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Colour change and proteolysis of skim milk during high pressure thermal–processing

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ABSTRACT

The effect of high pressure–thermal processing (HPTP), in the range of 0.1–600 MPa and 100–140 °C for up to 60 min, on colour change and proteolysis of reconstituted skim milk (10% w/w) was investigated. The kinetic results showed that colour change (ΔE_{ab}) and proteolysis (determined by chromatography) increased with both increasing temperature and pressure. The apparent reduction of free amino groups in skim milk, indicating sugar conjugation to milk proteins/peptides, was accelerated with increasing temperature, but decelerated with increasing pressure (at constant temperature) at higher temperature. The milk's colour changed drastically at 400 MPa where most of the milk proteins formed coagulates and left the solutions nearly translucent. Mathematical models describing the kinetics of colour change, proteolysis, and free amino acids reduction as a function of pressure and temperature are proposed.

1. Introduction

Untreated raw milk is highly perishable and often contains food-borne bacterial pathogens. Thus, selling of raw milk is prohibited or at least restricted to direct commercialisation at the farm in most countries. Sterilisation and long shelf life of milk is often achieved through ultra high temperature (UHT) processing for a few seconds at or above 135 °C; however, canning of milk products at 120 °C up to 30 min is still practiced in the dairy industry (Kessler, 1996).

Milk experiences colour change during thermal processing. Firstly, an increase in lightness occurs due to denaturation of β-lactoglobulin and its conjugation to κ-casein (Burton, 1994). At the early stage of the Maillard reaction in milk, lysine conjugates with lactose and results in colourless compounds. These compounds convert into coloured products including melanoidins during the final stage of the Maillard reaction (Burton, 1994).

Compared to other heat induced changes of milk systems (e.g., Maillard reaction and disulfide-bonded aggregation), only a few studies have been carried out on heat induced proteolysis. One of the reasons is the lack of high precision analytical techniques for identifying the proteolysis products and determining their concentrations (Morales and Jiménez-Pérez, 1998). Most studies on thermal proteolysis of milk proteins focus on casein since casein is more heat stable than whey protein and more available in milk (Guo et al., 1989). Casein degrades during severe heating (i.e., $\geqslant$120 °C) and results in peptide fractions, mostly from α_{s1}-casein followed by κ-casein (Hustinx et al., 1997; Gaucheron et al., 1999). Whey proteins strengthen their hydrophobic bonding during heating to 60 °C. They unfold and denature during heating between 60 and 100 °C. Further heating to 140 °C results in breakdown of disulfide bonds and reduction of covalent cross-links (de Wit and Klarenbeek, 1984).

High pressure–thermal processing (HPTP) combines high pressure (up to 800 MPa) and heating (above 60 °C) has been considered for sterilisation and shelf life extension of foodstuff due to its ability to inactivate bacterial spores at reduced heat and thereby preserving desirable functional properties of foods better than conventional thermal processing (Heinz and Buckow, 2010). Accelerated and homogeneous heating and cooling of food occurs during HPTP due to the increase and decrease in temperature accompanying the physical compression and decompression of the product. This facilitates uniform heating of all food and also reduces the need for excessively long heating times and often results in improved food quality attributes, such as flavour, texture, nutrient content, and colour, compared with conventional thermal processing (Grauwet et al., 2012).

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Studies addressing the effect of HPTP on the quality of dairy products are still limited (Devi et al., 2013). In contrast to heated milk, the research on visual change of high pressure (applied at ⩽40 °C) treated milk focused on its light scattering properties (i.e., lightness and turbidity) rather than its tristimulus colour variables (i.e., Lab system). Reduced lightness and turbidity of milk upon high pressure processing (HPP) is usually associated with changes of casein micelle conformation (Huppertz et al., 2002). The processing temperature (4–40 °C) during HPP, particularly at moderate pressures (200–300 MPa), affects the lightness/turbidity of milk. The differences in the optical properties of milk after HPP at 250 MPa at various temperatures (4–40 °C) might be due to temperature-dependent casein micelle dissociation (during pressurisation) or reassociation (during pressure release), or combination of both (Gaucheron et al., 1997; Orlien et al., 2006). Under HPTP conditions, a more complex interaction amongst milk constituents (e.g., proteins and lactose) is likely to take place which can result in the milk's concomitant colour change due to Maillard reaction products (Jaeger et al., 2010).

To the best of our knowledge, studies focusing on the quality of bovine milk as affected by HPTP at higher temperatures (i.e., ⩾100 °C) are not available. Therefore, the aim of this kinetic study was to investigate and predict proteolysis in skim milk, interaction between milk proteins with lactose, and consequences to skim milk colour during HPTP and heat-only treatments at 100–140 °C.

2. Materials and methods

2.1. Material

Skim milk powder (SMP), produced with low-heat, was purchased from Tatura Milk Industries Ltd. (Tatura, VIC, Australia). The composition according to the manufacturer was as follows: 33.6% protein, 0.7% fat, 2.8% ash, 3.6% moisture, and 59.3% lactose. A solution of 10% w/w in deionised water gave a pH value of 6.7.

