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COLLOIDAL STABILITY OF MILK WITH VARIOUS CONTENTS OF FAT SUBJECTED TO HIGH-PRESSURE HOMOGENIZATION

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The research was aimed at determining the effect of high-pressure homogenization (100 MPa) on the stability of proteins and equilibrium of mineral salts in skim milk and milk standardized to fat content of 2% and 4%.

High-pressure homogenization of skim milk evoked a slight increase in the content of protein compounds sedimenting during ultracentrifugation and in a degree of their hydratation. In turn, homogenization of milk with the standardized fat content affected a decrease in the content of proteins in milk plasma as well as in the quantity of sedimenting proteins and in the degree of their hydrations, both observed to intensify along with a higher fat content. In addition, the study demonstrated partial denaturation of whey proteins, an increase in the level of soluble forms of calcium and phosphorus salts, as well as a decrease in heat stability and time of rennet coagulation upon high-pressure homogenization. The changes were observed to intensify along with an increasing content of fat in milk.

INTRODUCTION

A traditional method applied for milk preservation is heat treatment, *e.g.* thermization, pasteurization or sterilization. In constant pursuit for improving the quality of milk and dairy products of high interest are methods of preservation of milk and dairy products alternative to the thermal method. One of them is high-pressure homogenization conducted based on a dynamic effect of pressures around 100 MPa on a product, with an additional factor being temperature which increases along with a pressure rise by 2-2.5°C per each 10 MPa [Popper & Knorr, 1990].

Thiebaud *et al.* [2003] demonstrated the effect of high-pressure homogenization on decreasing and unifying the size of fat globules, on an increase in milk temperature in respect of its initial value, and on the inactivation of microorganisms along with a pressure rise. Thus, the high-pressure homogenization is a process that increases the stability of milk both from the viewpoint of emulsion stability (an increase in the degree of fat dispersion) and microbiological one (reduction of bacterial count). Owing to this, its application enables eliminating pasteurization from the production process of dairy products, *e.g.* drinking milk [Hayes *et al.*, 2005] or some types of cheese. However Smiddy *et al.* [2007] postulate that preservation of milk through high-pressure homogenization is insufficient to obtain microbiological quality typical of drinking milk with elongated shelf life (ESL). A practical application of high-pressure homogenization in technology of milk, ESL milk in particular, is far more effective when coupled with conventional heat processes.

The application of high pressures during homogenization elicits a number of significant changes in particular constituents of milk, thus affecting its structure and properties. During preservation of dairy products by means of high temperatures of key significance is the stability of the colloidal phase determined, to the greatest extent, by dynamic equilibrium of milk proteins as well as form of calcium and phosphorus salts. Apart from increase in the degree of fat dispersion, homogenization modifies the arrangement and equilibrium of components of the colloidal phase of full fat milk since the surface-active fractions of milk proteins participate in reconstruction of membranes of homogenized fat globules. The impact of homogenization on milk proteins and susceptibility of homogenized milk to coagulating agents is linked, most of all, with their adsorption on the surface of milk fat increasing upon that process [McCrae *et al.*, 1994]. Owing to the fact that homogenization affects also proteins of skim milk [Garcia-Risco *et al.,* 2002; Sandra & Dalgleish, 2005], the objective of the presented study was to compare effects of high-pressure homogenization on the stability of components of the colloidal phase in plasma of skim milk and that of milk standardized to fat content of 2% and 4%.

MATERIALS AND METHODS

The experimental material was skim milk and milk standardized to fat content of 2% and 4% (control samples) and milk subjected to high-pressure homogenization at 100 MPa using a PANDA SN–3439 Niro Soavi homogenizer. Temperature of milk samples examined was measured before

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(60±1°C) and after the homogenization process. The experiment was conducted in six replications.

Analyses covered determinations of: the content of protein compounds (acc. to Kjeldahl's method) in milk and in plasma separated from fat during centrifugation [Cano-Ruiz & Richter, 1997], the content of protein compounds sedimenting during ultracentrifugation (68,000×*g*, 35 min, 35°C), degree of proteins hydratation acc. to Thompson *et al.* [1969], and degree of whey proteins denaturation (precipitation at pH 4.6). Simultaneously, samples of milk and supernatant were determined complexometrically for the content of calcium [Satia & Raadsveld, 1969] and colormetrically for the content of phosphorus [Swartling & Mattson, 1954]. Susceptibility of milk to coagulating agents was analysed based on determination of its heat stability (HS) as the time of thermal coagulation at a temperature of 140°C acc. to White & Davies in modification of Kruk *et al.* [1979] and time of rennet coagulation (RCT) [Alais & Jolles, 1964].

The results obtained were analysed statistically by means of the one-way analysis of variance conducted in StatSoft Inc. Statistica v. 7.0 software.

