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2004 J. Phys.: Condens. Matter 16 S1067

(http://iopscience.iop.org/0953-8984/16/14/017)

INSTITUTE OF PHYSICS PUBLISHING JOURNAL OF PHYSICS: CONDENSED MATTER

J. Phys.: Condens. Matter **16** (2004) S1067–S1070 PII: S0953-8984(04)74925-5

The effect of high pressure on nitrogen compounds of milk

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Received 21 January 2004
Published 26 March 2004
Online at stacks.iop.org/JPhysCM/16/S1067
DOI: 10.1088/0953-8984/16/14/017

Abstract

The effect of pressurization at different pressures (from 200 to 1000 MPa, at 200 MPa intervals, $t_{\text{const.}} = 15$ min) and periods of time (from 15 to 35 min, at 10 min intervals, $p_{\text{const.}} = 800$ MPa) on the changes of proteins and nitrogen compounds of skimmed milk was studied.

The pressurization caused an increase in the amount of soluble casein and denaturation of whey proteins. The level of nonprotein nitrogen compounds and proteoso-peptone nitrogen compounds increased as a result of the high-pressure treatment. These changes increased with an increase in pressure and exposure time. High-pressure treatment considerably affected the changes in the conformation of milk proteins, which was reflected in the changes in the content of proteins sedimenting and an increase in their degree of hydration.

1. Introduction

Widespread heat treatment in the dairy industry can cause losses of some milk components. Therefore, alternative methods have received much interest, including the application of high pressures for milk and dairy product preservation. High-pressure technology affords potential opportunities in the preservation of raw dairy materials and products as well as in the creation of products with modified sensory and nutritional properties [1]. The application of high pressures in the technology of drinking milk produces a sterile product while maintaining the sensory properties typical for fresh milk. An additional advantage of this preservation method

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is that it can be carried out in packaging. This prevents reinfection and eliminates the process of aseptic packaging.

Under high pressures, the hydrophobic and electrostatic bonds in milk are broken and the majority of milk components are affected. In milk technology, the molecular state of its proteins has a particular importance.

Pressurization induces structural changes in milk proteins which result in the unfolding of polypeptide chains, aggregation, polymerization, precipitation, coagulation or gelling. The mechanism of protein conformation changes under high pressures is determined by protein type, pH, ionic strength and pressurization parameters, i.e. applied pressures, temperature and exposure time.

Further studies into the effect of high pressure on the nitrogen compounds system in milk will complement the current understanding of the modifications of milk properties, which is important from the technological point of view.

The objective of the present work was to examine the influence of pressurization on the changes in nitrogen compounds in skimmed cow's milk.

2. Material and methods

In the experiment, the cow's milk was pressurized at 200 to 1000 MPa at 200 MPa intervals ($t_{\text{const.}} = 15$ min; $T_{\text{const.}} = 20\,^{\circ}\text{C}$), and for periods of time ranging from 15 to 35 min, at 10 min intervals ($p_{\text{const.}} = 800$ MPa; $T_{\text{const.}} = 20\,^{\circ}\text{C}$).

In the examined skimmed milk samples, the following determinations were carried out: protein hydration degree according to Thompson *et al* [10] (ultracentrifugation at 68 000 g for 35 min at 37 °C), the number of protein compounds sedimenting during ultracentrifugation (Kjeldahl's method), casein content in the supernatant (soluble casein) (precipitation in the isoelectric point), the degree of whey protein denaturation (precipitation of casein with whey proteins in pH $= 4, 6$, expressed as a percentage of the contents of the corresponding raw milk), and the level of proteose-peptone nitrogen and nonprotein nitrogen (PPN + NPN) [11].

The content of the nondenatured whey proteins of the milk was determined by high-performance liquid chromatography (HPLC) of the filtrate left after precipitation of casein with denatured whey proteins in pH $= 4, 6$ [7, 8, 12].

The quality of the obtained chromatograms was evaluated by comparing the retention time for α-lactoalbumin, β-lactoglobulin A, β-lactoglobulin B, and immunoglobulin in standard samples with the retention time in the examined samples. The amounts of investigated whey proteins were calculated by comparing the surface area of their peaks for standard and examined samples, after their integration with regard to the concentration of these whey proteins in the standard samples. The standard samples of α-lactoalbumin, β-lactoglobulin A, and β-lactoglobulin B were obtained from Sigma, and immunoglobulin—from Merck. The pH of the milk was controlled during the experiment.

3. Results and discussion

The effect of high-pressure treatment on the changes in some physico-chemical properties of milk are presented in table 1.

The studies showed that high pressure with the applied parameters induced a slight increase in the pH of skimmed milk by 0.04, from 6.65 to 6.69 (data not shown).

High-pressure treatment considerably affected the changes in the nitrogen compounds, and this depended on the pressure and the length of exposure. Pressurization resulted in an increase in the amount of soluble casein, which constituted about one third of total casein in milk at pressures from 800 to 1000 MPa.

Table 1. The effect of high-pressure treatment on changes in nitrogen compounds in milk.

Treatment		Prot.$_{sed.}$ (%)	Hydrat. (gH_2O/ g prot.$_{sed.}$)	Casein solv. (%)	Degree of whey prot. denat. (%)	PPN + NPN (%)
Control sample		2.46	1.98	0.15	—	0.034
Pressurized						
$t = 15$ min	200 MPa	1.99	1.99	0.66	18.5	0.033
	400 MPa	2.00	2.46	0.72	35.7	0.041
	600 MPa	2.04	2.58	0.78	51.1	0.043
	800 MPa	2.10	2.65	0.78	62.7	0.044
	1000 MPa	2.16	2.60	0.84	78.3	0.047
$p = 800$ MPa	15 min	2.10	2.65	0.78	62.7	0.044
	25 min	2.11	2.74	0.79	67.7	0.045
	35 min	2.12	2.65	0.79	73.1	0.046

Pressurization, depending on the applied parameters, degrades casein micelles to smaller forms or leads to their reaggregation accompanied by a change in milk turbidity [2, 6].

