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Formation of Lipid Oxidation and Isomerization Products during Processing of Nuts and Sesame Seeds

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The aim of the present study was to quantify some nutritional and safety quality parameter changes that take place in nuts (roasting) and sesame seeds (dehulling, roasting, milling, and sterilization) during processing. Such evaluation was based on chemical analysis of various indicators of lipid alteration in raw and processed pistachios, almonds, peanuts, and tahina. Lipid oxidation was assessed by the evolution of lipid oxidation products including hydroperoxides, *p*-anisidine, and thiobarbituric acid reactive substances, as well as carboxymethyllysine (CML) and *trans* fatty acids (tFAs). All these parameters were significantly affected by the different processing stages, especially by roasting and sterilization (tahina). Nut roasting and sesame heat treatment increased the primary (hydroperoxides) and secondary (aldehydic compounds) lipid oxidation products, with the *p*-anisidine value reaching 6−11.5 and thiobarbituric acid reactive substances 3−5 mg/kg (equiv of malondialdehyde) in the different end products. In addition, roasting led to the formation of CML (between 12.7 and 17.7 ng/mg) and tFAs (between 0.6 and 0.9 g/100 g) in nuts and tahina, which were absent in the raw material. Roasting parameters appear as the critical factor to control to limit the CML and tFA formation in the final product.

KEYWORDS: Lipid oxidation; carboxymethyllysine; *trans* fatty acids; nuts; sesame seeds

INTRODUCTION

Roasted nuts and seeds are very appreciated worldwide as snack foods and appetizers. In Mediterranean countries, and particularly in Lebanon, they are an intrinsic part of the diets, and their consumption is evaluated at approximately 50 (g/person)/day as indicated by a diet survey conducted on Lebanese adults living in the capital, Beirut (not published).

From a general point of view, thermal treatment improves the food microbiological safety, digestibility, sensory qualities, and shelf life of food products. Roasting is the main step of nut and sesame processing, a process specifically designed to enhance the flavor, color, and texture of nuts and sesame. Compared to raw nuts, the roasted product has a delicate, uniquely nutty, and widely appreciated taste. Roasting also allows inactivation of enzymes and destroying of undesirable microorganisms (*1*). Moreover, roasting promotes the production of potentially beneficial compounds, with antioxidant properties related to the formation of phenole-type structures and/or the chelating properties of melanoidins (*2*). However, the roasting process of nuts and sesame seeds also induces the development of undesirable reactions that may cause loss of nutritional value, as well as formation of potentially toxic compounds.

The major oxidative reaction in heat-treated PUFA-rich foods such as nuts and seeds is lipid peroxidation (LPO) (*3*). Lipid oxidation in foods almost exclusively concerns unsaturated fatty acids and is essentially autocatalytic, explaining increasing reaction rates with time (*3*). Nuts and sesame seeds contain high amounts of unsaturated fatty acids, making them very sensitive to rancidity (*3*). Severe heat treatment of lipid-rich foods may also induce the isomerization of double bonds, leading to the formation of *trans* fatty acids (tFAs) (*4*). The secondary lipid oxidation products, namely, the aldehydic compounds formed during peroxidation, are prone to formation of lipoxidation end products during the Maillard reaction by nucleophilic attack of the carbonyl compound on the free amino groups of amino acids or proteins (*5*). Carboxymethyllysine (CML), a well-known advanced Maillard product, is a useful stable indicator of the reaction (*6*).

All these compounds were described as possibly exerting adverse effects in humans. Advanced peroxidation products have

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been described as cytotoxic and proatherogenic (*7*). *trans* fatty acids are considered to alter the HDL/LDL ratio, increasing the cardiovascular risk, and to affect intrauterine human growth (*4*). CML has been found to promote oxidative stress and microinflammation as a result of its interaction with the receptors for advanced glycation end products (*8*). Consequently, the roasting process, which can promote the formation of such undesirable compounds, needs to be precisely controlled to allow limitation of their concentration while the other objectives regarding organoleptic and microbiological quality are reached.

Controlling the quality of heat-processed foods involves the selection of pertinent indicators. Many indicators are currently used to assess the extent of lipid oxidation in foods, such as hydroperoxides, thiobarbituric acid reactive substances (TBARs), and *p*-anisidine value as primary and secondary oxidation products (*9*). Each assay gives information about different stages of oxidation and cannot be used alone. For example, the hydroperoxide level indicates the initial products of LPO, and it is only applicable to monitor peroxide formation in the early oxidation stage. During the course of oxidation, the hydroperoxide level reaches a maximum and then declines as a result of its breakdown into secondary oxidation products. Measuring these secondary LPO products can be achieved by the global *p*-anisidine value (pAV) based on a chemical reaction between α- and *β*-unsaturated aldehydes and *p*-anisidine under acidic conditions and subsequent measurement of the optical density of the resulting yellow adduct at 350 nm or by the TBA test, which is more selective for malondialdehyde (MDA), a short and volatile reactive aldehyde produced during advanced peroxidation of highly polyunsaturated FAs. Although both tests are valid determinants of secondary oxidation products, the pAV measures a more extensive assortment of nonvolatile compounds and compounds from more advanced secondary hydroperoxide oxidation (*10*). Even if aldehydes are no longer detectable with the TBA test, the anisidine value may still indicate that the fat has been oxidized because the residues detected are not volatile (*10*). Regarding the Maillard reaction, CML has been proposed as a useful and stable indicator of severely heat treated foods (*6*).

In this study we measured the levels of primary (hydroperoxides) and secondary (MDA and nonvolatile aldehydes) lipid oxidation products, CML, the product of interaction between carbonyl compounds and lysine in proteins, and fatty acid *trans* isomerization products in industrially processed nuts and sesame seeds. Moreover, the evolution of each indicator in sesame seeds was modeled as a function of the roasting time. The purpose of this study was to evidence the process critical steps regarding the quality deterioration according to such indicators and to assess the extent of the modification observed in real industrial samples.