RESULTS AND DISCUSSION

The effect of homogenization on milk components depends on the pressure applied and temperature of milk during the process. In the case of the high-pressure homogenization, one of its effects exerted on milk is a rise of its temperature, increasing along with an increasing fat content of milk. At an ambient temperature of 18°C, after homogenization the temperature of skim milk and milk standardized to fat content of 2% and 4% reached: 65°C, 67°C and 69°C, respectively. This is, however, inconsistent with literature data, according to which an increase in temperature of milk as a result of homogenization accounts for *ca*. 18°C [Hayes & Kelly, 2003; Thiebaud *et al.,* 2003] and 12°C [Sandra & Dalgleish, 2005] per each 100 MPa. It could have been due to different experimental conditions, among others, type and yield of a homogenizer, volume of a milk sample, temperature of milk before homogenization, and ambient temperature.

Results of a study into the effect of high-pressure homogenization on selected characteristics of the colloidal phase of skim milk and milk standardized to fat content of 2% and 4% were presented in Table 1.

The evaluation of the effect of high-pressure homogenization on changes in the forms of protein compounds and mineral salts occurring in milk with different contents of fat was conducted based on results of the one-way analysis of variance and presented in Table 2.

High-pressure homogenization of skim milk resulted in a slight, statistically insignificant, increase in the content of protein sedimenting during ultracentrifugation (2.7%), which constituted 65.7% and 67.5% of milk plasma protein before and after the process, respectively. In turn, homogenization of milk with fat content of 2% and 4% affected a decrease in the content of milk plasma proteins by 18% and 32%, respectively, and in the content of sedimenting proteins by *ca*. 6% (statistically significant changes at $p<0.05$) and by *ca*. 21% (significance of differences at $p<0.01$). Those changes are linked with the adsorption of protein compounds of milk

TABLE 1. Effect of high-pressure homogenization on changes in the form of protein compounds and mineral salts occurring in milk with various contents of fat (mean values, n=6).

Specification	Non-homogenized milk with fat content:			Homogenized milk with fat content:		
	0.05%	2%	4%	0.05%	2%	4%
Total protein (%)	3.32	3.31	3.29	3.32	3.31	3.29
Plasma protein (%)	3.32	3.17	3.14	3.32	2.61	2.13
Sedimenting protein (%)	2.18	2.11	2.06	2.24	1.98	1.63
Hydratation (H_2O/g pellet)	2.13	2.12	2.09	2.24	1.91	1.87
Denaturation degree of whey proteins (%)	0	0	0	8.3	9.5	12.3
Soluble calcium (g/100g Ca_{total})	33.0	32.9	32.9	37.3	38.1	39.4
Soluble phosphorus (g/100g P_{total})	30.9	30.9	31.9	35.6	36.8	39.5

TABLE 2. One-way analysis of variance of results obtained in the study on the effect of high-pressure homogenization on changes in the forms of protein compounds and mineral salts occurring in milk with various contents of fat.

Parameters	Milk with fat content of:					
	0.05%		2.0%		4.0%	
	F	p	F	p	F	p
Sedimenting protein	2.58	0.139	8.11*	0.017	116.05**	<0.001
Hydratation of sedimenting proteins	1.60	0.234	4.51	0.060	8.27*	0.017
Soluble calcium	9.60*	0.011	29.68**	<0.001	18.36**	0.002
Soluble phosphorus	18.20**	0.002	21.33**	0.006	28.37**	<0.001

n = 6; $F_{tab.}$ = 4.96; *difference statistically significant at $p<0.05$; $F_{tab.}$ = 10.04; **difference statistically significant at $p<0.01$.

plasma on the extending surface of homogenized fat globules [Kiełczewska *et al.,* 2004].

The content of sedimenting proteins in respect of the total protein in plasma of milk with standardized fat content was observed to increase from *ca*. 66% before homogenization to *ca*. 76% after the process, which has been confirmed in results reported by Kiełczewska *et al.* [2006]. The increase in the content of sedimenting protein in total protein of milk plasma after homogenization is, probably, affected by a change in the size of casein micelles. Results of a study by Garcia-Risco *et al.* [2002] showed that two-stage conventional homogenization affected a decrease in the size of casein micelles in full fat and skim milk. Sandra & Dalgleish [2005] did not demonstrate any effect of homogenization at a pressure of 41 MPa on the size of casein micelles of reconstituted skim milk, whereas an increase of pressure applied over the range of 114-186 MPa contributed to a reduction of their sizes. In addition, those authors reported on the effect of high-pressure homogenization on dissociation of casein (κ, α_{s1}, α_{s2}), most likely as a result of disintegration of casein micelles. In contrast, Hayes & Kelly [2003] did not observe any effect of homogenization conducted under chill conditions at a pressure of ≤150 MPa on changes in the size of casein micelles, whereas a small reduction of micelles (5%) was observed upon subjecting skim milk to pressure treatment at 200 MPa. The increase in the content of sedimenting protein in respect of plasma protein in homogenized full fat milk could have been due to both micellar casein and non-sedimenting proteins, *i.e.* submicellar casein and whey proteins, adsorbed during homogenization on the surface of fat globules which – owing to their size – remained in the plasma. Very small fat globules are characterised by a higher thickness as compared to that of plasma due to a very high protein-to-fat ratio, thereby they sediment during ultracentrifugation [Walstra & Jennes, 1984].

