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## Effect of high pressure homogenisation and heat treatment on physical properties and stability of almond and hazelnut milks

Neus Bernat <sup>a</sup>, Maite Cháfer <sup>a</sup>, Julia Rodríguez-García <sup>b</sup>, Amparo Chiralt <sup>a</sup>, Chelo González-Martínez <sup>a, \*</sup>

<sup>a</sup> Institute of Food Engineering for the Development, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain <sup>b</sup> Research Group of Food Microstructure and Chemistry, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain

#### A R T I C L E I N F O

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#### ABSTRACT

The effect of high pressure homogenisation (HPH) and heat treatments on physicochemical properties and physical stability of almond and hazelnut milks was studied. Vegetable milks were obtained and homogenised by applying 62, 103 and 172 MPa (MF1, MF2 and MF3, respectively). Untreated and MF3 samples were also submitted to two different heat treatments (85 °C/30 min (LH) or 121 °C/15 min (HH)). Physical and structural properties of the products were greatly affected by heat treatments and HPH. In almond milk, homogenised samples showed a significant reduction in particle size, which turned from bimodal and polydisperse to monodisperse distributions. Particle surface charge, clarity and Whiteness Index were increased and physical stability of samples was improved, without affecting either viscosity or protein stability. Hazelnut beverages showed similar trends, but HPH notably increased their viscosity while change their rheological behaviour, which suggested changes in protein conformation. HH treatments caused an increment of particle size due to the formation oil droplet-protein body clusters, associated with protein denaturation. Samples submitted to the combined treatment MF3 and LH showed the greatest stability.

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#### 1. Introduction

In the last few years, the population ratio demanding vegetablebased products is growing, either because of the increasing problems related with the intolerances to Cow's milk (Fiocchi et al., 2010) or because of changes in the food preferences. As a consequence of new consumer tendencies, food industries are currently producing new nutritionally improved products with added value. Vegetable-based beverages are included in these new products, which are available at any supermarket as an alternative to dairy products, with an increasing consumer acceptance.

There is a wide variety of vegetable-based beverages, although most of the research activity has been focused on those obtained from soy. For soy "milk", studies into the physicochemical characterization, the effects of processing, the application of new

\* Corresponding author.

E-mail address: cgonza@tal.upv.es (C. González-Martínez).

technologies, such as electric pulses and ultra-high homogenisation pressures have been carried out (Cruz et al., 2007; Li, Chen, Liu, & Chen, 2008).

Research dealing with the use of non-soy vegetable milk is still scarce and most of it is related with the nutritional quality of such products. In this sense, almond and hazelnut beverages have been used as an alternative to milk in lacto-intolerant people, pregnant women and celiacs, due to their high levels of calcium, phosphorous and potassium (Eroski Foundation, 2007; Luengo, 2009). These nuts have low sodium content and an equilibrated mono-unsaturated fatty acid-polyunsaturated fatty acids ratio, which define the products which are healthy for people with heart disease (Mateos, 2007). They are also considered helpful for maintaining cholesterol at healthy levels due to their high content of antioxidant compounds which contributes to heart disease prevention (Fraser, Bennett, Jaceldo, & Sabaté, 2002; Jenkins et al., 2008; Kris-Etherton, Hu, Rose, & Sabaté, 2008; Tey et al., 2011).

Vegetable based beverages are emulsified products where the nut fat is dispersed in an aqueous phase and where the rest of the components play different roles in the product stability. The different process steps, such as homogenisation and heat treatments usually produce changes in the arrangement of components,





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*Abbreviations:* CLSM, confocal laser scanning microscopy; DSC, differential scanning calorimetry; HH, high heat; HPH, high pressure homogenisation; pl, isoelectric point; LH, low heat; MF1, homogenisation at 62 MPa; MF2, homogenisation at 103 MPa; MF3, homogenisation at 172 MPa; WI, whiteness index.

thus leading to modifications in the particle size, colour, viscosity and physical stability of the product. These physicochemical modifications have to be known to efficiently control the process and to implement the necessary improvements in the production lines. The most commonly used homogenisation pressures in the food industry range between 20 and 50 MPa, although much higher pressures are used in high pressure homogenisation (HPH) processes with some advantages: the deflocculation of clusters of primary fat globules (Floury, Desrumaux, & Lardières, 2000) and uniform dispersion of agglomerates, the changes in protein conformation (Pereda, Ferragut, Quevedo, Guamis, & Trujillo, 2009), the increase in emulsion viscosity (Desrumaux & Marcand, 2002) and stability and the microbial inactivation (Cruz et al., 2007; Diels, Callewaert, Wuytack, Masschalk, & Michiels, 2005; Pereda, Ferragut, Guamis, & Trujillo, 2006; Smiddy, Martin, Huppertz, & Kelly, 2013, 2007).

The objective of the present study is to analyse the effect of heat treatments and high homogenisation pressures on the physical properties and stability of almond and hazelnut beverages (nut milks) in order to define processing conditions which ensure the product quality and stability.