2.2. Sample preparations

Reconstituted skim milk at 10% w/w was used throughout the study and was prepared daily and separately for each experiment. SMP was dispersed in deionised water under stirring for 2 h at room temperature and allowed to fully hydrate overnight at 5 °C. Milk solutions were transferred into 2.0 mL crimp vials (#5181-3375, Agilent Technologies Inc., Santa Clara, CA, USA) and sealed with an 11 mm silver aluminium crimp FEP/rubber cap (#5181-1210, Agilent Technologies) prior to thermal processing. For HPTP, milk solutions were filled into 1.0 mL cryogenic vials (#5000–1012, Nalgene, Rochester, NY, USA).

2.3. Isothermal–isobaric treatments

Milk solutions were treated under isothermal/isobaric conditions at temperatures ranging from 100 to 140 °C and pressures of 0.1–600 MPa up to 60 min to accurately follow and understand selected biochemical reactions in milk. Heat-only treatments were performed in a temperature-controlled shaking glycerol bath (#SWB20, Ratek, Boronia, VIC, Australia). Heating of the glass vials to target temperature took approximately 1 min. Following treatment, the samples were immediately cooled in an iced water bath and chemical analysis was performed within 4 h.

HPTP experiments under isothermal conditions were performed using a multi-vessel high pressure unit (#U111, Unipress, Warsaw, Poland) as described previously (Buckow et al., 2011). The target temperature was varied between 100 and 130 °C and the pressure of 200, 400 or 600 MPa was applied. Application of 200 or 400 MPa at 130 °C was not feasible as the removal of (flexible) plastic containers containing boiling milk was not possible. Skim milk was filled with no airspace into cryogenic vials. The cap of a duplicate vial was pierced with a needle to insert a thermocouple. Samples were sealed and stored at approximately 4 °C before they were placed into the pressure vessels heated to target temperatures. Pressurisation was started when the sample reached a temperature which would result in the target temperature after compression heating. The compression rate was set to 21 MPa/s to achieve an operational pressure of 600 MPa in less than 30 s. Pre-heating times were very short (<60 s) but vary slightly depending on the applied pressure–temperature conditions. Treatment time was started as soon as isobaric and isothermal conditions were reached. A data acquisition system (2700 Integra multimeter, Keithley Instruments, Cleveland, OH, USA) connected to the high pressure multi-vessel apparatus U111 software for data acquisition (Version 2.1c, Unipress, Warsaw, Poland) was used to monitor the pressure and temperature history of each sample.

The initial time (0 min) was defined as the time point when target temperature and/or pressure were reached, followed by immediate decompression and/or cooling in an iced water bath. The initial colour, initial area (A_0), and initial free amino group concentration (C_0) were defined as the colour, area, and concentration, respectively, found in the samples at 0 min. Area (A) refers to the area under the peptide chromatograms in the HPLC spectrum and was calculated using mathematical integration. All experiments were performed at least in duplicate.

2.4. Colour measurement

Colour of milk samples was determined at room temperature using a chromameter (Konica Minolta CR-300, Morinuchi, Tokyo, Japan) and recorded in CIE-Lab tristimulus system. Milk was placed in a glass cuvette, inserted into a black chamber (provided by Konica Minolta), and connected to the chromameter. This arrangement provided 90° angle of observation. Standard illuminant D65 was used as the light source. Colour measurement was taken in triplicate for each sample and average values were used. The colour difference between milk before and after the treatment was expressed as colour difference (ΔE_{ab}), which was calculated using Eq. (1).

$$\Delta E_{ab} = \sqrt{[(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2]} \quad (1)$$

where L is the lightness, a is the green–red co-ordinate, while b is the blue–yellow co-ordinate.

2.5. HPLC analysis

Prior to separation of peptides on HPLC, all samples were adjusted to 2% w/v trichloroacetic acid (TCA), by addition of 167 μL of 8% w/v TCA (#200-927-2, AnalaR, Merck Millipore, Kilsyth, VIC 3137, Australia) to 500 μL of milk sample. Short time (3 × 5 s) homogenisation (IKA Labortechnik T8 Ultra Turrax Disperser, Staufen, Germany) at pulse No. 2 was applied on coagulated samples before 500 μL was taken for acid precipitation. The precipitated large peptides and intact milk proteins were removed by centrifugation (25,000 × g for 20 min at 4 °C) and the TCA soluble peptides were filtered through a 0.45 μm filter (#2165, GRACE Davison Discovery Sciences, Rowville, VIC 3178, Australia) before application to the column. HPLC was performed using an automated Thermo Finnigan Surveyor Plus system (San Jose, CA, USA) fitted with a widepore C18 reversed phase Aeris column (particle size 3.6 μm, pore size 300 Å, 150 mm × 2.1 mm; Phenomenex, Lane Cove, NSW 2066, Australia) and guard column (10 mm × 2.1 mm, Phenomenex). The column temperature was maintained at 35 °C.