

ALMOND MILK PRODUCTION AND STUDY OF QUALITY CHARACTERISTICS

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Abstract

Almond milk production at pilot plant and basic characterization were performed. The study included the effect of 0.03 % lecithin addition to the raw product and pasteurization (90 °C, 15 s) with two step homogenization (180 MPa and 40 MPa), resulting in the comparison of four different almond milks: raw product (PB), raw product with lecithin (PBL), pasteurized product (PH) and pasteurized product with lecithin (PHL). Compositional characteristic of almond milks showed the proximate composition similar to those found in the market. The effect of lecithin addition is to improve product stability and these differences were observed at days 2 and 7 of storage at 4 °C and 25 °C. Total counts and Micrococccaceae were reduced by 2.21 and 3.64 log cycles at pasteurized almond milk while no viable Enterobacteriaceae nor yeast and mould remained in any pasteurized milk samples. Pasteurized almond milk inactivated lipoxygenase activity compared to untreated almond milk. The formations of oxidized substances (malondialdehyde and lipid hydroperoxides) in almond milk were all greatly reduced by pasteurization.

Keywords: Almond milk, Chemical stability, Homogenization, Microbial quality, Physical stability

1. Introduction

Recently, demands by consumers for vegetable milk have experienced a noticeable increase due to some problems of milk protein allergenicity and healthy life (Donkor *et al.*, 2007). Considerable attention has been given to soy and almond milk due to their good nutritional value and functionality (Watkins, 2005). However it has been reported that soy milk has high oxidation potential that contributes to rancidity (Kumar *et al.*, 2006), while, almond milk showed interesting compositional attributes to investigate. One of the reasons for the popularity of almond milk was its high added-value foodstuff. Almond is chiefly valued due to its balanced composition in protein content and fat (Jenkins *et al.*, 2002), fiber, vitamin and minerals and no lactose content (Spiller and Miller, 2003). This was reported by many almond species such as Indian almond (Agurbiade *et al.*, 2011), Turkey almond (Omar *et al.*, 2011) and almond exotic (Lima *et al.*, 2007). Almond milk is an oil-in-water emulsion with the dispersed phase constituted by complex protein dispersion and oil droplets. Principally, emulsions are thermodynamically unstable systems and they tend to breakdown over time due to a number of different physicochemical mechanisms, which may occur concurrently such as creaming, flocculation, coalescence, phase inversion and/or Ostwald ripening (McClements, 2005). Emulsion stability is one of the most important factors governing the shelf-life of colloidal foods. For that reason, energy input through shaking, stirring, homogenizing, or spray processes are needed to get a fine emulsion with precise properties of texture and a high degree of stability (Desrumaux and Marcand, 2002). Homogenization operation diminishes

fat globule size in order to prevent skimming and coalescence of fat during the storage of food emulsions. Proteins in almond composition, act as emulsifier, which is fundamental for obtaining almond milk. However, depending on the pressure applied at the homogenization operation, volume fraction of oil droplets could be too much high related to the protein fraction, and thus to protect oil-water interface generated in the homogenization step. Emulsifiers such as lecithin are among the most commonly used emulsifiers for food processing due to their biocompatibility and functional properties (Dickinson, 2003). It facilitates the formation of emulsion and improves their stability by reducing the oil-water interfacial tensions and by forming a protective layer around the fat droplets to prevent them from aggregating (Guzey and McClements, 2006). Almond milk is highly nutritious medium and it favors growth of spoilage and pathogenic microorganisms. It also contains high concentrations of polyunsaturated fatty acids susceptible to oxidative and hydrolytic rancidity which produces undesirable volatile compounds and off-flavors (Wang *et al.*, 2008). Therefore, thermal treatments are applied to almond milk in order to extend its conservations (Hayes *et al.*, 2005). Previous study showed pasteurization can also be used to reduce some undesirable substances as lipoxidase that promote to rancidity (Athisaya *et al.*, 2003). Nowadays, consumers demand fresh and safe foods with characteristics that give them greater value. For this reason, technologists investigate and develop foods with minimal processing, to increase their durability in thermal microbiological, sensory and nutritional while maintaining the final product as close as possible to those of fresh product. Ultra high pressure homogenisation (UHPH) is an alternative process that is been investigated as new treatment for liquid food pasteurisation (Vachon *et al.*, 2002). UHPH contrary with conventional homogenisation used in the dairy and food industry imply much higher pressures. Pressure applied in UHPH treatments up to 350MPa is involved in destroying microbial cells and reducing particle size in colloidal food (Diels *et al.*, 2005a). This is because application of UHPH in liquid emulsions, such as almond milk, could have several beneficial results. This study is a preliminary work in the context of UHPH treatment of almond milk. The aim of the present work was to develop a base almond milk product for further UHPH application and study their main characteristics of quality (compositional, physicochemical, biochemical and microbiological) comparing raw and pasteurized products.