The dissociation of particular casein fractions has the following course: $\beta \rightarrow \chi \rightarrow \alpha_{s1} \rightarrow \alpha_{s2}$-casein; this is closely related to the level of the phosphate residues in casein and the transformation of colloidal calcium phosphate into a dissolved form [6].

Milk exposure to high pressure resulted in the denaturation of milk whey proteins at a higher degree if a higher pressure and longer intervals were applied. The level of nonprotein nitrogen compounds and proteose-peptone nitrogen compounds increased with increasing pressure and time.

The conformational changes in proteins resulting from high-pressure treatment are reflected in the changes in the content of proteins sedimenting during ultracentrifugation as well as in the increase in their degree of hydration. It was found out that the content of proteins sedimenting at 200 MPa decreased; however, when the pressure was higher their content increased, irrespective of time exposure.

Consequently, the effect of this process on an increase in sedimenting protein hydration results from higher casein hydration, which is connected with an increase in the degree of micelle dispersion. Another factor which affects the increase in sedimenting protein hydration is water retention by denaturated whey proteins associated with casein.

The results of the present studies showed that the effect of high-pressure treatment on the amount of nondenatured whey protein depends on its type (figure 1).

The biggest changes were noted in β-lactoglobulin, whose denaturation degree amounted to 20% as a result of a pressure of 200 MPa and increased with higher pressure and longer exposure time, reaching maximum values ranging from 80% to 90%. High-pressure treatment induced smaller changes in α-lactoglobulin and immunoglobulins. The denaturation of α-lactalbumin (about 10%) was noted only as a result of a pressure of 400 MPa, while higher pressures caused further denaturation changes which reached a value of 40%. Alpha-lactoalbumin is more resistant to denaturation by pressure than beta-lactoglobulin due to the presence of four disulfide bonds. Immunoglobulins denaturated at each applied pressure, and their denaturation degree was 17% in milk subjected to a pressure of 200 MPa, increasing with higher pressure and longer intervals up to 36%.

The experimental results are partially confirmed by literature. The application of high pressures causes denaturation of whey proteins with denaturation of β-lactoglobulin beginning at pressures over 100 MPa, while α-lactoalbumin and serum albumin are resistant to pressures below 500 and 400 MPa, respectively [4, 5]. On the other hand, studies into the effect of

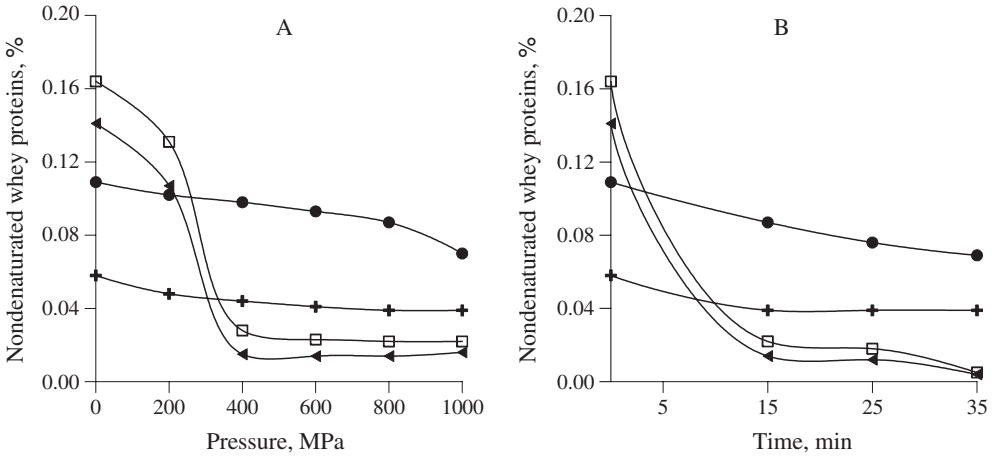

Figure 1. The effect of pressure (A) and time of pressurization (B) on the amount of nondenatured whey proteins: ●—α-lactoalbumin; □—β-lactoglobulin A; ▲—β-lactoglobulin B; +—immunoglobulin.

high pressures on immunoglobulins concerned goats milk, and showed their stability under pressures of 300 MPa and their 35% denaturation under 500 MPa [3].

Pressurization modifies the protein quarternary and tertiary structure through breaking the hydrophobic and electrostatic bonds (ionic and polar). On the other hand, hydrogen bonds that particularly stabilize the protein secondary structure can be strengthened or broken, depending on the value of the applied pressure. The peptide bonds that stabilize the protein primary structure are not broken because high pressure applied to milk at room temperature does not affect covalent bonds [9].

The findings of the experiment allow us to draw the conclusion that high-pressure treatment induces a dynamic equilibrium disturbance of milk proteins and, consequently, may exert an influence on their molecular stability and related milk liability to coagulating agents.

Acknowledgment

This paper has been prepared with financial support from the Commission of the European Communities, specific RTD programme 'Quality of Life and Management of Living Resources', QLK1-2002-00401 'Warmia and Mazury Dairy Excellence Center'.

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