Food Chemistry 176 (2015) 433–440

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

# Compositional and biochemical changes during cold storage of starter-free fresh cheeses made from ultra-high-pressure homogenised milk

A. Zamora, B. Juan, A.J. Trujillo *

*Centre Especial de Recerca Planta de Tecnologia dels Aliments (CERPTA), XaRTA, XiT, MALTA Consolider Group, Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain*

ARTICLE INFO

*Article history:*
Received 17 January 2014
Received in revised form 21 October 2014
Accepted 17 December 2014
Available online 24 December 2014

*Keywords:*
Fresh cheese
Ultra-high pressure homogenisation
Proteolysis
Lipolysis
Oxidation

ABSTRACT

The aim of the present study was to evaluate the effects of using ultra-high pressure homogenisation (UHPH) on the composition and biochemistry of starter-free fresh cheeses and to monitor their evolution during cold storage as an alternative to conventional treatments applied in the production of fresh cheese such as conventional pasteurisation and homogenisation–pasteurisation. Although both homogenisation treatments increased cheese moisture content, cheeses from UHPH-treated milk showed lower moisture loss during storage than those from conventionally homogenised–pasteurised milk. Lipolysis and proteolysis levels in cheeses from UHPH-treated milk were lower than those from conventionally treated milk samples. Although, oxidation was found to be the major drawback, in general terms, high quality starter-free fresh cheeses were obtained from UHPH-treated milk.



## 1. Introduction

Fresh cheeses are ready for consumption immediately after processing of the curds. Biochemical changes during cheese ripening involves three primary events, i.e., glycolysis, lipolysis and proteolysis, the products of which are modified via various biochemical reactions. Glycolysis and related events are caused by living microorganisms, while lipolysis and proteolysis are catalysed mainly by enzymes from rennet, milk or bacteria (Fox & McSweeney, 2004). In fact, during cold storage of starter-free fresh cheeses, such changes are undesired. Moreover, possible biochemical changes provoked in the milk through processing, previous to the cheese-making, could drastically alter the characteristics of the final product (Fox & McSweeney, 2004).

Milk for the manufacture of starter-free fresh cheeses is generally pasteurised at 70–80 °C for 15–40 s resulting in fresh cheeses with short shelf life. As an alternative, ultra-high pressure homogenisation (**UHPH**) has been proven to inactivate microorganisms and, especially, food-borne pathogens in milk (Pereda, Ferragut, Quevedo, Guamis, & Trujillo, 2007). Some studies on the cheese-making characteristics of UHPH-treated milk have focused on the rennet coagulation properties. On the contrary to conventional treatments, UHPH enhances the coagulation properties of milk by decreasing the rennet coagulation time by ~20% and increasing both the curd firming rate and the gel firmness (Zamora, Ferragut, Jaramillo, Guamis, & Trujillo, 2007; Zamora, Ferragut, Quevedo, Guamis, & Trujillo, 2012).

A part from the sensory characteristics, two main aspects are of major importance for the quality of starter-free fresh cheeses: syneresis or amount of whey expelled during storage and microbiological quality. Since the general trend in the commercialisation of such products involves longer shelf-life, both handicaps have to be tackled. From a technological point of view, UHPH treatment could be an alternative to conventional treatments of milk for fresh cheese production by obtaining longer shelf-life through the reduction of microbial growth and the prevention of excessive syneresis (Zamora, Ferragut, Quevedo, et al., 2012). Moreover, UHPH triggers textural changes in fresh cheeses, i.e., higher firmness, lower deformability and higher water retention, which could be explained by the reduction of fat globule size and the incorporation of caseins and whey proteins at the milk fat globule membrane (Zamora, Ferragut, Juan, Guamis, & Trujillo, 2011). Indeed, UHPH alters protein–protein interactions within rennet curds (Zamora, Trujillo, Armaforte, Waldron, & Kelly, 2012). UHPH also provokes a drastic reduction of fat globule size with a concomitant increase of their specific surface area which was overcome by the adsorption of non-native proteins of the milk fat globule membrane

