Food Research International 72 (2015) 74–79

Contents lists available at ScienceDirect

Food Research International

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

Skim milk protein distribution as a result of very high hydrostatic pressure

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ARTICLE INFO

Article history:
Received 23 December 2014
Received in revised form 27 February 2015
Accepted 10 March 2015
Available online 17 March 2015

Keywords:
Very high hydrostatic pressure
Whey proteins
Casein
Milk protein distribution
Lactoferrin
Secretory IgA

ABSTRACT

This work studies the micellar size and the distribution of caseins, major and minor whey proteins in different fractions of skim milk treated up to 900 MPa for 5 min. Transmission electron microscopy showed that the smallest casein micelles were formed around 450 MPa with no variations at higher pressures. The changes found in micellar size correlated with the concentration of soluble casein, because treatments at 250 MPa significantly enhanced the level of non-sedimentable casein while, between 700 and 900 MPa, there were no further increases with respect to lower pressures. There was a severe β-lactoglobulin (β-Lg) denaturation at pressures ≥700 MPa, which reached 77–87%. α-Lactalbumin (α-La) was stable up to 550 MPa, but it denatured at higher pressures. The content of soluble lactoferrin (Lf) decreased with pressure, particularly from 550 to 800 MPa, while that of secretory IgA (sIgA) progressively decreased from 250 up to 700 MPa. Our results indicated that treatment of milk at very high pressures, from 700 to 900 MPa, did not reduce micellar size nor released more soluble casein with respect to treatments at lower pressures (250–550 MPa). However, these treatments led to a severe denaturation of the whey proteins, in particular of β-Lg and the minor proteins Lf and sIgA. The possibility of using high hydrostatic pressure to obtain a soluble milk fraction with a casein and whey protein composition similar to that of human milk is discussed.

1. Introduction

The knowledge on high pressure technology has increased steadily over the past twenty years, allowing a great development mainly oriented towards food preservation. This went along with an exponential increase in the number of high pressure units capable of reaching 650 MPa in the food industries. More specifically, in 2009, 132 pieces of industrial high hydrostatic pressure equipment were installed worldwide, which made possible the marketing of a wide range of pressurized products, such as juices, guacamole, seafood products, and cold meats (Bermúdez-Aguirre & Barbosa-Cánovas, 2011; Tonello, 2011). On the other hand, several technological advances have also contributed to the availability of new pilot-scale equipment that can reach higher pressures, up to 1400 MPa (Bermúdez-Aguirre & Barbosa-Cánovas, 2011).

High pressure treatments cause substantial modifications to milk proteins and to the mineral balance of milk which affect its technological properties and the quality of dairy products (for reviews, Huppertz, Fox, De Kruif, & Kelly, 2006; López-Fandiño, 2006a,b). Pressure produces denaturation of whey proteins and affects casein micelle structure, causing micellar disruption and reaggregation and releasing soluble casein particles (Huppertz, Fox, & Kelly, 2004; López-Fandiño, de la Fuente, Ramos, & Olano, 1998; Needs, Stenning, Gill, Ferragut, & Rich, 2000). These events, whose extent depends on the process (intensity, duration and temperature), as well as on the calcium content, pH and protein concentration, influence the rennet and acid coagulation properties of milk and change the characteristics of the resulting cheeses and yogurts (Anema, 2008a; Anema, Lowe, & Stockmann, 2005; Huppertz & De Kruif, 2006). Furthermore, pressure-induced changes on whey proteins and caseins may allow the production of protein fractions of nutritional interest or improve the ability of milk proteins to act as vehicles for the encapsulation and delivery of bioactive compounds (Bravo, Molina, & López-Fandiño, 2012; Yazdi et al., 2013). The effect of the pressure level up to 600 MPa on those changes has been studied in depth, but much less is known on the outcomes of higher pressures. This work studies the micellar size and the distribution of caseins, whey proteins and minor proteins in different fractions of skim milk treated up to 900 MPa for short treatment times (5 min) that allow a high product throughput.

Abbreviations: α-La, α-lactalbumin; β-Lg, β-lactoglobulin; CE, capillary electrophoresis; Lf, lactoferrin; sIgA, secretory IgA; SP, pH 4.6 soluble fractions; US, ultracentrifugation supernatants.

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http://dx.doi.org/10.1016/j.foodres.2015.03.014
0963-9969/

2. Materials and methods

2.1. Samples

Raw bovine milk from Holstein Friesian cows, supplied by a local dairy farm, was warmed up to 37 °C for 30 min and skimmed by centrifugation at 3000 ×*g* and 20 °C for 30 min, followed by filtration through glass wool to remove fat particles.

2.2. High pressure treatment

Skim milk was submitted to 250, 450, 550, 700, 800 and 900 MPa for 5 min in a TE 9000 equipment (Thiot Ingenierie, NC Hyperbaric, Bretenoux, France–Burgos, Spain) with silicon oil as pressure transmitting fluid. The initial temperature of the chamber and samples was 15 °C and the temperature increase, measured in the pressure transmitting fluid, as a result of the pressure treatment was 6.5 °C/100 MPa. Pressure was raised at a rate 6 MPa s^{-1} and released in 30 s. After the pressure treatments, samples were stored overnight at 4 °C before protein fractionation. High pressure experiments were repeated 4 times (in duplicate on two different days, with milk from two different batches). The analytical determinations were carried out at least in duplicate.