#### 2. Materials & methods

#### 2.1. Preparation of almond and hazelnut milks

Nut beverages were produced by soaking and grinding *Prunus amygdalus* L. dulcis almonds and *Corylus avellana* hazelnuts, supplied by Frutos Secos 3G S.L. (Valencia, Spain). The extraction was carried out in Sojamatic 1.5 (Sojamatic<sup>®</sup>; Barcelona, Spain), equipment specifically designed for the production of vegetable beverages, with a nut-water ratio of 8:100. This equipment carries out both the nut grinding and the solid particles' retention throughout a filter. The manufacturing process takes 30 min at room temperature, in which both grinding and filtering were performed discontinuously every two minutes. The milky liquid obtained was used as control sample (untreated).

#### 2.2. High pressure homogenisation and heat treatments

High pressure homogenisation (HPH) treatments were carried out in a high pressure homogeniser M-110P model (Microfluidics International Corporation, Newton, MA, USA) by applying 62, 103 and 172 MPa (samples MF1, MF2 and MF3 respectively). Some samples were submitted to a low temperature heat treatment (LH) at 85 °C for 30 min by using a temperature-controlled water bath (Precisdig, JP-Selecta; Barcelona, Spain) and to a high temperature heat treatment (HH), 121 °C for 15 min in an autoclave (Precisdig, JP-Selecta; Barcelona, Spain). The heat treatment conditions chosen were those in which the destruction of all vegetative cells and enzymes are ensured (Walstra, Wouters, & Geurts, 2006). Samples submitted to heat treatment were the control samples (LH and HH samples) and those homogenised at 172 MPa (MF3LH and MF3HH samples).

#### 2.3. Characterization of chemical composition

The quantification of moisture, ash, fat content, proteins and sugars was carried out in the nut milks. Fibre content was estimated by means of the difference in terms of component percentages. Almond beverages were freeze-dried (ioalfa-6 freezedryer; TELSTAR, Terrassa, Spain) prior to the analysis. AOAC Official Methods were chosen to determine water, total fats and total nitrogen contents (AOAC 16.006, AOAC 945.16 and AOAC 958.48, respectively) (Horwitz, 2000). Total sugars and ashes were obtained following the protocols suggested by Matissek, Schnepel, and Steiner (1998). All the determinations were performed in triplicate.

#### 2.4. Characterization of physical and structural properties

#### 2.4.1. pH and density

Measurements were carried out at 25  $^{\circ}$ C using a pH-meter GLP 21+ (Crison Instruments S.A., Barcelona, Spain) and a digital densitometer DA-110 M (Mettler Toledo, Barcelona, Spain), respectively. These determinations and those described below were carried out in triplicate.

#### 2.4.2. Particle size distribution and $\zeta$ -potential

Analysis of the particle size distribution was carried out using a laser diffractometer Mastersizer 2000 (Malvern Instruments Ltd, Worcestershire, UK). The Mie theory was applied by considering a refractive index of 1.33 and absorption of 0.1. Samples were diluted in de-ionised water at 2,000 rpm until an obscuration rate of 10% was obtained.  $D_{32}$  (surface weighted mean diameter) and  $D_{43}$  (volume weighted mean diameter) were obtained. The volume-weighted average diameter is sensitive to the presence of large particles, whereas the surface-weighted average diameter is more sensitive to the presence of small particles.

 $\zeta$ -potential was determined at 20 °C by using a Zetasizer nano-Z (Malvern Instruments Ltd, Worcestershire, UK). Samples were diluted to a fat droplet concentration of 0.4 g/100 mL using a phosphate buffer 0.02 mol/L solution. The Smoluchowsky mathematical model was used to convert the electrophoretic mobility measurements into  $\zeta$ -potential values.

#### 2.4.3. Rheological behaviour

The rheological behaviour of nut milks were characterized by using a rotational rheometer (HAAKE Rheostress 1, Thermo Electric Corporation, Karlsruhe, Germany) with a sensor system of coaxial cylinders, type Z34DIN Ti. The shear stress ( $\sigma$ ) was measured as a function of shear rate ( $\dot{\gamma}$ ) from 0 to 112 s<sup>-1</sup>. The up and down curves were obtained, taking 1 min to rise and 1 min to fall. The Herschel-Bulkey model (Equation (1)) was fitted to the experimental points to determine the flow behaviour index (n), consistency index (K) and yield stress ( $\sigma_y$ ) by using a non-linear procedure. Apparent viscosities were calculated at 100 s<sup>-1</sup>.

$$\sigma = \sigma_y + K_\gamma^n \tag{1}$$

#### 2.4.4. Optical properties

Colour coordinates were measured from the infinite reflection spectrum in a Spectrum-colorimeter CM-3600 d (MINOLTA Co, Osaka, Japan). CIE  $L^*a^*b^*$  coordinates were obtained using illuminant D65/10° observer. Colour of samples was characterized as to Lightness ( $L^*$ ), Chrome ( $C^*_{ab}$ ), hue ( $h^*_{ab}$ ) and Whiteness Index (WI) as defined in Equations (2)–(4). Colour difference ( $\Delta E$ ) between treated and untreated samples was also calculated by using Equation (5).

$$C^* = \sqrt{a^{*2} + b^{*2}} \tag{2}$$

$$h_{ab*} = \arctan(b^*/a^*) \tag{3}$$

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}}$$
(4)

$$\Delta E = \sqrt{(\Delta L^{*})^{2} + (\Delta a^{*})^{2} + (\Delta b^{*})^{2}}$$
(5)

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