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Analytical Methods

A novel, fast and accurate chemiluminescence method for measuring lipoxidation in almonds and almond-based products during processing and storage

Sanaa Tazi, Antoine Puigserver, El Hassan Ajandouz *

Biosciences-ISM², UMR 6263, CNRS – Université Paul Cézanne-Aix Marseille III, Case 342, Faculté des Sciences et Techniques de Saint Jérôme, 13397 Marseille, France

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ABSTRACT

An analytical method for investigating lipoxidation processes based on soft extraction and chemiluminescence (CL) detection methods was developed and tested on almond and almond-derived foodstuffs. The TBARS method, which detects any carbonyls generated by lipoxidation, was used for comparative purposes. When developing the present method, the classical chloroform–methanol procedure was first used to extract the CL reacting substances initially generated, and solubilizing agents were then screened for optimal extraction. In the detection stage, cytochrome C was used as a reference substance for screening the CL signal enhancers. The method presented here was based on an extraction step with a dimethylsulfoxide/Gum Arabic mixture, and hemin was used to enhance the luminescence signal. This method accurately detects the lipoxidation processes triggered by the physical and chemical treatments of all kinds which are applied during almond processing. The simultaneous use of the present method and the TBARS method gave a broader picture of the chemical interactions involved, including the lipoxidation process.

1. Introduction

The almond is a useful nut which is known to be beneficial to human health, due to the subtle combination of nutrients and micronutrients it contains (Jenkins et al., 2002; Spiller & Miller, 2003). Its world production usually exceeds 1.5 million tons, and the main regions involved in its production are California and the Mediterranean area, especially Spain and Italy. It is used in various formulations and proportions for human dietary purposes and in lower levels in the pharmaceutical and cosmetic sectors.

The lipid content of almonds ranges from about 40% to 60%, depending on the genetic variety and the environmental conditions. Oleic acid, linoleic acid and linolenic acid account on a mass basis for about 70%, 20% and less than 1%, respectively (Abdallah, Ahumada, & Gradziel, 1998; Askin, Balta, Tekintas, Kazankaya, & Balta, 2007; Romojaro, Riquelme, Gimenez, & Liorante, 1988). The lipid fraction also includes lipid soluble vitamins and pigments, such as carotenoids and phenolic compounds. The lipid components tend to undergo changes during almond storage and processing (García-Pascual, Mateos, Carbonell, & Salazar, 2003; Harris, Westcott, & Henick, 1972; Rizzolo, Senesi, & Colombo, 1994; Zacheo, Cappello, Gallo, Santino, & Cappello, 2000; Zacheo, Cappello, Perrone, & Gnoni, 1998).

As with most high-lipid nuts, foodstuffs and dietary ingredients in general, the deterioration of almonds results largely from lipoxidation, which is enhanced by chemical and physical factors such as the degree of unsaturation, water activity, temperature, light, and oxidants such as transition metals. Reducing substances are therefore commonly used as additives in the food industry and other sectors. There exist a large range of analytical methods for monitoring the initial and advanced stages of the lipoxidation process (Benzie, 1996; German, 1999; Wheatley, 2000). However, consumers still require the deterioration due to lipid oxidation to be controlled for reasons such as the appearance, organoleptic acceptance and toxicity of the products, as well as their nutritional value.

As far as studies on almonds are concerned, the various stages in the oxidation reactions have been monitored using the peroxide value method (Baiano & Del Nobile, 2005; García-Pascual, Mateos, Carbonell, & Salazar, 2003; Rizzolo et al., 1994) and spectrophotometric methods based on either the specific UV absorbance (Baiano & Del Nobile, 2005; Rizzolo et al., 1994) or visible TBARS detected (Zacheo et al., 1998, 2000). The TBARS method actually detects the pool of carbonyls generated by lipoxidation and other chemical processes, such as the non enzymatic browning reactions (NEB). To our knowledge, luminescence detection methods, which can also be used to measure peroxides (Sharov, Kasamanov, & Vladimirov, 1989; Yasuda & Narita, 1997), have never previously been tested in the case of almonds. The main problem here in fact involves the use of organic solvents for extracting the apolar peroxides generated during the initial stages of these reactions,

* Corresponding author. Tel.: +33 4 91 28 81 36; fax: +33 4 91 28 84 40.
E-mail address: el-hassan.ajandouz@univ-cezanne.fr (E.H. Ajandouz).