Homogenization elicited denaturation of whey protein in the range of 8.3-12.3%, observed to increase along with an increasing content of fat. Garcia-Risco *et al.* [2002] demonstrated the effect of conventional homogenization on denaturation of whey proteins of milk heated to 40°C. In turn Hayes & Kelly [2003] did not observe denaturation of those proteins in milk subjected to high-pressure homogenization at a temperature of *ca*. 7°C. Whereas, applying that process on milk with an initial temperature of 45°C affected denaturation of β-lactoglobulin and, to a lesser extent, of α-lactalbumin [Hayes *et al.,* 2005].

The increasing degree of whey protein denaturation demonstrated in the reported study along with an increasing fat content of homogenized milk is likely to result from the effect of process temperature and is additionally caused by precipitation (at pH 4.6) of whey proteins, both the denaturated and undenatured ones, adsorbed with casein on the surface of fat globules. The fat globules with plasma proteins adsorbed on their surface behave similarly to large casein micelles and participate in reactions typical to them [Walstra & Jennes, 1984]. Thus, preferential adsorption of large molecules of proteins on the surface of homogenized fat globules [Walstra & Jennes, 1984], in that case of β-lactoglobulin, as well as its lower heat resistance as compared to α-lactalbumin, lead to a higher degree of β-lactoglobulin denaturation.

High-pressure homogenization caused a negligible, statistically insignificant, increase (*ca*. 5%) in the hydratation degree of casein of skim milk, which may be a consequence of enhanced dispersion of casein micelles and, additionally, mechanical retention of water by aggregated whey proteins of homogenized milk. As a result of the homogenization process, the degree of hydratation of sedimenting proteins in the standardized milk was subject to a decrease by *ca*. 10%, yet results of the statistical analysis demonstrated the significance of differences at a level of $p<0.05$ only in the case of milk with fat content of 4%.

In addition, the study demonstrated an increase in the level of soluble salts of calcium (*ca*. 13%) and phosphorus (*ca.* 15%) in the homogenized skim milk. In turn, in the homogenized milk with fat content of 2% and 4% the content of soluble calcium was observed to increase by *ca.* 16% and 20%, whereas that of soluble phosphorus by *ca*. 19% and 24%, respectively. Changes in levels of those components occurring as a result of high-pressure homogenization were statistically significant at $p<0.01$. An exception was the content of soluble calcium in skim milk, the level of which before and after homogenization differed significantly at $p<0.05$.

The increased contents of calcium and phosphorus in milk serum, observed in this study, are likely to result from the effect of homogenization on changes in the colloidal phase that led to dissociation of colloidal calcium phosphate. Those changes included an increase in the degree of casein micelles dispersion and release of casein fractions κ, α_{s1}, and α_{s2} to the liquid phase in homogenized skim milk [Sandra & Dalgleish, 2005] as well as disintegration of micelles to subunits and their adsorption on the surface of homogenized fat globules in full fat milk [Dalgleish *et al.,* 1996].

The changes occurring in milk upon the process of high--pressure homogenization resulted in the shortening of time of heat coagulation (HS) and rennet coagulation time (RCT). What is more, the changes were observed to intensify along with an increasing content of fat in milk (Figure 1).

TABLE 3. One-way analysis of variance of results obtained in the study on the effect of high-pressure homogenization on changes in heat stability and rennet coagulation time of milk with various contents of fat.

Parameters	Milk with fat content of:					
	0.05%		2.0%		4.0%	
	F	p	F	p	F	p
Heat stability	1.73	0.536	25.58**	<0.001	26.19**	<0.001
Rennet coagulation time	2.18	0.170	4.50	0.060	36.71**	<0.001

n = 6; $F_{tab.}$ = 4.96; *difference statistically significant at $p<0.05$; $F_{tab.}$ = 10.04; **difference statistically significant at $p<0.01$.