MATERIALS AND METHODS

Samples and Processing Conditions. Three types of roasted nuts (pistachios, almonds, and peanuts) commonly consumed in Lebanon and sesame seed products (tahina) were sampled at different steps of processing in the industrial plant of most representative local manufacturers. The products were processed following the flow diagram presented in **Figure 1**. At the level of the sesame seed roasting step (F), samples were collected every 15 min at three different places of the roaster (to take into account the possible heterogeneity of the roaster) for kinetic analysis. The product temperature and the moisture content during roasting were also recorded. Pistachios (*Pistachia vera* L.; from Iran), peanuts (*Arachis hypogaea* L.; from China), and almonds (*Amygdalus communis* L.; from the United States) were roasted at 190 °C for 10 min, 180 °C for 10 min, and 180 °C for 8 min, respectively.

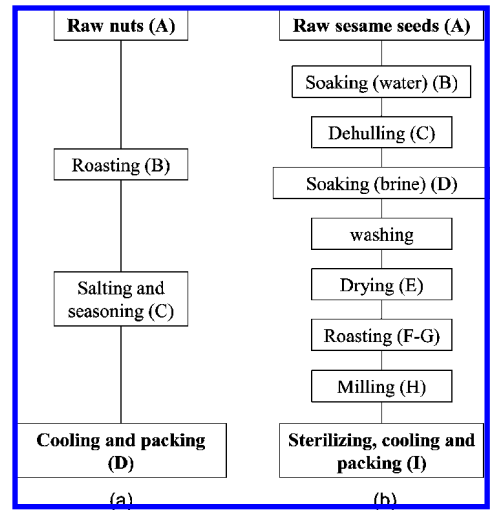

Figure 1. Flow diagram of the nuts and tahina manufacturing process showing the different stages studied: (**a**) nuts (A, raw material; B, roasting; C, seasoning; D, commercial end product), (**b**) tahina (A, raw material; B, soaking in water; C, dehulling; D, soaking in brine; E, drying; F and G, roasting (F, after 15 min of roasting; G, after 2 h of roasting); H, milling; I, sterilized end product).

Manufacture of Tahina. The sesame seeds, a white variety (*Sesamum indicum* L.) originating from Sudan, were sieved and then soaked in water for 2 h. The soaked seeds were stained off and passed through a peeler. The hulls and other foreign materials were separated by soaking in brine (180−200 g/L). The seeds were stained off and washed with water to remove the salt. The seeds were then left to dry at ambient temperature and passed through a roaster (115 °C for 2 h) (steam-heated jacketed open drum with continuous stirring). The roasted sesame seeds were then sieved and ground by stone mills to a viscous paste which was sterilized (100 °C for 30 min), then filled into plastic containers, and marketed as tahina.

Moisture Content Determination. Moisture content was determined according to the ISO 665-2000 method (*11*), by drying of 10 g of ground sample in an oven at 103 °C until a constant weight was reached.

Lipid Extraction. Oil was cold extracted from the samples according to the method described in Standard NF V03-030 (*12*) using hexane/2-propanol (3:2, v/v). After extraction, the hexane layer was removed and dried under nitrogen, and the pure oil was weighed to calculate the percentage yield.

Assessment of Lipid Oxidation. *Hydroperoxides.* Hydroperoxides were analyzed by an indirect colorimetric method based on the oxidation of iron(II) to iron(III) by hydroperoxides and subsequent formation of a brown chromophore in the presence of xylenol orange, according to the method of Jiang et al. (*13*). A 100 ± 5 mg sample of oil extract was diluted in 10 mL of 1-propanol, and then 2400 μL of this solution was mixed with 600 μL of a methanolic reaction mixture of xylenol orange and iron(II) sulfate in H_2SO_4 (94%) and BHT (1%), before reaction for 30 min at room temperature. The solution was filtered through a nylon filter (Cluzeau) before reading of the absorbance at 560 nm using a Unicam UV−vis spectrophotometer. Quantification used a calibration curve with H_2O_2. The results are expressed as millimoles of hydroperoxides per 100 mg of fat.

p-Anisidine Value. The pAV was measured according to the method described by Labrinea et al. (*14*). A quantity of 1 g of oil extract was dissolved with 15 mL of propanol. A 300 μL sample of this solution was mixed with 1.9 mL of *p*-anisidine solution in propanol (0.039 mol/L) and 0.7 mL of glacial acetic acid. The absorbance was measured at 350 nm using a Unicam UV−vis spectrophotometer. The pAV is defined by convention as 100 times the absorbance measured in a 1 cm cuvette of a solution resulting from the reaction of 1 g of fat with a 100 mL mixture of solvent and reagent.