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especially at factories dealing with almonds and other ingredients, where the lipoxidation process needs to be controlled.

Calissons are a typical French almond-based confectionery product marketed in the south of France: they are based on peeled almonds, crystallized melon and a mixture of glucose and sucrose. No additives are used in these delicacies, and the optimum shelf life under ambient temperature conditions is 9–10 months. However, some early rancidity sometimes occurs, and it was decided to investigate the causes of this deterioration. For this purpose, it was necessary to develop an accurate and sensitive method of analysis which could be used *in situ* at plants in the processing and packaging areas.

An analytical method based on soft extraction and chemiluminescence detection was therefore developed. This method is suitable for monitoring peroxidation processes in the early stages of almond processing. The experimental approach is described and discussed here up to the final stage in the procedure, which involves the use of a mixture of Gum Arabic (GA) and dimethylsulfoxide (DMSO) for extracting the CL reactive substances (CLRS) and that of hemin as a luminescence signal enhancer. This method was used to screen the peroxidation occurring during the almond processing and Calisson production processes under both normal and abnormal conditions. The TBARS method was used to obtain an overall analytical view of the chemical interactions in which lipoxidation is involved.

2. Material and methods

2.1. The samples

The samples used here were provided by the Confiserie du Roy René, Aix-en-Provence, France: non peeled and peeled almonds; almond powder; almond pastes (P1: peeled almonds + crystallized melon, in equal masses; P2: peeled almonds + crystallized melon + sugar syrup (glucose + sucrose)); stored paste (after 1-month at the factory under ambient light, temperature and water activity conditions); end product Calissons. The samples selected at the factory were analyzed immediately or on the following days (after being stored in the laboratory in the dark at room temperature under ambient water activity conditions).

2.2. TBARS method

Two TBARS methods were first tested, which were previously used to measure lipoxidation processes in a model system (Matsumura et al., 2003) and in almonds (Zacheo et al., 1998). Both methods gave fairly similar results, and the latter method was then adapted for use with almonds and their derivatives. Typically, in 2 mL Eppendorf tubes, the sample (0.3 g) was blended (by performing four 15 s sequences followed by 15 s rest periods) in 1 mL trichloroacetic acid (0.3 M) using an Ultraturax DHX520 blender (Labomoderne, France), and the homogenate was centrifuged for 10 min at 14,000 rpm. The homogenous intermediate phase was collected (500 μL using a Hamilton syringe) and centrifuged again for 2 min at 9000 rpm. The sample (400 μL) was then mixed with 400 μL thiobarbituric acid (TBA: 34 mM) in Pyrex capped tubes and the mixture was bubbled with nitrogen before being heated at 100 °C for 15 min. After cooling the preparation in ice, the absorbance was measured at 532 nm, using a Shimadzu CPS-240A Spectrophotometer. When necessary, the preparations were diluted to obtain absorbance values below 1.5. Control assays were performed on samples not containing almond extracts.

2.3. CL-developed method

2.3.1. Chemistry and use of CL

Chemiluminescence is based on the measurement of the light emitted during oxidation/reduction events in a given medium using a reaction initiator such as luminol and signal enhancers such as cytochrome C. The oxido-reduction triggered by the initiator is monitored as long as luminescence is emitted, as described by Pastorino, Maiorino, & Ursini, 2000 (Fig. 1). Alkaline conditions are necessary to obtain an optimum CL signal, and the detection can be coupled (Wheatley, 2000) or not (Stepanyan, Arnous, Petrakis, Kefalas, & Calokerinos, 2005; Zamburlini et al., 1995) with a separation system. CL-based methods have been used with both aqueous (Lind, Merenyi, & Ericksen, 1983; Nieman, 1989) and lipid (Yasaei, Yang, Warner, Daniels, & Ku, 1996) systems.