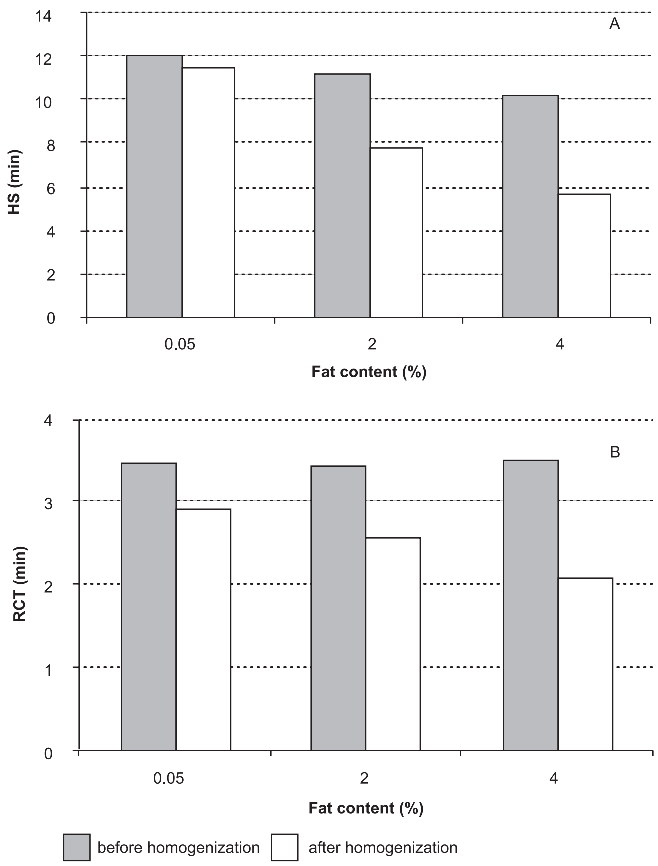

FIGURE 1. Effect of homogenization on heat stability – HS (A) and rennet coagulation time – RCT (B) in milk with various contents of fat.

Results of the one-way analysis of variance conducted for the effect of high-pressure homogenization on changes in the resistance of proteins of skim milk and milk standardized to fat content of 2% and 4% as affected by coagulating factors, *i.e.* high temperature and rennet, were presented in Table 3.

Heat stability of milk standardized to fat content of 2% and 4% was decreasing as a consequence of homogenization (changes statistically significant at $p<0.01$), maximally by 45% in the case of milk with fat content of 4%. High-pressure homogenization was also shown to affect the shortening of rennet coagulation time of milk with fat content of 2% (changes statistically insignificant) and maximally by 41% in the case of milk with fat content of 4% (changes statistically significant at $p<0.01$). It has been confirmed in a previous study by Kiełczewska *et al.* [2000], who demonstrated the effect of high-pressure homogenization conducted under pressure range of 20–140 MPa on decreasing heat stability and shortening rennet coagulation time of full fat milk. A smaller, statistically insignificant range of changes in heat stability (decrease by <5%) and rennet coagulation time (shortening by <16%) was observed in the case of skim milk.

Data obtained for the changes in heat stability of homogenized milk as affected by its fat content have, in part, confirmed results of experiments conducted by Sweetsur & Muir [1983]. They showed that homogenization at pressures up to 31 MPa did not affect heat stability of skim milk, whereas had a tangible effect on destabilization of proteins of full fat milk.

The observed tendency of changes in rennet coagulation time upon the homogenization process is consistent with findings of Hayes & Kelly [2003], who demonstrated the effect of conventional and high-pressure homogenization on statistically significant shortening of the time of enzymatic coagulation of full fat milk. In turn, Sandra & Dalgleish [2007] observed diminished time of rennet coagulation upon high--pressure homogenization also in skim milk. They explained those changes by previously observed [Sandra & Dalgleish, 2005] dissociation of fraction κ from the surface of casein micelles as a consequence of milk homogenization. In contrast Walstra [1980] did not observe any changes in the rennet coagulation time of skim milk as a result of conventional homogenization. In the case of full fat milk, the location of milk proteins, casein in particular, on the surface of homogenized fat, accompanied by a small contribution of fraction κ, leads to their increased availability, which in turn facilitates coagulation of milk.

The results obtained in the reported study, supported by the statistical analysis, as well as the above discussion enable concluding that homogenization of full fat milk, to a greater extent than that of skim milk, caused destabilization of components of the colloidal phase, *i.e.* proteins and mineral salts, which has been reflected in changes in parameters of technological usability, *i.e.* heat stability and rennet coagulation time.

CONCLUSIONS

1. High-pressure homogenization results in a decrease in protein content of milk plasma, including also protein subject to sedimentation, as well as in changes of the hydratation degree of sedimenting protein, with the changes being more intensified along with an increasing fat content of milk.
2. The effect of high-pressure homogenization of the colloidal phase of milk is reflected in changes of its mineral balance towards an increase in the level of soluble forms of calcium and phosphorus.
3. High-pressure homogenization contributed to a diminished resistance of milk proteins to coagulating factors, *i.e.* high temperature and rennet, to the extent determined by the content of fat in milk.

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