



Transglutaminase treatment of thermally and high pressure processed milk: Effects on the properties and storage stability of set yoghurt

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ABSTRACT

The objectives of this work were to study the effect on the quality parameters of set yoghurt made from high pressure (HP) and transglutaminase (TGase) treated milk (separately or in combination), and to determine the shelf life of this product. Yoghurt samples made from HP– in combination with TGase-treated milk exhibited the highest values of firmness, lowest values of whey separation and similar values of acidity with the other samples. All yoghurt samples made from HP-treated milk, (with or without subsequent TGase treatment), exhibited a creamier perception than the ones from thermally-treated milk. Yoghurt samples prepared by the conventional procedure were judged as unacceptable after the fifth week of storage due to an intense syneresis and separation of the coagulum from the cup. Overall, HP and TGase treatment of milk can be a useful tool for the dairy industry to achieve products of improved structure and desirable sensorial characteristics.

Industrial relevance: In order to achieve a tight and compact structure of yoghurt, solid fortification and/or addition of stabilizers is needed. Transglutaminase and high pressure treatment of milk (when applied individually or in combination) can be alternative treatments of milk to produce yoghurt with improved textural and sensorial characteristics, without dependence on costly solid fortification.

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1. Introduction

The texture of yoghurt is determined by the composition of the milk base and the method which is used for its production. Any alteration related to the above parameters can lead to a significant modification to the structure and texture of the final product (Lankes, Ozer, & Robinson, 1998). Many techniques have been introduced in order to improve the body and structure of yoghurt, mainly involving modification of milk composition by fat replacement with starches, gelatin, gums or carrageenan (Keogh & O'Kennedy, 1998; Teles & Flôres, 2007).

High pressure (HP) processing has been proposed as an alternative method of modifying the functionality of milk components, such as caseins and whey proteins, while it can lead to significant inactivation of pathogenic and spoilage microflora of milk, with limited effect on indigenous milk enzymes and its quality characteristics and nutritional value (Huppertz, Smiddy, Upadhyay, & Kelly, 2006; Law et al., 1998; Lopez-Fandino, de la Fuente, Ramos, & Olano, 1998; Trujillo et al., 2008; Trujillo, Capellas, Saldo, Gervilla, & Guamis, 2002; Moatsou et al., 2000). It has been demonstrated that HP treatment of the milk base used for the production of yoghurt, alters the quality parameters of the final product (Huppertz et al., 2006; Hemar, Liu, Meunier, & Woonton, 2010; Lanciotti, Vannini, Pittia, & Guerzoni, 2004). The rate of

acidification of HP-treated milk is higher than of thermally treated milk. Moreover, HP-treated milk coagulates at higher pH value and yoghurt prepared from HP-treated milk exhibits lower amounts of whey separation and increased gel strength (Ferragut, Martinez, Trujillo, & Guamis, 2000; Harte, Amonte, Luedecke, Swanson, & Barbosa-Cánovas, 2002; Harte, Luedecke, Swanson, & Barbosa-Cánovas, 2003; Needs, Capellas, et al., 2000; Needs, Stenning, Gill, Ferragut, & Rich, 2000).

Transglutaminase (TGase) is an enzyme able to introduce covalent cross-links in proteinaceous systems by catalyzing acyl transfer reactions between the g-carboxamide group of peptide or protein bound glutamine (acyl donor) and primary amines (acyl acceptor) including the e-amino group of lysine residues. When the e-amino group of protein bound lysine reacts as an acyl acceptor, intra-molecular and/or intermolecular cross-links (isopeptide bonds) are formed, resulting in the polymerization of proteins (Dickinson, 1997). Among milk proteins, caseins can be easily cross-linked by TGase due to their flexible and, little or no secondary structure, while whey proteins are not efficiently cross-linked due to their globular compact structure (de Jong & Koppelman, 2002; Lorenzen, 2002; O'Connell & de Kruif, 2003; Sharma, Zakora, & Qvist, 2002). Although, κ -casein was found to be the most susceptible to cross-linking in unheated milk followed by β -casein (Sharma, Lorenzen, & Qvist, 2001), it was shown that heat pre-treatment of milk enhances protein cross-linking by TGase treatment (Kulozik, Tolkach, Bulca, & Hinrichs, 2003; Rodríguez-Nogales, 2006a, 2006b) and, the used concentration of TGase is an important factor in the physicochemical characteristics and textural attributes of the

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final product (Kuraishi, Yamazaki, & Susa, 2001; Ozer, Kirmaci, Oztekin, Hayaloglu, & Atamer, 2007).

Thermal pre-treatment of milk can enhance protein cross-linking by TGase (Kulozik et al., 2003; Rodriguez-Nogales, 2006a, 2006b). TGase treatment of milk can be applied prior or during the fermentation of the milk. In the first case the enzyme should be subsequently inactivated, usually by a thermal treatment step (Kuraishi et al., 2001) or by chemical inhibition, i.e. with N-Ethylmaleimide (NEM) (Jacob, Noebel, Jaros, & Rohm, 2011). The utilization of TGase in dairy products results in altered firmness, viscosity, water holding capacity, stability, fermentation capacity and mechanical properties of the gel (Boenisch, Huss, Lauber & Kulozik, 2007; Cancino, Fuentes, Kulozik, & Boenisch, 2006; Farnsworth, Li, Hendricks, & Guo, 2006; Guyot & Kulozik, 2010; Jaros, Partschfeld, Henle, & Rohm, 2006; Lorenzen, Neve, Mautner, & Schlimme, 2002; Ozrenk, 2006).