2.3.2. Development and optimization of the method

The aim was to develop an analytical method which could be used routinely at the factory participating in this study. Using

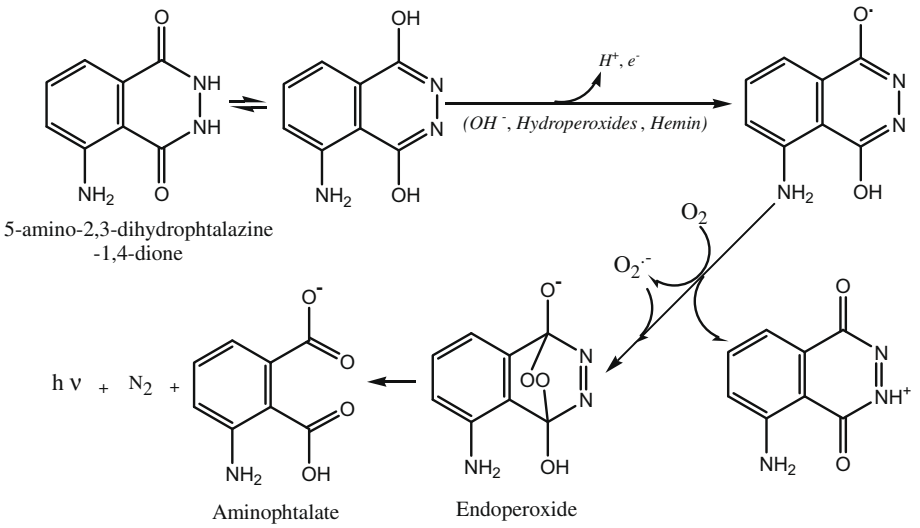

Fig. 1. Chemistry of the chemiluminescence based on luminol oxidation by hydroperoxides. Under alkaline conditions, luminol, which is in equilibrium with the enolic form, is oxidized by hydroperoxides and hemin into the corresponding radical. The superoxide generated under these conditions therefore reacts with the luminol radical, forming an endoperoxide, which is decomposed into aminophtalate and nitrogen, emitting a photon. Adapted from Pastorino, Maiorino, and Ursini (2000).

almond-based samples from the factory and luminol, various combinations of solubilizing agents, and CL enhancers were therefore screened to obtain an optimum CL signal.

2.3.2.1. Extraction. The classical Bligh and Dyer procedure involving extraction in chloroform/methanol (Bligh & Dyer, 1959) was first used, and the results obtained were taken as reference values for the luminescence signals. The extraction efficiency of the CLRS was then tested in the case of "soft" apolar solvents, which means solvents suitable for use at the factory, especially in terms of their toxicity. The organic solvents tested included ethanol, methanol, propanol and DMSO, in combination or not with emulsifiers (members of the Tween and Span families, and Gum Arabic). Since poor quantitative and qualitative responses were obtained with ethanol and the Span and Tween emulsifiers tested, these data are not shown here.

In the extraction procedure used, the samples (0.3 g) were blended as described above in the solubilizing mixture and centrifuged for 5 min at 5000 rpm. Depending on the solubilizing solution used, two to three homogeneous phases (lower, intermediate and upper) were visible in the Eppendorf tube. The homogenized phases were aliquoted (by collecting at least 50 μL using a Hamilton syringe) and used for luminescence measurements. Details are given in the tables and figures.

2.3.2.2. Analysis. Each sample (10 μL) was mixed (in a 10 s vortexing step) with luminol and an enhancer in 190 μL Glycine buffer (0.1 M, pH 10) placed in a 1 mL polystyrene tube. The tube was then placed in the detection chamber of the luminometer and the signal was integrated for 30 s. The blank used in the control assays contained no almond or almond-based products. This kinetic procedure, as well as the buffer and the volumes sampled, were previously optimized (data not shown). The cytochrome C enhancer was used with Bligh and Dyer's extraction procedure and served as the reference enhancer during the optimization of the extraction procedure, and then compared with Fe-EDTA, iron chloride and hemin using the optimum extraction procedure. The concentrations of luminol and the enhancers used are given in the legends to the figures. Stock solutions of luminol/enhancer in Glycine buffer were stored at −80 °C and used after being appropriately diluted. The CL values of hydrogen peroxide were used to check the stability of the stock solutions and compared with those obtained using almond and almond-derived foodstuffs. The Lucy Luminometer (Yelen, Ensuès-La-Redonne, France) was used to measure the luminescence.

The CLRS and TBARS measurements were repeated four times and the values were expressed relative to one gram of almond. CL was expressed in relative light units (RLU), whereas absorbance units at 532 nm (AU) were used with the TBARS method. The CL values recorded were divided by 10^5 to obtain an appropriate scale on which these data could be easily compared with the TBARS data.