* Corresponding author at: Tecnologia dels Aliments, Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain. Tel.: +34 93 581 32 92; fax: +34 93 581 20 06.
*E-mail address:* toni.trujillo@uab.es (A.J. Trujillo).

http://dx.doi.org/10.1016/j.foodchem.2014.12.070
0308-8146/

(**MFGM**), i.e., whey proteins and partially dissociated casein micelles (Zamora, Ferragut, Guamis, & Trujillo, 2012).

Since alterations in the protein-fat interactions may affect, either enhancing or inhibiting, the biochemical reactions that take place during storage and fresh cheeses form UHPH-treated milk present longer shelf life, the present study aimed at evaluating the effects of using UHPH, as an alternative to conventional treatments applied in the production of starter-free fresh cheese, on the evolution of the composition and biochemistry, i.e., proteolysis, lipolysis and oxidation, during cold storage.

## 2. Materials and methods

### 2.1. Milk supply and treatment

Standardised raw bovine milk samples were treated as described by Zamora, Ferragut, Quevedo, et al. (2012). UHPH treatment at 300 MPa at an inlet temperature of 30 ± 0.5 °C (high-pressure homogeniser model FPG11300, Stansted Fluid Power Ltd., Harlow, UK) was compared with conventional treatments, i.e., pasteurisation (**PA**; at 80 °C for 15 s) and homogenisation–pasteurisation (**HPA**; homogenisation at 60 °C with 15 and 3 MPa at first and second valves, respectively, followed by pasteurisation at 80 °C for 15 s).

### 2.2. Fresh cheese production

Starter-free fresh cheeses were produced as described by Zamora, Ferragut, Quevedo, et al. (2012) and were analysed on the day after production (day 1) and the expiring day (day 13 for PA- and HPA-cheeses, day 19 for UHPH-cheeses). Cheeses made from UHPH-treated milk were also analysed on day 13 in order to be compared with cheeses from conventional treatments.

### 2.3. Cheese composition

Cheeses were analysed in duplicate for fat (ISO, 1975) and in triplicate for total solids (**TS**; IDF, 2004) and total nitrogen (**TN**; IDF, 2002). Moisture content (100 − TS) and total protein (TN × 6.38) were calculated. Salt in cheese was determined in triplicate by chloride analysis (Corning 926 Chloride Analyzer, Sherwood Scientific Ltd., Cambridge, UK) following the procedure in the manual of the chloride titrator, and expressed on a dry basis.

### 2.4. Proteolysis

Water-soluble extracts were prepared according to the method of Kuchroo and Fox (1982). Water-soluble nitrogen at pH 4.6 (**WSN**), expressed as percentage of TN, was determined in duplicate by the Dumas combustion method (IDF, 2002). Total free amino acids (**FAA**) were determined in triplicate on the water soluble extracts by the cadmium–ninhydrin method described by Folkertsma and Fox (1992).

pH 4.6-insoluble fractions recovered during the WSN extraction were washed three times with 1 M sodium acetate buffer (pH 4.6), and the remaining fat was eliminated by washing with dichloromethane-sodium acetate buffer (1:1 v/v). The final protein precipitate was then lyophilised. Analysis of individual proteins in cheese was performed by capillary electrophoresis.

Capillary electrophoresis was performed in triplicate following the method of Recio and Olieman (1996) with an Agilent CE instrument (Agilent Technologies, Waldbronn, Germany) controlled by Chemstation software (Agilent). Protein separation was carried out with a fused-silica capillary column (BGB Analytik, Essen, Germany) of 0.6 m × 50 μm interior diameter with an effective length of 50 cm, by applying a linear voltage gradient from 0 to 20 kV in 3 min at 45 °C, followed by a constant voltage of 20 kV. Electrophoregrams were obtained at 214 nm and designation of capillary electrophoresis peaks was carried out by comparing the electrophoregrams with those of pure standards (Sigma–Aldrich, St Louis, MO, USA) and those of Recio, Pérez-Rodríguez, Ramos, and Amigo (1997).