2. Methods

2.1 Production of almond milk

Grinded almond seed used in this study was kindly provided by Nectina, S. A. (Valencia, Spain). To obtain the vegetable milk 4% (w/w) almond in water at 80 °C was poured in a mixing tank and subsequently milled in a colloidal mill (E. Bachiller, B. S. A. Barcelona, Spain) at 80 °C followed by filtration to separate the liquid phase. The almond milk obtained was the base product (PB), which was homogenized in two step valve homogenizer (Niro Soavi, Model X68P, Parma, Italy) at 180 MPa and 40 MPa, and treated by pasteurization (90 °C, 15 s) in an indirect tubular system UHT equipment (Finamat heat exchanger, Model 6500/010, Gea Finnah GmbH, Anhaus, Germany). Lecithin (0.03 % w/w) was added during grinding process for production of almond milk with lecithin. Almond milk samples were collected and stored at -20 °C for physical and chemical analyses. Microbiological analysis was performed on the same day after treatment. Analyses were made on four individual productions of almond milk; raw almond milk or base product (PB), raw almond milk with lecithin (PBL), pasteurized almond milk without lecithin (PH) and pasteurized almond milk with lecithin (PHL). Before treatment, all equipments were disinfected by circulating a diluted mixture (20 %) of peracetic acid and hydrogen peroxide (P3-Oxonia Active, Ecolab Hispanic Portuguese, Barcelona, Spain) for 15 min at 30 °C.

2.2 Composition analysis

Analysis of fat content was made following Soxhlet method (AOAC, 2000) using petroleum ether for 6 hours. Protein content was determined using Khjedahl method (IDF, 2002). Moisture and ash content were analyzed by the reference AOAC Method, carbohydrate was estimated by difference (Pearson, 1976). pH of milk was measured using a MicropH Model 2001 (Crison, Barcelona, Spain). Analyses were performed in triplicate.

2.3 Physical stability

Samples were stored at 4 °C and room temperature with addition of 0.04 % sodium azide (NaN₃). Thirty ml of almond milk were put in flexible plastic tubes (32 mm diameter, 115 mm length) and centrifuged (Centrifuge Sigma 4K15, Postfach, Germany) for 45 min at 3000 rpm at 20 °C. Results were expressed as % of solids deposited after centrifugation. Physical stability of almond milk was measured on days 2 and 7 after processing. Analyses were performed in duplicate.

2.4 Microbial analysis

Decimal dilutions in peptone water solution were for microbial enumeration. Microbial analysis of almond milk was determined using different mediums. Total bacteria count were determined in Plate Count Agar (PCA) incubated at 30 °C for 48 h. *Enterobacteriaceae* counts were determined in Violet Red Bile Glucose Medium (VRBG) incubated at 37 °C for 24 h. To assess spore and mould counts, Rose-Bengala Chloranfenicol (RBGA) medium was used. *Micrococccaceae* count was analyzed using Manitol Salt Agar (MSA) while determine the presence and, in case of positive test, the number of *Bacillus cereus* Selective Agar (BA) containing Polimixin B was used and confirmed with commercial kit for easy identification “Microgen Bacillus-ID” (Microgen Bioproducts, UK). Sample was inoculated using “The Eddy Jet Spiral Plating System” (IUL Instruments, Germany). Colonies counting were measured using a “Flash & Grow Colony Counter System”, (IUL Instruments, Germany). All the mediums and respective supplements were purchase at Oxoid Ltd., Basingstoke, Hampshire, UK, and ensembles following the Manual Oxoid (Oxoid Ltd. 1995, Spain) Values were measured in duplicate.