3. Results and discussion

3.1. CL-based on methanol–chloroform extraction and TBARS methods

Fig. 2 shows the CL values (a) obtained after extraction using Bligh and Dyer's classical procedure and using luminol and cytochrome C as the initiator and enhancer, respectively, along with the TBARS values (b).

Both CL and TBARS values started to increase as soon as the almonds were peeled and subsequently reflected the effects of the physical and chemical treatments applied during the Calisson manufacturing process. The increase observed in the TBARS values obtained with almond paste was due to the carbonyl pool contributed by the caramelized sugars added, and the 50% additional increase in the Calisson product can be attributed to the carbonyls generated by NEB reactions, which can be expected to be enhanced by the heat treatment applied to almond paste during Calisson manufacture (Ajandouz, Desseaux, Tazi, & Puigserver, 2008). Likewise, the CL values increased during the processing, and an unexpectedly high signal was detected in the ground almond, which was also observed in the TBARS values, indicating in the latter case that lipoxidation occurs at advanced stages in the processing of this food ingredient. The use of ground almond instead of peeled almond as a Calisson ingredient was subsequently stopped at the Confiserie du Roy René. It is also worth noting that both the CL and TBARS methods discriminated clearly between the successive samples corresponding to various stages in the processing scheme, and between rancid and non rancid Calisson.

However; although the CL method based on Bligh and Dyer's extraction procedure run in parallel with the TBARS method and made it possible to follow more clearly the behaviour of the peroxide pools in the various stages in the manufacturing process, neither the TBARS method nor the CL method is suitable for installation in processing and packing areas.

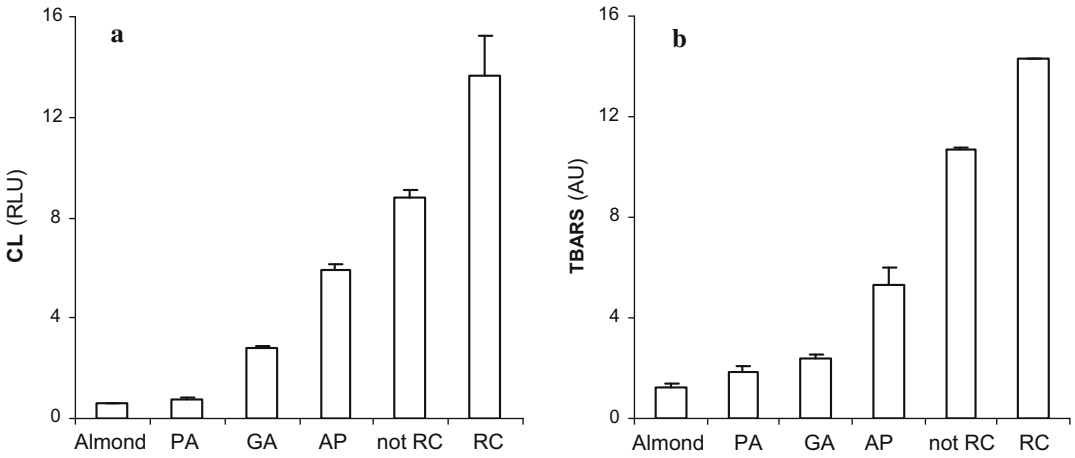

Fig. 2. CL values based on the extraction in methanol–chloroform (a) and TBARS values (b) in almonds and almond-based products. Luminescence was determined after extracting the lipids from the samples in a chloroform/methanol mixture and using luminol (1 mM) and cytochrome C (20 mM). PA: peeled almonds, GA: ground almonds, AP: almond paste, RC: rancid Calisson. The CL values given in this figure and the following figures have been divided by 10^5, as mentioned in the text, to facilitate comparisons with the TBARS data.

3.2. Optimization of the CL method

Fig. 3 shows the CL monitoring data obtained after the extraction step with some solubilizing mixtures. All four combinations made it possible to discriminate fairly clearly between the samples corresponding to various stages in the processing, and between rancid and non rancid Calisson. Note that the maximum signal obtained was about four-fold lower than that obtained after extraction in a methanol–chloroform mixture, and it can be assumed that similarly reacting peroxides were extracted. In fact, one does not need to extract all the reacting substances to be able to monitor the relative development of lipoxidation in processed foods or any other medium. As regards the solubilization efficiency of the mixtures tested, the combination propanol–DMSO–water was found to be the most efficient, followed by DMSO–GA, propanol–DMSO–GA and propanol–GA. As regards the discriminative power, the combination DMSO–GA was found to be by far the best, while the other three combinations were all found to have much lower discrimination efficiencies.