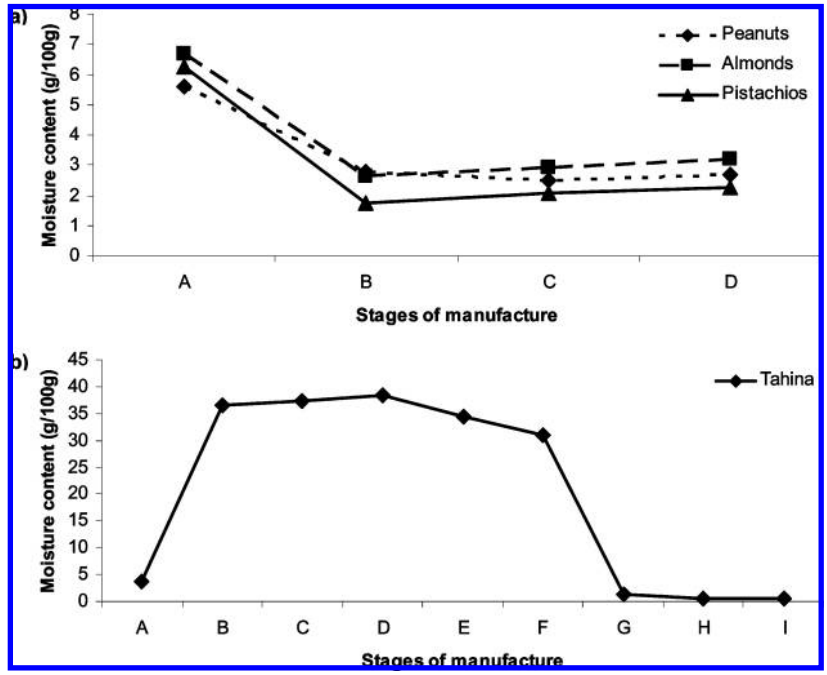

Figure 2. Change in the moisture content of nuts and tahina during the processing stages of (**a**) nuts (A, raw material; B, roasting; C, seasoning; D, commercial end product) and (**b**) tahina (A, raw material; B, soaking in water; C, dehulling; D, soaking in brine; E, drying; F and G, roasting (F, after 15 min of roasting; G, after 2 h of roasting); H, milling; I, sterilized end product). Data are averages of three measurements.

Thiobarbituric Acid Reactive Substances. TBAR values were determined using the TBA method described by Genot (*15*). For extraction, 2 g of the sample was homogenized together with 16 mL of a 5% (w/v) aqueous solution of trichloroacetic acid (TCA) containing 100 μL of freshly prepared BHT in ethanol (1 mg/mL) by using a homogenizer set at 20 000 rpm for 15 s. After filtration, 2 mL of extract was mixed with 2 mL of TBA solution (20 mol/L) in stoppered test tubes, and the test tubes were immersed in a 70 °C water bath for 30 min and then rapidly cooled in ice. After cooling, the absorbance of the reaction solutions was read at 532 nm using a Unicam UV−vis spectrophotometer against a blank containing 2 mL of TCA and 2 mL of TBA reagent. The results are expressed as milligrams of MDA equivalents per kilogram (or parts per million) of food sample using a molar extinction coefficient of 1.56×10 (*5*) $M^{-1} \cdot cm^{-1}$ for malondialdehyde. The sensitivity of the method is 0.37 mg of MDA equiv/kg of food sample.

Analysis of *trans* Fatty Acids. Fatty acids and their *trans* isomers were determined in the oil extracts as their methyl esters by gas chromatography. For the preparation of fatty acid methyl esters (FAMEs), 50−100 mg of extracted oil were accurately weighed and treated with 0.2 mL of methanolic NaOH (2 mol/L) in stoppered tubes under vigorous agitation after addition of 1 mL of heptane containing the internal standard heptadecanoic acid (1.91 mg/mL). The tubes were then heated at 50 °C in a water bath for 20 s and shaken for 10 s. A 400 μL portion of methanolic HCl (1 mol/L) was added, and the tubes were shaken vigorously and left to stand until separation of the layers. The FAME-containing supernatant was diluted 15 times and transferred to glass vials for further analysis. Quantitative analysis of FAMEs was performed with a gas chromatograph (Perkin-Elmer, Autosystem XL) equipped with a flame ionization detector (FID). A BPX 90 capillary column (30 m length × 0.25 mm i.d. × 0.25 μm film thickness) from SGE was used. The temperature program was as follows: initial temperature 145 °C (1 min holding), raised to 170 at 10 °C/min, held for 10 min, raised further to 230 at 15 °C/min, and then held for 4 min. The injector and detector temperatures were 270 and 280 °C, respectively. The carrier gas was hydrogen at a flow rate of 0.7 mL/min. All samples were injected in duplicate. Chromatograms were recorded using ChromQuest software. Methyl esters were identified by comparing their retention times to those of a FAME mix purchased from Sigma. Additionally, *trans*-octadecenoic acid (elaidic acid) and *cis*,*trans*-9,12- and *trans*,*trans*-9,12-octadecadienoic acid were purchased from Alltech. Fatty acid methyl esters in the samples were quantified by comparing the area under their peaks in the chromatogram to that of methyl heptadecanoate (derived from the internal standard).

CML Quantification. CML was quantified by GC−MS−MS according to the method of Charissou et al. (*16*), after methylation of the carbonyl groups and acylation of the amine residues. The solid food samples were crushed, homogenized, and then defatted with two successive extractions by a hexane/methanol (2/1, v/v) solution. The samples were hydrolyzed in 6 N HCl at 110 °C for 18 h. The hydrolyzed samples were derivatized after addition of the internal standard cycloleucine (CL) (10 μg/100 mg of sample) by thionyl chloride in methanol. The CML was quantified by selected ion monitoring using a FOCUS GC (Thermo Electron Corp.) gas chromatograph coupled to a PolarisQ ion trap mass detector. The injection was made in the split mode at 250 °C. A 30 m × 0.25 mm × 0.25 μm DB5-MS capillary column was used. The temperature program was as follows: initial temperature 70 °C (2 min hold), raised at a rate of 5 °C/min to 260 °C and further at a rate of 15 °C/min to 290 °C, and then held for 5 min. The carrier gas was helium at a flow rate of 1.5 mL/min. The mass spectrometer was operated in the electron impact mode at 70 eV. $m/z = 392$ as a parent ion was isolated using an energy of 0.90 V. The ions generated were $m/z = 374$ (25%), 360 (100%), 332 (40%), and 206 (10%). Data were collected and integrated with Xcalibur software (Thermo Electron Corp.). The limits of quantification are 0.3−0.9 ng/mg of dry matter.

Statistical Analysis. The results were expressed as the mean values and standard errors (SEs). Analysis of variance (ANOVA) was included in the data treatment to study the variation of the results, and Tukey's mean separation test was included to evaluate significant differences among the means ($P < 0.05$). All analyses were performed in duplicate or triplicate. Statistical analyses of the data were carried out using Statistica 7 StatSoft software. Kinetic modeling was done on MATLAB 7, The MathWorks, Inc. software.