The combined treatment of milk with HP and TGase applied individually or simultaneously before producing gels acidified with glucono- δ -lactone (GDL) has been studied by Anema, Lauber, Lee, Henle, and Klostermeyer (2005). It was shown that the combined treatment of TGase under HP conditions resulted in gels with lower gelation time, higher gelation pH and higher storage modulus (G') values than those obtained by HP or TGase treatment individually, suggesting an increased level of cross-linking of the milk proteins when milk is treated with TGase under HP compared with treatment under atmospheric pressure conditions.

In yoghurt production practice in order to achieve a final gel of increased firmness, demanded by the current consumer trends, milk powders, including non-fat dry milk or milk protein concentrates, are blended with the milk during the standardization procedure. The total solids content of milk can also be increased by evaporation under vacuum and membrane processing and by adding to the milk base stabilizers, like pectin or gelatin, to enhance yoghurt texture, and prevent wheying-off (Tamime & Robinson, 1999). However, the use of stabilizers, can lead to over-stabilization (gelatinous texture) or under-stabilization (weak texture and whey separation), while in some countries regulations do not allow the use of stabilizers for plain (unsweetened) yoghurt (Lee & Lucey, 2010).

The replacement of milk fortification with protein concentrates or stabilizers with HP and/or TGase treatment of milk and milk proteins to achieve products with improved structure needs further exploration in view of a potential commercial application. The objectives of this study were a) to evaluate the effect of HP or TGase treatment or the combined treatment of milk on the quality attributes of set yoghurt at different levels of added protein, and b) to investigate the effect of the above treatments of milk on the shelf life of set yoghurt during refrigerated storage, to determine whether such a product could be produced and be commercially viable.

2. Materials and methods

2.1. Sample preparation

Homogenized milk of different levels of protein content (3.00%, or standardized to 3.45 and 3.70%) was provided directly from the plant of a leading dairy company. The standardization of the protein content of milk from the initial level of 3.00% was achieved by adding external protein, with a ratio of 50:50 caseins to whey proteins. After standardization of milk proteins, the casein:whey protein (CWP) ratio for each milk was equal to 80:20, 76.5:23.5 and 74:26 for milks of 3.00, 3.40 and 3.75% protein content, respectively. The fat content of milk was standardized to 4.0%. Both protein and fat content standardization were performed by the dairy company. Homogenization was carried out in a two stage industrial homogenizer at 150/50 bar. Milk was either subjected to thermal (85 °C for 30 min) or HP (600 MPa/55 °C for 10 min) treatment.

2.2. HP treatment

HP treatments were performed using a laboratory-scale HP system with a maximum operating pressure of 1000 MPa (Food Pressure Unit FPU 1.01, Resato International BV, Roden, Netherlands), consisting of an HP unit with a pressure intensifier, an HP vessel of 1.5 L volume and a multi-vessel system consisting of six vessels of 42 mL capacity each. All HP vessels are surrounded by a water circulating jacket connected to a temperature control system. The pressure-transmitting fluid used was polyglycol ISO viscosity class VC 15 (Resato International BV, Netherlands). Milk samples of 750 mL volume were put into multi-layer (PP, foil, PE) packaging and placed in the 1.5 L chamber for processing. The desired value of pressure was set and, after pressure build-up (approximately 20 MPa s⁻¹), the pressure vessel was isolated; this point defined the zero time of the process. Pressure of the vessel was released after a preset time interval (10 min pressurization time) by opening the pressure valve (release time <3 s). The initial temperature increase during pressure build-up (about 3 °C per 100 MPa) was taken into consideration in order to achieve the desired operating temperature. Pressure and temperature were constantly monitored (intervals of 1 s) and recorded during the process. All samples were pressurized at 600 MPa and 55 °C for a process time of 10 min. After processing, samples were kept to overnight storage at 4 °C until use.

2.3. Enzymic cross-linking with transglutaminase (TGase)

Thermally and HP-treated milk was heated to 42 °C, inoculated with TGase at a concentration of 2.2 U/g protein (ACTIVA YG, Ajinomoto, 100 U/g transglutaminase activity). Incubation of milk with the enzyme was carried out into 2 L sterile beakers, which were placed into a water-bath of controlled temperature (43 ± 0.2 °C) for 180 min. The milk was then rapidly heated at 80 °C for 1 min in order to inactivate the enzyme and cooled down to the fermentation temperature. Samples not treated with TGase were also incubated at 42 °C for 180 min, then heated at 80 °C for 1 min and cooled down to the fermentation temperature.

2.4. Yoghurt preparation

Milk was heated to fermentation temperature and inoculated with commercial starter culture. The starter culture was prepared as a 1:5 (w/v) dilution of freeze-dried YC-X11 culture (Christian Hansen, Denmark) in commercial UHT skim milk and maintained in cool storage until inoculation. The inoculated milk was divided into UV-sterile plastic cups and sealed with foil. Incubation at 42 °C was followed until pH reached 4.75. After fermentation, yoghurts were transferred to a refrigerator at 4.8–5.1 °C and stored until testing. For comparison of the applied treatments in milk, tests were performed three days after the production of the samples. For shelf life study, samples were tested in weekly intervals and as zero time it was considered the day after the production of the yoghurts.

2.5. Fermentation time

A 0.001 precision pH meter (AMEL 338, AMEL Instruments, Italy) was used for pH measurements during fermentation procedure. Fermentation time is defined as the time needed for milk coagulum to reach pH value of 4.75, which is a common pH value of commercial set yoghurt and was recommended by the Greek dairy industry.

2.6. Study on the quality attributes of yoghurt

2.6.1. Microbiological analysis

Yoghurt sample of 10 g was transferred to a sterile stomacher bag with 90 mL sterilized Ringer solution (1.15525, Merck, Germany) and was homogenized for 60 s with a Stomacher (BagMixer® interscience,

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