Commonly used luminescence enhancers were then screened using the DMSO–GA extraction procedure, with a view to obtaining optimum CL detection (Fig. 4). Fairly clear-cut differences were observed between the samples with all the enhancers tested. The enhancing values (with/without enhancer) were in the 3–6 and 3–24 range in the case of almond paste and Calisson, respectively. Ferric chloride was found to be the least efficient enhancer, whereas hemin was the most efficient, taking into account both the CL enhancing effect and the ability to discriminate between the various steps in the manufacturing process. It should also be noted that the day-to-day standard deviations were lower with hemin than with the other enhancers tested (data not shown).

On the other hand, as shown in Fig. 4, the luminescence value obtained with fresh Calisson (9 RLU per gram of almond) was fairly similar to that of 0.1 mM of H_2O_2 (12 RLU), and one might be tempted to express the CL values on the basis of the H_2O_2 equivalent, as has sometimes been done in the literature. However, H_2O_2 may not accurately reflect the oxidant power of a given lipid-rich medium because other parameters such as the solubility and reactivity may be involved; in line with this idea, it can be seen from Fig. 4 that the relative H_2O_2/fresh Calisson response varied depending on the enhancer used.

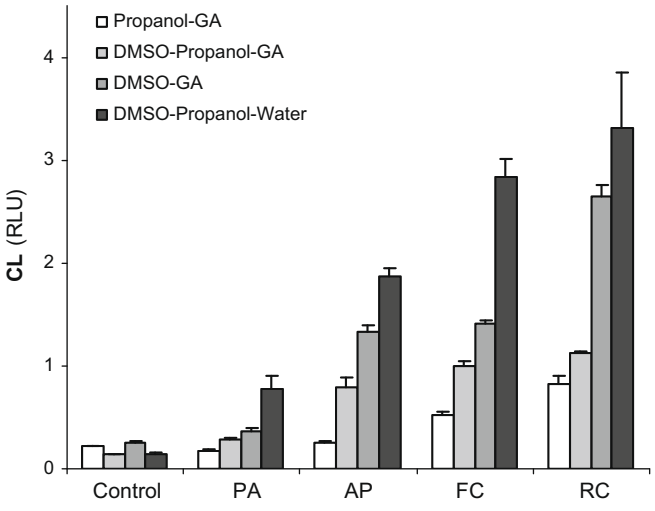

Fig. 3. Alternative extraction-based CL values obtained with peeled almonds and almond-based products. CL values determined after performing lipid extraction using the combinations of solubilizing agents specified in the text. The cytochrome C and Gum Arabic concentrations used were 0.4 mM of water and 5 mg/mL of water, respectively. The volume ratios used and the phases sampled for CL analysis were as follows: DMSO/propanol/water, 1:1:1, lower phase; DMSO/propanol/GA, 1:1:1, upper phase; propanol/GA, 2:1, lower phase; DMSO/GA, 2:1, intermediate phase. The meaning of the abbreviations PA, AP and RC is the same as in Fig. 2; FC: fresh Calisson; control: samples containing no almond.

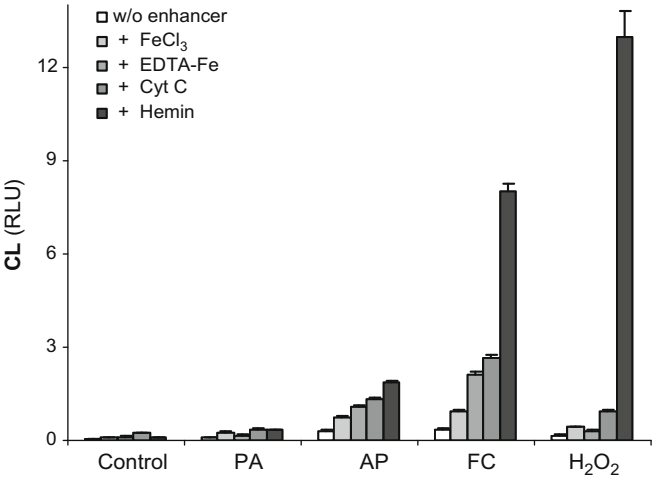

Fig. 4. "Soft" extraction CL values obtained with almond-based foodstuffs and hydrogen peroxide, depending on the CL enhancer used. Extraction of the CLRS was performed in DMSO–GA, using luminol (1 mM) and an enhancer (0.4 mM). Control: samples not containing any almond.