RESULTS AND DISCUSSION

Evolution of Moisture Content during Processing. Figure 2 shows the decrease of nut and tahina moisture content during processing. In nuts (**Figure 2a**), roasting led to a significant loss in moisture content (63%, 52%, and 51% loss in pistachios,

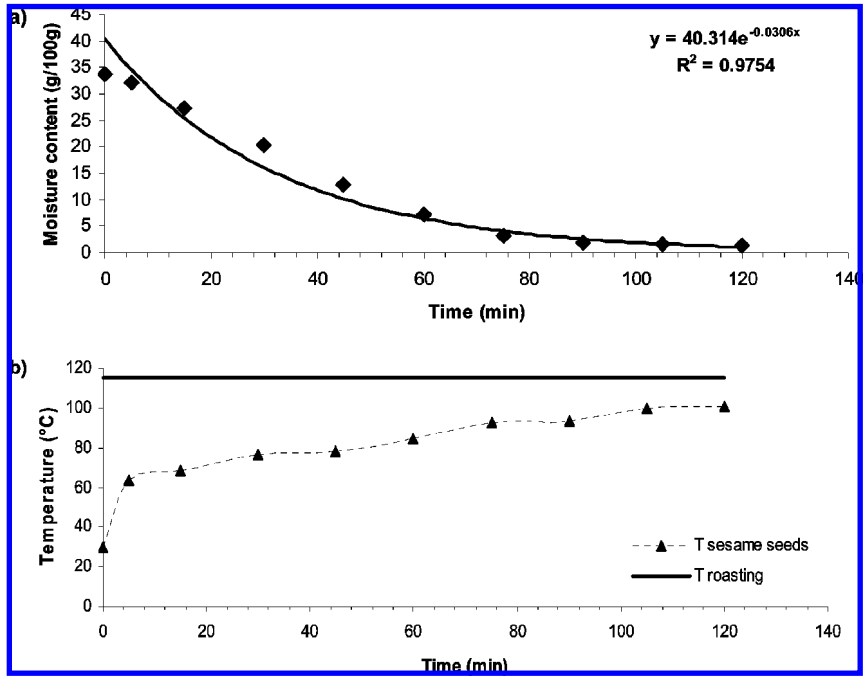

Figure 3. Modeling of the (**a**) moisture content decrease (continuous line, exponential fitting curve of moisture content evolution) and (**b**) temperature increase during roasting of sesame seeds. Data are averages of three measurements.

almonds, and peanuts, respectively). In tahina (**Figure 2b**), heating induced in the end product 85% water loss from the raw sesame seeds. During roasting of sesame seeds, water loss was around 96% between the beginning and the end of roasting. The evolution curve in **Figure 3a** shows that the roasting process induces an almost complete drying off of the water still present in sesame seeds before roasting. The dehydration started within the first 5 min of the roasting process, increased linearly until 75 min, and then proceeded with decreasing drying rate, which is typical for the final drying period. Exponential fitting provided a satisfactory description ($R^2 = 0.97$) of the moisture content evolution (**Figure 3a**). Similar results were reported by Kahyaoglu and Kaya (*17*) during roasting of sesame seeds.

Product Temperature Evaluation during Roasting of Sesame Seeds. The product temperature fluctuates only at the very beginning of the roasting process with a relative standard deviation (RSD) of 6%. At later stages, the roasting process is much more reproducible (RSD of 1−2.5%) at different places of the batch.

Figure 3b shows the mean temperature profile of sesame seeds during 120 min of roasting at 115 °C. The fast temperature increase during the first 5 min was followed by a progressive stabilization. During the whole roasting time, the sesame seed temperatures did not fully reach the inlet temperature. A plateau of 100 °C was reached after 105 min, which was held till the end of the roasting process.

Effect of Processing on Lipid Oxidation Changes. *Oxidative Quality of Raw Material.* Both raw nuts and sesame seeds used in this study had low initial hydroperoxide values (between 0.02 and 0.25 mmol/100 mg of fat). However, they showed relatively high initial *p*-anisidine (between 4.3 and 5.4) and TBAR (between 0.65 and 1.8 mg of MDA/kg) values compared to those of other studies (0.28 and 0.41 mg of TBARs/kg in raw and roasted almonds, respectively (*18*); pAV between 2 and 4 in fried salted peanuts (*19*)), meaning that oxidation was taking place during the storage period before processing. This provides evidence that the hydroperoxide value does not always reflect the past history of the food. It is only used as an indicator of the initial stages of oxidation since hydroperoxides decompose rapidly during storage. Therefore, the hydroperoxide value may not necessarily be indicative of the actual extent of lipid oxidation; instead, TBARs and pAV, which are cumulative measures over a period of time, are often used.

Peanuts had significantly higher initial TBAR values as compared with almonds and pistachios, despite similar pAVs, which may be due to their higher linolenate content. Linolenic acid contains two inner allylic methylene groups; thus, double bonds in Cl8:3 are twice as likely to shift and form a conjugated diene than C18:2, yielding more malondialdehyde (*20*). The TBA test is more sensitive than the *p*-anisidine value to evidence LPO when applied to food samples rich in linolenic acid (*9*).