2.3. Electron microscopy

Unpressurized and pressurized skim milk samples were examined with a transmission electron microscope JEOL sem-1010 (JEOL Ltd., Tokio, Japan) operated at 80 Kv in the Electron Microscopy Center "Luis Bru" (Universidad Complutense de Madrid, Spain). Samples were prepared according to Garcia-Risco, Recio, Molina, and Lopez-Fandiño (2003). Micellar diameters were measured manually with the program MeasureIT (Olympus Soft Imaging Solutions GmbH, Münster, German) on 16.1 × 21.6 cm sections of the photographs taken at 40,000× magnifications.

2.4. Protein fractionation

Ultracentrifugation supernatants (US) were obtained by ultracentrifugation of milk at 100,000 ×*g* and 20 °C for 1 h in a Beckman L70 preparative ultracentrifuge (Beckman Instruments Inc., San Ramon, CA), using a type 70 Ti rotor. The fractions soluble at pH 4.6 (SP) were obtained by drop-wise addition of 2 M HCl under continuous stirring and, after being allowed to stand for 20 min at room temperature, they were centrifuged at 4000 ×*g* and 20 °C for 30 min and the supernatants were filtered through Whatman n° 40 filter paper.

2.5. Protein content

The total protein content of samples was determined by the Kjeldahl method, according to the reference procedure published by the International Dairy Federation (Standard 20B, 1993) to determine total nitrogen, which was multiplied by 6.38 to obtain the protein content.

2.6. Capillary electrophoresis

Samples were analyzed by capillary electrophoresis (CE) following Recio and Olieman (1996). Separations were performed using a Beckman P/ACE System 2050 and a TSP-coated fused-silica capillary (BGB Analytik Vertrieb, Schlossboeckelheim, Germany) of 57 cm (effective length of 50 cm), 0.50 μm i.d., and a slit opening of 100 × 800 μm. Electromigrations were run at 45 °C with a linear gradient from 0 to 25 kV in 3 min, followed by a constant voltage at 25 kV during 47 min. Injection time was 60 s and detection was at 214 nm. Protein identification was carried out according to Recio, Amigo, Ramos, and López-Fandiño (1997).

2.7. Determination of lactoferrin and secretory IgA

Lactoferrin (Lf) and secretory IgA (sIgA) were quantified by sandwich ELISA with commercial kits from Bethyl Laboratories Inc. (Montgomery, USA), following the instructions of the manufacturer.

2.8. Statistical analysis

The statistical analysis of the data was carried out by one-way analysis of variance (ANOVA). The least significant difference test (LSD), considering confidence levels of 95%, was applied to determine significant differences among the pressure treatments. All statistical analyses were carried out using the Statgraphic Plus program for Windows (Manuscript Inc., 1999).

3. Results

Fig. 1 shows the transmission electron micrographs of skim milk submitted to pressures between 250 and 900 MPa. Mean micellar diameters, which ranged from 17 to 255 nm in the unpressurized milk (on average 84 nm), were reduced by high pressure, reaching a minimum at 450 MPa (Fig. 2). Pressurized micelles were more round in shape and homogeneous in size as compared with the unpressurized ones and those treated at 250 MPa. At pressures ≥450 MPa the maximum micellar diameter was very similar and smaller than 115 nm (on average between 45.97 and 53.65 nm).

As shown in Table 1, the content of proteins non-sedimentable by ultracentrifugation at 100,000 ×*g* (US) was higher in milk treated at 250 MPa than in unpressurized milk and decreased afterwards, although there were no statistically significant differences in the range of 450 and 900 MPa. As an example, Fig. 3 illustrates the protein pattern of the US from unpressurized milk and milk samples treated at 700–900 MPa as analyzed by CE. Determination of CE peak areas indicated that the content of non-sedimentable caseins increased significantly following treatments at 250 MPa of pressure ($P < 0.05$), and then much more gradually up to 700 MPa, while the whey proteins, α-lactabumin (α-La) and β-lactoglobulin (β-Lg), progressively lost solubility due to pressure-induced denaturation (Table 2).

Whey protein denaturation amounted to, approximately, 50% at pressures of 800 and 900 MPa, as reflected by the determination of the protein content of the fractions soluble at pH 4.6 (SP, Table 1). CE analysis of the SP fractions (data not shown) indicated that β-Lg was more rapidly denatured (from 12% at 250 MPa to 77% at 700 MPa and 87% at pressures ≥800 MPa) than α-La at all the pressures assayed (from 0% at pressures ≤550 MPa to 42% at 900 MPa).

Thus, while the protein content of the US was comparable in the unpressurized milk and in samples treated at pressures ≥550 MPa (Table 1), the casein and whey protein compositions of these fractions were very different because of two overlapping events: the solubilization of caseins and the denaturation of whey proteins. As a result, the ratio of whey proteins to caseins in the US decreased with pressure to reach, approximately, 20% of the value of US of the unpressurized milk in the US of milk treated at 900 MPa (Table 2). Casein solubilization induced by high pressures, together with the fact that denaturation of β-Lg was faster than that of α-La, made it possible to select the treatment conditions that provided US whose protein composition resembled that of human milk. On the one hand, the protein content of the US from the pressurized samples (that ranged between 12.9 and 10.2 g/L, Table 1) was similar to that of human milk (averaging approximately 9.4 g/L, Manso, Miguel, & López-Fandiño, 2007). On the other hand, as shown in Table 2, pressures of 450 and 550 MPa yielded US with ratios of whey proteins to caseins ranging from 42:58 to 39:61, as compared to 13:87 in the skim milk.

We also looked at the effect of high pressure on the minor whey proteins with nutritional relevance, Lf and sIgA (Fig. 4). The fraction soluble at pH 4.6 showed the same Lf content than the skim milk while, in the