3.3. Screening of almond during the manufacturing process

Selected samples of almonds and almond products were analyzed on several dates spaced 2.5-months apart (Fig. 5). The values recorded in July show that the luminescent signals increased steadily from raw almonds to 3-month old Calisson. No significant increases occurred from P1 to P2, as the added sugars did not contribute any additional peroxides. However, a dramatic increase (13-fold) in the signal was observed in the 1-month stored paste, and the Calissons made with this paste (SPC) gave the highest signal. The values obtained with Calissons known to be rancid were mid-way between those obtained with the 3-month old Calisson and with SPC.

Six weeks later, the CL values increased markedly (3- to 4-fold) in the fresh and 3-month old Calissons, but only slightly (up to 30%) in the rancid Calissons and in Calissons manufactured from the stored paste. With the other samples, except for P2 (which showed a 2.3-fold increase), only a fairly small CL increase (of up to 25%) occurred between July and September. The fact that only a slight change was observed with samples RC1, RC2 and SPC suggests that the pool of CLRS had reached a maximum in these older samples, whereas in the fresh and 3-month old Calissons and the pastes, the values were still moving towards that maximum. Note that the paste stored in the processing area (SP) behaved like the Calissons made with that paste and the rancid Calissons, which suggests the presence of a peroxide load during the 1-month storage period under air and light in the processing area. The CL of the properly packed parent paste stored for 2.5-months at the laboratory in the dark showed only a 2.3-fold increase in comparison with the 13-fold increase observed in SP (Fig. 5). This shows the importance of the effects of light, temperature and probably water activity on the peroxide production rates, and means that the paste should not be stored for long periods before the Calissons are manufactured.

The same samples were subsequently analyzed using the TBARS method (Fig. 6). Fairly predictable values were obtained. The signal increased due to the additional sugar syrup loading the carbonyl

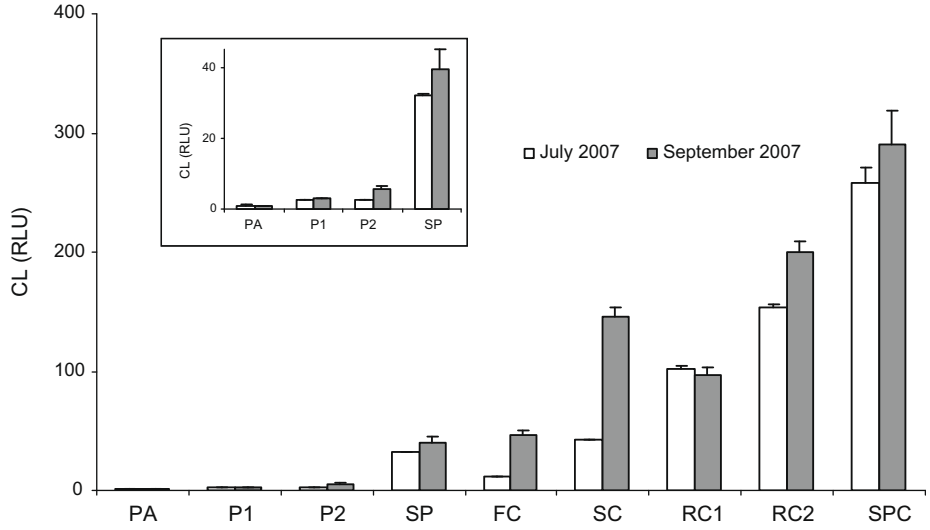

Fig. 5. CL behaviour observed in almond-based products, depending on the processing and storage conditions. PA: peeled almonds, P1: peeled almond + crystallised melon, P2: peeled almond + crystallized melon + sugar syrup, SP: stored paste, SPC: Calisson product from SP, FC: fresh Calisson, SC: 3-month stored Calisson, RC: rancid Calissons.