Evolution of Hydroperoxides during Processing. At temperatures above 100 °C, hydroperoxides, the primary oxidation products, appear only transitorily and decompose rapidly into a multitude of volatile and nonvolatile products (*21*). Alcohols, saturated aldehydes, α,β-unsaturated aldehydes, and epoxy compounds have been reported as major secondary oxidation products (*21*). In the conditions of this study, hydroperoxides are only formed transitorily. This was reflected in the amount of hydroperoxides measured, which did not reach elevated values in the end products. **Figure 4** shows the changes in hydroperoxides during the manufacture processing of nuts (**a1**) and tahina (**b1**). In nuts, roasting provoked an increase in the amount of hydroperoxides, indicating that this stage induces the initiation of lipid oxidation. The level of hydroperoxides decreased in the following stages (C and D) as a result of hydroperoxide decomposition. The couple time/temperature of roasting was a critical factor in the formation of lipid hydroperoxides, as pistachios (190 °C/10 min) presented the highest level of hydroperoxides immediately after roasting, followed by peanuts (180 °C/10 min) and then almonds (180 °C/8 min). In tahina, the level of hydroperoxides increased after dehulling as a result of a possible slight heating due to friction, causing the initiation of the oxidation process followed by a continuous increase during the next steps. A maximum was reached at the beginning of roasting, as the severe heat treatment of this process

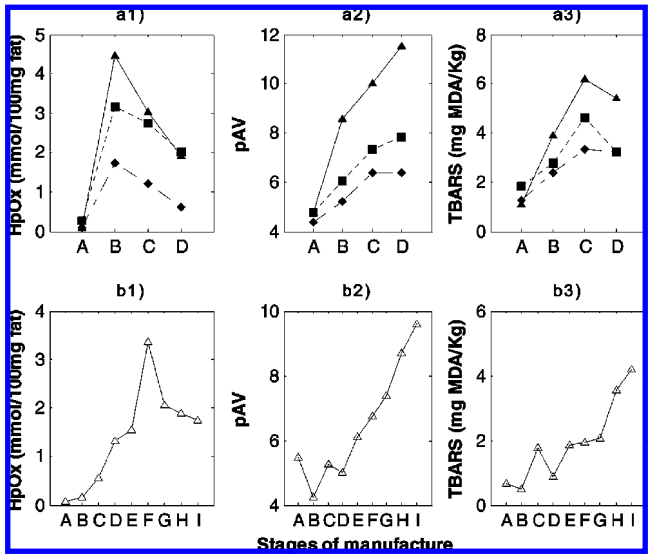

Figure 4. Lipid oxidative deterioration during the stages of processing of (**a**) nuts (pistachios, solid line; almonds, dashed−dotted line; peanuts, dotted line) and (**b**) tahina: hydroperoxides (**a1** and **b1**), pAV (**a2** and **b2**), and TBARs (**a3** and **b3**). Data are averages of two measurements.

step was associated with rapid decomposition of hydroperoxides (**Figure 4b1**). It is noteworthy that this pattern could not be ascertained during roasting of nuts because sampling throughout the roasting step was not possible.

Evolution of the pAV during Processing. The pAV measures high molecular weight saturated and unsaturated carbonyl compounds in triacylglycerols. The pAV increased for all nuts during roasting (**Figure 4a2**). For almonds which contain less PUFAs than other nuts (22% versus 28% and 31% in pistachios and peanuts, respectively, data not shown), this increase was much slower than for the other nuts. This is not surprising as the response for this test depends not only on the amount of aldehydic components but also on their structure. It has been shown that the reaction is more intense with diunsaturated aldehydes than with monounsaturated and saturated aldehydes (*22*). Moreover, a double bond in the carbon chain conjugated with the carbonyl double bond increases the molar absorbance 4−5 times. This means that α,*β*-unsaturated aldehydes will contribute more to the pAV than saturated and other unsaturated aldehydes (*22*). The fact that oxidative degradation of oleic acid forms less α,*β*-unsaturated aldehydes than do linoleic and linolenic acid (*22*) explains the lower response observed in almonds. Pistachios had significantly higher pAVs as compared with almonds and peanuts, which may be due to the more drastic roasting parameters used as compared to those of the other nuts (higher roasting temperature than for peanuts and higher roasting time/temperature than for almonds), together with a lower antioxidant (total polyphenols and tocopherols) profile of pistachios (data not shown). In tahina (**Figure 4b2**), pAV increased throughout the processing manufacture, except in two stages (B and D), where the pAV decreased.

Evolution of the TBARs during Processing. Production of the TBARs in nuts and tahina is presented in **Figure 4**. Pistachios had again significantly higher TBAR values as compared with almonds and peanuts (**Figure 4a3**), for the same reasons as explained above for the pAV. It was observed that TBARs in nuts reached a maximum (stage C) and then declined, perhaps due to further reaction of carbonyl compounds, especially MDA, with proteins during roasting (*23*). In tahina (**Figure 4b3**), TBARs increased throughout the processing, except in two stages (B and D), where the TBARs decreased.

The different patterns observed for the pAV and TBARs in nuts reveal the cumulative nature of high molecular weight aldehydes measured by the pAV, as the latter continuously increased during roasting and even after, even though heat treatment was stopped. On the contrary, TBAR values decreased after roasting of nuts because of the highly reactive nature of MDA. Since oxidation rates tend to be slower during these stages (C and D) than during roasting as heat treatment was stopped, the rate of decomposition of MDA overtakes the rate of formation, resulting in such a decay. This difference was not observed in sesame seeds; the pAV and TBARs followed the same pattern even after roasting, as a heat treatment was still applied in the following stages (grinding and sterilization), promoting the continuous formation of MDA.

Parallel Evolution of Hydroperoxides, the pAV, and TBARs during Roasting of Sesame Seeds. **Figure 5** shows the time course of hydroperoxides, the pAV, and TBARs during roasting of sesame seeds. Hydroperoxide formation exhibited a bell-shaped curve as a function of time. Such evolution is typical of the transient hydroperoxide formation step, which results from both the formation and degradation rates. The level of hydroperoxides increased fast and sharply almost immediately after the beginning of roasting, until a maximum was reached at 30 min (3.5 mmol/100 mg of fat), before a continuous decrease was observed. Some studies (*24*) showed that hydroperoxides reached a plateau at a certain time of thermal treatment, indicating that a steady state has been obtained. Tilbury and Miller (*24*) found similar results when heating linoleic acid at 60 °C. In our case, the roasting temperature continuously increases during the roasting process (**Figure 3b**), preventing the steady state from being reached.

