.3 ^b	ANTIOXIDANT
	30	3.8 ± 0.1 ^a	2.8 ± 0.2 ^b	2.9 ± 0.3 ^a	1.4 ± 0.1 ^b	
	60	3.9 ± 0.2 ^a	2.9 ± 0.1 ^b	2.5 ± 0.4 ^a	1.6 ± 0.1 ^b	
	120	5.1 ± 0.2 ^a	3.2 ± 0.4 ^b	2.93 ± 0.03 ^a	1.72 ± 0.03 ^b	
60	10	3.5 ± 0.1 ^b	6.0 ± 0.4 ^a	3.0 ± 0.2 ^a	1.6 ± 0.3 ^b	ANTIOXIDANT
	30	4.4 ± 0.2 ^b	7.5 ± 0.1 ^a	4.2 ± 0.1 ^a	2.9 ± 0.2 ^b	
70	10	3.7 ± 0.1 ^b	6.5 ± 0.1 ^a	3.2 ± 0.2 ^a	1.8 ± 0.1 ^b	ANTIOXIDANT
	30	7.6 ± 0.1 ^b	10.5 ± 0.1 ^a	4.4 ± 0.1 ^b	6.9 ± 0.1 ^a	
	60	8.5 ± 0.3 ^b	11.5 ± 0.1 ^a	5.6 ± 0.3 ^b	8.4 ± 0.3 ^a	
100	30	8.1 ± 0.1 ^b	10.9 ± 0.1 ^a	5.1 ± 0.2 ^b	7.2 ± 0.2 ^a	PROOXIDANT
	60	9.7 ± 0.1 ^b	12.1 ± 0.5 ^a	6.5 ± 0.3 ^b	8.7 ± 0.4 ^a	

Mean ± SD of triplicate.

* Values in a row for walnuts or almonds followed by different letter indicate significant differences (P<0.05) between peeled and unpeeled nuts.