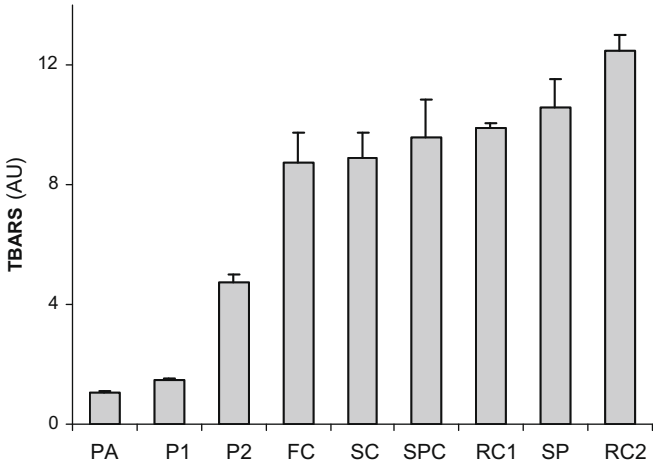

Fig. 6. TBARS values in various processed and stored almond-based samples. Abbreviations are as in Fig. 5.

pool, which was detected by the TBARS method. A marked further increase was then observed in the resulting Calissons (FC) due to the heat treatment applied during the final step. However, only a moderate increase was found to occur between non rancid Calissons (FC and SC) and rancid ones (RC1 and RC2); and interestingly, the stored paste and the Calissons made with this paste showed intermediate TBARS values, which suggests that the TBARS pool in the stored paste was already maximum. It should also be noted that neither the stored paste nor the Calissons made with this paste showed any abnormal taste or odour on the date of analysis, based on the sensory tests conducted at both the Laboratory and the Factory.

4. Conclusion

The analytical method presented here is a fast, sensitive and inexpensive method. All the data obtained on the almonds in various stages of processing were consistent with the expected effects of the physical and chemical treatments applied. The extraction procedure was carried out using microtubes and can be easily installed at the Factory, taking into account the recommendations of Material Safety Data Sheet reports regarding DMSO, as updated in 2006 (http://msds.chem.ox.ac.uk/ME/methyl_sulfoxide.html). This method makes it possible to clearly discriminate between the levels of lipoxidation products present throughout the production process, and the almond paste stored for excessively long periods gave the expected peroxide peaks.

The TBARS method yielded clearly detectable responses to the carbonyls added and generated in almond paste and the Calissons made with this paste, but discriminated less efficiently between the samples in terms of the rates of lipoxidation. In addition, as far as the sector in question here is concerned, the TBARS method is not practical to use in lipid-rich foodstuff processing areas; whereas the analytical method we have developed provides a comfortable means of discrimination, which can be adapted for detecting the peroxides present in either *in vivo* or *in vitro* samples for basic or applied research purposes. However, for both purposes, the TBARS method can be used in addition to CL to obtain a broader picture of chemical interactions involving lipoxidation.

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References

Abdallah, A., Ahumada, M. H., & Gradziel, T. M. (1998). Oil content and fatty acid composition of almond kernels from different genotypes and California production regions. *Journal of the American Society for Horticultural Science, 123*, 1029–1033.

Ajandouz, E. H., Desseaux, V., Tazi, S., & Puigserver, A. (2008). Effects of temperature and pH on the kinetics of caramelisation, protein cross-linking and Maillard reactions in aqueous model system. *Food Chemistry, 107*, 1244–1252.

Askin, M. A., Balta, M. F., Tekintas, F. E., Kazankaya, A., & Balta, F. (2007). Fatty acid composition affected by kernel weight in almond (*Prunus dulcis* (Mill.) D.A. Webb.) genetic resources. *Journal of Food Composition and Analysis, 20*, 7–12.

Baiano, A., & Del Nobile, M. A. (2005). Shelf life extension of almond paste pastries. *Journal of Food Engineering, 66*, 487–495.

Benzie, I. F. F. (1996). Lipid peroxidation: A review of causes, consequences, measurement and dietary influences. *International Journal of Food Sciences and Nutrition, 47*, 233–261.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *The National Research Council of Canada, 37*, 911–917.

García-Pascual, P., Mateos, M., Carbonell, V., & Salazar, D. M. (2003). Influence of storage conditions on the quality of shelled and roasted almonds. *Biosystems Engineering, 84*, 201–209.