The pAV and TBARs began to increase significantly after 30 min, when the temperature at the seed surface reached 80 °C, whereas the hydroperoxide concentration dropped. This increase was linearly proportional to the temperature increase ($R^2 = 0.98$ for both the pAV and TBARs). TBAR levels and the pAV in roasted sesame seeds were in good agreement ($R^2 = 0.98$, $n = 30$). However, the rate of formation of *p*-anisidine was steeper than that of TBARs as shown in **Figure 5**. This is again indicative of the cumulative nature of high molecular weight aldehydes measured by the pAV and the reactive nature of MDA measured by TBARs as explained before.

In conclusion, changes in lipid oxidation indicators (hydroperoxides, pAV, TBARs) reveal significant LPO in nuts and sesame seeds during heat processing. The above results also suggest that the indicators of the secondary peroxidation step, *p*-anisidine and TBAR values, are a better index of nut and sesame seed oxidation in the end product than the hydroperoxide value. However, the hydroperoxide value provides a sensitive indication of the initial oxidation rates. Accordingly, the levels of hydroperoxides indicated that pistachios were more prone to oxidation than almonds and peanuts, and peanuts more than almonds. This was confirmed by the results of the secondary oxidation products *p*-anisidine and TBARs. Finally, the particular resistance of almonds against oxidation (revealed by the lowest *p*-anisidine and TBAR values) compared to that of other nuts may be explained by the high level of MUFAs, especially oleic acid, and the high polyphenol and tocopherol (especially α-tocopherol) content (data not shown). Moreover, the highest polyphenol degradation rate was found in almonds, compared to other nuts, suggesting a more efficient antioxidant activity

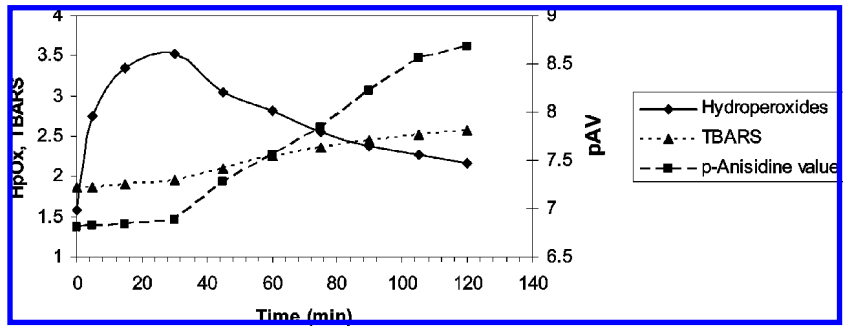

Figure 5. Time course patterns of hydroperoxide, *p*-anisidine, and TBAR formation during the roasting of sesame seeds. Data are averages of two measurements.