2.5 Oxidation analysis

Oxidation analysis was performed by measuring the presence of lipid hydroperoxides, Malondialdehyde and Lipoxygenase (LOX) activity. Lipid hydroperoxides concentration was determined spectrophotometrically at 500 nm (CECIL 9000, CECIL Instruments, Cambridge, UK) according to the method described by Zacheo *et al.* (1998). Malondialdehyde (MDA) analysis performed according to the procedure by Fenaille *et al.* (2001). Absorbance was measured using a CECIL 9000 spectrophotometer (CECIL Instrument, Cambridge, United Kingdom) to 521.5 nm. The spectrum of oxidative dissolution (MDA) was recorded from 400 to 650 nm to an absorbance of 240 nm speed min⁻¹ against the reaction of blank (reference). The third derivative was obtained by electronic differentiation of the spectrum and the quantification was performed using the calibration curve. Lipoxygenase (EC 1.12.11.12) kinetic analysis was performed following the procedure described by Axelrod *et al.* (1981) with some modifications made by Van der Ven *et al.* (2005). Spectrophotometric measures at 234 nm were made in continuous mode, using

CECIL 9000 spectrophotometer (CECIL Instruments, Cambridge, United Kingdom). To express the LOX activity of the sample, a unit of enzyme was taken as equivalent to an increase absorbance at 0.01 min^{-1} . All enzyme determinations were done in triplicate.

2.6 Statistical analysis.

One-way analysis of variance (ANOVA) was used to compare mean values between samples. Differences were considered to be significant at $p < 0.05$. All statistical analysis was performed with SAS 9.1 software (SAS Inc, 2005) using *Student Newman Keuls* (SNK) to identify where the possible differences are.

3. Results

In this study, different almond milk products were produced with lecithin (0.03 %) added and without lecithin, which were subsequent homogenized and pasteurized. Then, the study was made in four almond milk products: raw almond milk or base product (PB), raw almond milk with lecithin (PBL), pasteurized almond milk without lecithin (PH) and pasteurized almond milk with lecithin (PHL). The chemical composition of almond seed and almond milks is shown in Table 1.

Table 1: Mean (\pm SD) values (w/w %) of chemical composition in almond milk

	<i>Almond Seed</i>	<i>PB</i>	<i>PBL</i>	<i>PH</i>	<i>PHL</i>
Dry matter	94.95 \pm 0.21	3.51 \pm 0.22 ^a	3.88 \pm 0.04 ^c	3.36 \pm 0.15 ^b	3.54 \pm 0.05 ^d
Fat	56.76 \pm 0.98	1.82 \pm 0.11 ^b	2.19 \pm 0.18 ^a	1.06 \pm 0.13 ^d	1.49 \pm 0.19 ^c
Protein	20.39 \pm 0.23	0.90 \pm 0.01 ^a	0.93 \pm 0.01 ^a	0.80 \pm 0.04 ^c	0.87 \pm 0.02 ^c
Ash	5.03 \pm 0.08	0.11 \pm 0.01 ^a	0.20 \pm 0.01 ^b	0.09 \pm 0.02 ^a	0.17 \pm 0.01 ^b
Carbohydrate	12.73 \pm 0.05	0.68 \pm 0.03 ^b	0.56 \pm 0.01 ^a	1.41 \pm 0.02 ^d	1.01 \pm 0.05 ^c

*Values within a row followed by the same letter are not significantly different $p > 0.05$.

La Rosa *et al.* (2000) found remarkable microbial loads (bacteria, moulds and yeasts), in fact genera belonging to *Enterobacteriaceae*, *Micrococcaceae* and *Bacillaceae* in almond product. Table 2 shows the reduction of the studied microbial populations caused by pasteurization treatment. Pasteurization reduced total counts by 2.22 and 2.40 log cfu/ml, in PH and PHL respectively, compared to raw products. *Enterobacteriaceae* reduced by 2.21 and 4.41 log cfu/ml in both pasteurized products.