German, J. B. (1999). Food processing and lipid oxidation. *Advances in Experimental Medicine and Biology, 459*, 23–50.

Harris, N. E., Westcott, D. E., & Henick, A. S. (1972). Rancidity in almonds: Shelf life studies. *Journal of Food Science, 37*, 824–827.

Jenkins, D. J. A., Kendall, C. W. C., Marchie, A., Parker, T. L., Connelly, P. W., Qian, W., et al. (2002). Dose response of almonds on coronary heart disease risk factors – Blood lipids, oxidized LDL, Lp(a), homocysteine and pulmonary nitric oxide: A randomized controlled cross-over trial. *Circulation, 106*, 1327–1332.

Lind, J., Merenyi, G., & Ericksen, T. E. (1983). Chemiluminescence mechanism of cyclic hydrazides such as luminol in aqueous solutions. *Journal of the American Chemical Society, 105*, 7655–7661.

Matsumura, Y., Egami, M., Satake, C., Maeda, Y., Takahashi, T., & Nakamura, A. (2003). Inhibitory effects of peptide-bound polysaccharides on lipid oxidation in emulsions. *Food Chemistry, 83*, 107–119.

Nieman, T. (1989). Detection based on solution-phase chemiluminescence systems. In J. W. Birks (Ed.), *Chemiluminescence and photochemical reaction detection in chromatography* (pp. 99–123). New York: VCH.

Pastorino, A. M., Maiorino, M., & Ursini, F. (2000). Kinetic analysis of lipid-hydroperoxides in plasma. *Free Radical Biology and Medicine, 29*, 397–402.

Rizzolo, A., Senesi, E., & Colombo, C. (1994). Studies on the storage of shelled and in-shell almonds. *Acta Horticulturae, 373*, 259–264.

Romojaro, F., Riquelme, F., Gimenez, J. L., & Liorante, S. (1988). Fat content and oil characteristic of some almond varieties. *Fruit Science Reports, 17*, 53–57.

Sharov, V. S., Kasamanov, V. A., & Vladimirov, Y. A. (1989). Selective sensitisation of chemiluminescence resulted from lipid and oxygen radical reactions. *Free Radical Biology and Medicine, 7*, 237–242.

Spiller, G. A., & Miller, A. (2003). Effects of plant-based diets high in raw or roasted almonds or roasted almond butter on serum lipoproteins in humans. *Journal of the American College of Nutrition, 22*, 195–200.

Stepanyan, V., Arnous, A., Petrakis, C., Kefalas, P., & Calokerinos, A. (2005). Chemiluminescent evaluation of peroxide value in olive oil. *Talanta, 65*, 1056–1058.

Wheatley, R. A. (2000). Some recent trends in the analytical chemistry of lipid peroxidation. *Trends in Analytical Chemist, 10*, 617–628.

Yasaei, P. M., Yang, G. C., Warner, C. R., Daniels, D. H., & Ku, Y. (1996). Singlet oxygen oxidation of lipids resulting from photochemical sensitizers in presence of antioxidants. *Journal of the American Oil Chemists' Society, 3*, 1177–1181.

Yasuda, M., & Narita, S. (1997). Simultaneous determination of phospholipids hydroperoxides and cholesterol ester hydroperoxides in human plasma by high-performance liquid chromatography with chemiluminescence detection. *Journal of Chromatography B: Biomedical Sciences and Applications, 693*, 211.

Zacheo, G., Cappello, M. S., Gallo, A., Santino, A., & Cappello, A. R. (2000). Changes associated with harvest ageing in almond seeds. *Lebensmittel-Wissenschaft and Technologie, 33*, 415–423.

Zacheo, G., Cappello, A. R., Perrone, L. M., & Gnoni, G. V. (1998). Analysis of factors influencing lipid oxidation of almond seeds during accelerated ageing. *Lebensmittel-Wissenschaft and Technologie, 3*, 6–9.

Zamburlini, A., Maiorino, M., Barbera, P., Pastorino, A. M., Roveri, A., Cominacini, L., et al. (1995). Measurement of lipid-hydroperoxides in plasma lipoproteins by a new highly sensitive "single photon counting" luminometer. *Biochimica et Biophysica Acta, 1256*, 233–240.