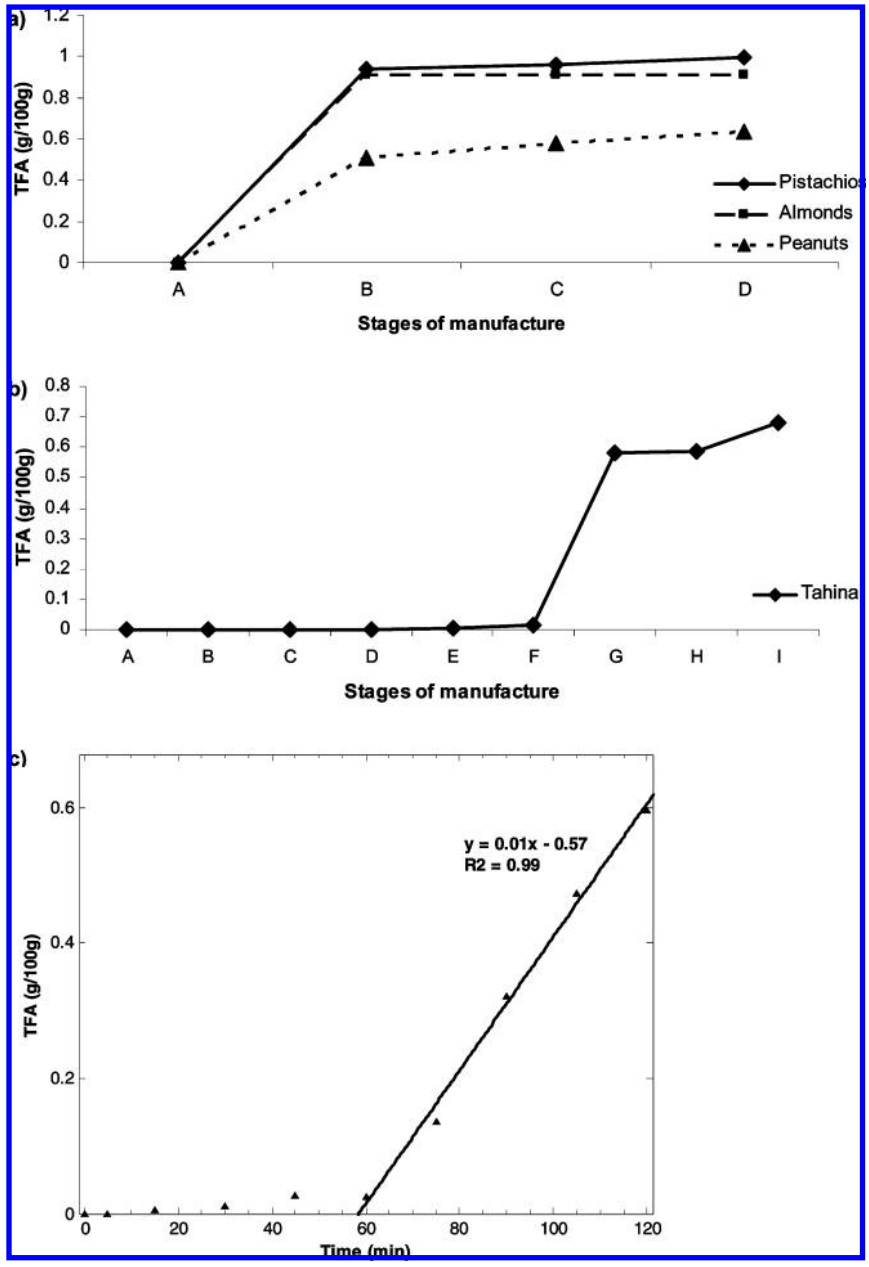

Figure 6. CML accumulation during the stages of processing of (**a**) nuts and (**b**) tahina and (**c**) during the roasting of sesame seeds (continuous line, exponential fitting curve of CML accumulation in sesame seeds during roasting). Data are averages of two measurements.

of the polyphenols present in almonds (data not shown). This stability was reflected by a lower PUFA degradation (data not shown).

Effect of Processing on CML and tFA Formation. *CML Formation during Processing.* CML was absent in the raw samples. **Figure 6** shows the formation of CML during the processing of nuts and tahina. In general, CML increased significantly with processing, providing values in the final product close to 17 ng/mg in pistachios and tahina, 15 ng/mg in peanuts, and 12 ng/mg in almonds. In nuts (**Figure 6a**),

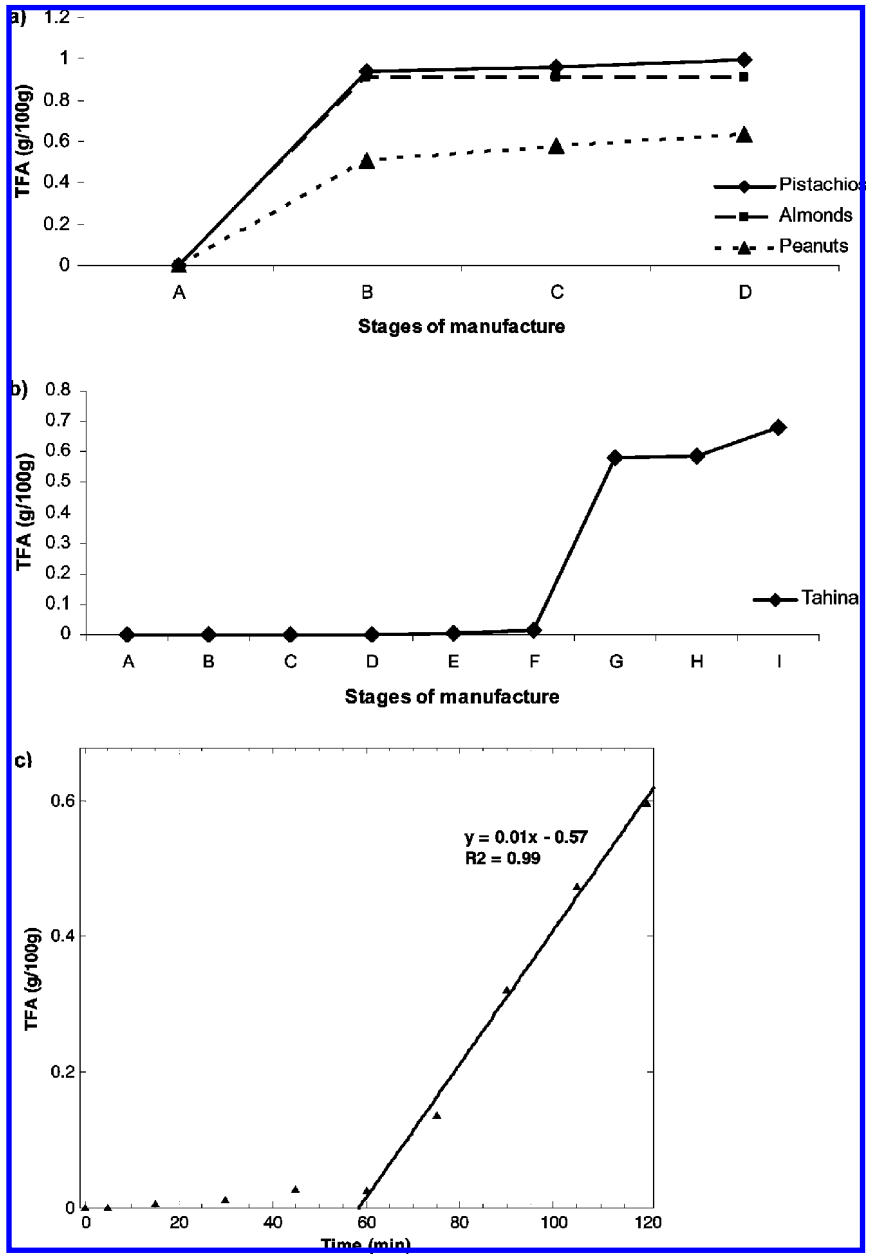

Figure 7. *trans* fatty acid accumulation during the stages of processing of (**a**) nuts and (**b**) tahina and (**c**) during the roasting of sesame seeds (continuous line, linear fitting curve of tFA accumulation in sesame seeds during roasting). Data are averages of two measurements.