Table 2. Microbial counts (expressed in log cfu/ml) of almond milk

	<i>Total counts</i>	<i>Spores</i>	<i>Yeasts and moulds</i>	<i>Enterobacteriaceae</i>	<i>Micrococccaeae</i>	<i>Bacillus cereus</i>
PB	6.98 ± 0.02	5.94 ± 0.02	6.80 ± 0.05	6.95 ± 0.04	6.30 ± 0.06	5.66 ± 0.04
PH	4.76 ± 0.01	3.63 ± 0.06	ND ^a	ND ^a	3.66 ± 0.04	3.81 ± 0.04
Reduction	2.22	2.31	6.80	6.95	3.64	1.85
PBL	6.29 ± 0.09	3.31 ± 0.05	3.09 ± 0.10	4.10 ± 0.004	5.31 ± 0.05	5.17 ± 0.09
PHL	3.89 ± 0.02	2.34 ± 0.04	ND ^a	ND ^a	2.18 ± 0.12	3.44 ± 0.21
Reduction	2.40	0.97	3.09	4.10	3.13	1.73

* ND indicates that colonies could not be detected.

Table 3. Mean and SD of stability index^A of almond milk

<i>Temperature</i>	<i>Treatment</i>	<i>PB</i>	<i>PBL</i>	<i>PH</i>	<i>PHL</i>
25 °C	Day 2	54.16 ± 0.18 ^a	39.94 ± 0.05 ^b	35.45 ± 0.21 ^c	33.79 ± 0.09 ^c
	Day 7	51.19 ± 0.21 ^a	37.57 ± 0.06 ^b	33.89 ± 0.19 ^c	31.84 ± 0.14 ^d
4 °C	Day 2	48.32 ± 0.55 ^a	30.75 ± 0.07 ^c	33.23 ± 0.11 ^b	27.78 ± 0.10 ^d
	Day 7	46.78 ± 0.18 ^a	30.20 ± 0.32 ^c	32.12 ± 0.12 ^b	25.54 ± 0.22 ^d

*^A Expressed as % w/w of particles sedimented after centrifugation.

* Different superscript letters within row indicate significant difference ($p < 0.05$)

After 7 days of storage, PB and PH stored at both, 25 °C and 4 °C, showed a layer of spontaneous precipitation on the bottom of the bottle, while the products added with lecithin did not show any kind of sedimentation during the storage. This observation is in accordance with the index of stability shown in Table 3, after centrifugation. In general, PB almond milk showed highest percentages of sedimented solids compared to PH, PHL, and PBL in all cases studied. Emulsion based products stored in 4 °C showed an increasingly index of stability.

Table 4. Average values of oxidation parameters in almond milk.

<i>Oxidation parameter</i>	<i>PB</i>	<i>PBL</i>	<i>PH</i>	<i>PHL</i>
LOX (U/L)	0.02 ^a	0.02 ^a	ND ^c	ND ^c
TBA (mg MDA/kg)	0.05 x 10 ^{-3a}	1.0 x 10 ^{-8b}	2.0 x 10 ^{-8c}	1.0 x 10 ^{-8b}
Lipid Hydroperoxides (POD)	0.125 ^a	0.118 ^b	0.096 ^c	0.046 ^d

* Values within rows followed by the same letter are significantly different $p < 0.05$

* LOX indicates the activity of Lipoxygenase enzyme

* TBA indicates the thiobarbituric acid method for determining free malondialdehyde (MDA)

* Lipid hydroperoxides indicates the oxidized product produced during oxidation.

After applying pasteurization treatment, LOX enzyme was not detected, thus indicating that there was a total inactivation. However, there were formation of primary oxidation product

(lipid hydroperoxides) and secondary product (MDA) in both pasteurized almond milk (PH and PHL). Based on the results, it can be concluded that the formations of oxidized products are very low. The values obtained from equivalent MDA in almond milk (0.05 mg MDA/kg) seem quite small.