### 2.5. Lipolysis

Assessment of lipolysis was carried out by qualitative and quantification analysis of free fatty acids (**FFA**). 1 g of cheese was mixed in a screw-capped plastic tube with 3 g of anhydrous $Na_2SO_4$, 0.3 mL of $H_2SO_4$ (2.5 M), and 20 μL of internal standard solutions (heptanoic acid 9.18 mg $mL^{-1}$, and decaheptanoic acid 8.00 mg $mL^{-1}$; Sigma–Aldrich). Then 3 mL of dry diethyl ether/heptane (1:1 v/v) were added, and the mixture was shaken for 20 s with a vortex mixer. After centrifugation at 230*g* for 2 min at 20 °C, the supernatant was transferred to a screw-capped glass tube containing 1 g of anhydrous $Na_2SO_4$. This operation was repeated twice, and the obtained supernatants were homogenised for 10 s using a vortex mixer.

Isolation of FFA from the ether/heptane extract was done using a solid phase extraction technique with aminopropyl column Speed NH2 500 mg 3 $mL^{-1}$ (Applied Separations, Allentown, PA, USA). The aminopropyl column was conditioned with 10 mL of heptane before the lipid extract was applied to the column. Then hexane/2-propanol (20 mL; 3:2 v/v) was used to eliminate glycerides, and finally, FFA were eluted with 5 mL of dry diethyl ether containing 2% formic acid. A direct injection of this solution (1 μL) was used for gas chromatographic analysis, as described below. Two independent extractions were carried out for each sample. All organic solvents were purchased from Panreac (Barcelona, Spain), while formic and sulphuric acids were acquired from Sigma Aldrich.

Analysis of FFA for each extraction was carried out by duplicate with an HP 6890 Series II gas chromatograph (Hewlett–Packard Inc., Wilmington, DL, USA) using a fused silica capillary column (30 m × 0.32 mm, 0.25 μm thickness, DB-FFAP-coated; J&W Scientific, Folsom, California, USA), and a flame-ionisation detector. Helium, hydrogen and synthetic air were the carrier gases. The initial column temperature of 75 °C was maintained for 1 min, then raised to 240 °C at a rate of 5 °C $min^{-1}$, and then held at 240 °C for 21 min. Injection of 1 μL of the extracted fraction was done in splitless mode and an injector temperature of 250 °C. The detector temperature was 300 °C. Each fatty acid was identified with reference to the retention time (**RT**) of standards (Sigma Aldrich).

Quantification was done with respect to the internal standards; heptanoic acid (C7:0; 17.674 min RT) was used for short-chain FFA (**SCFFA**; C4:0–C8:0) and medium-chain FFA (**MCFFA**; C10:0–C14:0), and decaheptanoic acid (C17:0; 36.289 min RT) for long-chain FFA (**LCFFA**; C16:0–C18:2). The relative areas were expressed on a dry basis (μg 100 $g^{-1}$ TS).

### 2.6. Lipid oxidation

Hexanal content of cheese, used as an indicator of lipid oxidation, was analysed by duplicate using a solid phase micro-extraction with an 85 μm CAR/PDMS fibre (Supelco, Bellefonte, PA, USA) in combination with gas chromatography–mass spectrometry (HP 6890 Series II gas chromatograph equipped with a HP 5973 mass selective detector, Hewlett–Packard Inc.). Preconditioning of the fibre and the micro-extraction of 1.5 g of cheese were performed for 1 h at 280 and 80 °C, respectively. Thermal desorption in the gas chromatograph injector was carried out in splitless mode at 280 °C for 1 min. A Supelcowax 10 capillary column