roasting was the step mostly responsible for CML formation. In tahina (**Figure 6b**), CML was also produced during the roasting process (G), reaching a value of 10 ng/mg, but its concentration still continued to increase during milling (H) (32.4% increase) and sterilization (I) (32.6% increase). **Figure 6c** shows the evolution of CML during the roasting of sesame seeds. After a lag time (about 60 min) where no significant CML was detected, CML increased exponentially as a function of time ($R^2 = 0.99$). The formation of CML did not start until the temperature of the sesame seeds reached about 85 °C, around 60 min, whereas carbonyl compounds already appeared at 30 min, indicating that perhaps the detection sensitivity for CML is lower than that of total carbonyl compounds. We found a high negative correlation ($R^2 = -0.96$) between CML accumulation and PUFA degradation in sesame seeds (data not shown) and a positive correlation between CML accumulation and lipid oxidation indices, *p*-anisidine and TBARs ($R^2 = 0.89$ and $R^2 = 0.84$, respectively). These results support the hypothesis that CML is mainly formed from PUFA oxidation products produced in heat-treated PUFA-rich food (*7*). Glyoxal, suggested to be a key substrate for CML formation (*25*), has been shown to be produced during methyl linoleate autoxidation (*26*). These data were later corroborated by diverse studies reporting glyoxal formation during UV irradiation of polyunsaturated fatty acids, oxidation of linoleic acid in an iron ascorbate model system (*26*), or arachidonic acid autoxidation (*5*). This dialdehyde is believed to be produced by further oxidation of α,β-unsaturated aldehydes formed during LPO (*26*). CML also showed satisfactory correlation when modeled exponentially over the moisture content ($y = 73.84e^{-1.688x}$, $R^2 = 0.94$). This indicates that the moisture content may be a critical factor in CML formation in roasted nuts and seeds, since glycoxidation and lipoxidation are promoted by low moisture contents (*27*).

trans Fatty Acid Formation during Processing. tFAs were absent in the raw samples. **Figure 7** shows the formation of tFAs during the processing of nuts and tahina. In general, tFAs increased significantly with processing, providing values in the

final product of around 0.95 g/100 g in pistachios, 0.5 g/100 g in peanuts, 0.9 g/100 g in almonds, and 0.6 g/100 g in tahina. In nuts (**Figure 7a**), roasting was the main step responsible for the development of tFAs with no further significant increase during the following stages as no further heat treatment was applied. Levels of tFAs remained stable following roasting since tFAs tend to be more stable during storage compared with FAs with *cis* bonds (*28*). In tahina (**Figure 7b**), tFAs appeared significantly after the roasting process (G), reaching a value of 0.58 g/100 g, and further increased during sterilization (I) (16.5% increase). **Figure 7c** shows the evolution of tFAs during the roasting of sesame seeds. After a lag time (75 min) where no significant tFA was detected, tFAs increased linearly ($R^2 = 0.99$) as a function of time. The formation of tFAs did not start until the temperature of the sesame seeds reached about 92.5 °C. At the end of the roasting process, the tFA content reached a value of 0.59 g/100 g, yielding a 4-fold increase in tFAs between 60 and 120 min of roasting.

On the basis of these results, we conclude that nuts and sesame processing induced significant lipid peroxidation and formation of neoformed contaminants (NFCs), such as CML and tFAs. The roasting step was the critical process, despite tahina sterilization further contributing to the final LPO product and NFC accumulation. Pistachios appeared to be the nuts mostly concerned by such reactions, due to the more drastic thermal conditions applied. No recommendation is proposed for NFCs up to date, except for tFAs (*4*). Although the tFA content was always lower than the recommended maximal limit, fixed at 1 g/100 g (*4*), the maximal levels found in this study were close to this limit (maximum of 0.94 g/100 g), and analysis of commercial products ($N = 20$) revealed that some products have reached the limit (data not shown).

Due to the potential negative health impact of LPO products, a better control of heat processing, namely, roasting, should be recommended. Nuts and seed products appear to be especially prone to those side reactions due to their high PUFA content, so that attention should be paid to the distribution of NFC levels in such products. Improvement of nuts and tahina safety implies optimization of the roasting conditions (nuts and tahina) and sterilization technology (tahina) without compromising their microbiological safety (*Salmonella* in tahina). Therefore, establishing the optimal roasting temperature/time is critical to balancing these effects while maintaining the desired organoleptic properties. In general, longer roasting residence times and moderate roasting temperatures (130−150 °C) are preferred for optimum oxidative stability, since it was found that the oxidation rate of roasted nuts increased considerably with increasing product temperatures, while process duration was only of minor influence (*29*). Moreover, roasting was shown to increase the size of intracellular pores, thereby increasing oxygen transfer during and after roasting, leading to oxidation over time. Thus, prevention of lipid oxidation in roasted nuts and seeds needs the integer tissue structure to be maintained (*29*). Recently, a two-step roasting process, using a mild temperature (∼130 °C) in the first step for plasticizing the product, followed by longer duration and higher but as low as possible temperatures for finishing roasting, was proposed to achieve this. The two-step roasting process kept the cell's pore size increase minimal, allowing a higher oxidative stability while maintaining desirable roasted flavor (*29*). Detailed investigations should be carried out to find optimal process conditions, together with information derived from sensory panels. Regarding roasting technology of sesame seeds, adoption of continuous, more efficient, and less time-consuming technologies, such as fluidized-bed roasting, is advisable (*30*). The cradle roasters with discontinuous action, as currently used for sesame roasting, have a low performance because of the characteristics of heat transfer to the seeds. The operation duration (approximately 2 h) and the immediate contact of the product and the air in the premise favor lipid oxidation and decrease the roasted sesame quality. With the continuous processes, the roasting duration can be sharply reduced to 25−30 min (*30*).

ABBREVIATIONS USED

BHT, butylated hydroxytoluene; CML, carboxymethyllysine; DM, dry matter; FID, flame ionization detector; LPO, lipid peroxidation; MDA, malondialdehyde; NFCs, neoformed compounds; pAV, *p*-anisidine value; RSD, relative standard deviation; SFAs, saturated fatty acids; TBA, thiobarbituric acid; TBARs, thiobarbituric acid reactive substances; TCA, trichloroacetic acid; tFAs, *trans* fatty acids.

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