4. Discussions

Composition of almond seeds expressed in w/w percentage: 94.95 ± 0.21 % dry matter, 20.39 ± 0.23 % crude protein, and 56.76 ± 0.98 % fat, 5.03 ± 0.08 % ash and 12.73 ± 0.05 % carbohydrate. Results obtained are similar to those reported by Agumbiade *et al.* (2006). Lecithin composed by phospholipids and has amphiphilic properties (Jenkins *et al.*, 2002) were added to increase stability in almond milk. Products composition presented quite similar values of components to those found in the market which had maximum values of 1 % protein and maximum values of 2 % fat.

Addition of lecithin led to higher content of protein and fat, thus increasing dry matter and ash content as also observed by Abbassy *et al.* (2004). Since lecithin composition present in phospholipids containing a coline group per molecule ($C_5H_{14}N$), obtained results are reasonable. In terms of lipid content, lecithin is extracted in the organic phase solvent during analysis procedure giving a total lipid value increased compared to the base products. However, in terms of protein composition, analytical procedure with previous mineralization step led to determine total nitrogen content; this is because protein values in almond milks containing lecithin are slightly estimated in excess. Lecithin acts as emulsifier, which improves the stability by reducing the oil-water interfacial tensions and by forming a protective layer around the fat droplets to prevent them from aggregating (Guzey & McClements, 2006a). Significant differences ($p < 0.05$) also were observed in lipid and protein content when heat treatment was applied to products, presenting always lower values. Probably these results are only due to manipulation of products during the heat treatment process, especially to the creaming of product into the tank in the previous pasteurization step.

In pasteurized product (PH and PHL), the yeast and moulds count were below the detection level in both treatments. Results obtained were similar to those obtained by Faid *et al.* (1995) where the presence of *Aspergillus flavus* found as a weak pathogen of almond. *Bacillus cereus* were only reduced by log 1.85 and 1.73 cfu/ml in PH and PHL. It might be due to the spores resistance to exposure at extreme heat (Prescott, 1993).

Addition of lecithin increased physical stability, probably by increasing particles dispersion (better humectability of hydrophobic coarse particles of almond). Pasteurization process led to more stable dispersions, probably due to the homogenization step previous to the heat treatment. By visually analyzing almond milk products, PBL and PHL produced a very thin layer of precipitate on the bottom of the bottles compared to the products stored at room temperature. Therefore, we concluded that addition of lecithin helps to stabilize the products. Lecithin facilitates the formation of emulsion and improves their stability by reducing the oil-water interfacial tensions and by forming a protective layer around the fat droplets to prevent them from aggregating. In fact, when adsorbed at the oil-water interface, these hydrocolloids can be even more effective than surfactants and proteins in conferring long-term emulsion stability due to the formation of a thicker and stronger secondary layer favoring electrostatic and steric stabilization (Nath *et al.*, 1983).

Almond milk contains polyunsaturated fatty acids (mainly linoleic and linolenic acids) which are suspected to oxidation (Nielsen *et al.*, 2002). Lipid oxidation refers to the reaction of

unsaturated fatty acids with molecular oxygen. Moreover, oxidation may take place by lipoxidase (LOX) enzymatic catalyzed pathway that promotes aldehydes production and off-flavours (Wang *et al.* 2000). After applying pasteurization treatment, LOX enzyme was not detected, thus indicating that there was a total inactivation. This thanks to composition of almond that contain antioxidant components that helps in minimizing lipid oxidation. Whereas the profile of fatty acid composition of almond milk could be closer to the fish for its high content of unsaturated fatty acids, the values obtained from equivalent MDA in almond milk (0.05 mg MDA/kg) seem quite small.

5. Conclusions

Pasteurization considerably reduced almond milk microbial load and producing a highly physically stable product with addition of lecithin. Result from oxidation analysis evidenced that pasteurization inactivated the LOX activity and reduced the formation of oxidized substances that contribute to odorless. This preliminary study demonstrates the potential of pasteurization to process almond milk as preservation and physical stability is achieved in this product. However, further work is needed for production of quality almond milk products.

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