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EFFECTS OF HIGH-PRESSURE HOMOGENIZATION ON THE PHYSICOCHEMICAL PROPERTIES OF MILK WITH VARIOUS FAT CONCENTRATIONS

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The effect of high-pressure (100 MPa) homogenization on some properties of the emulsion phase and colloidal phase of milk standardized to 2% fat or 4% fat were determined in the study. High-pressure homogenization caused a decrease in the size of fat globules and an increase in the level of protein compounds bound to the milk fat fraction, dependent upon the fat content of milk. The process resulted in an increase in the amount of ultracentrifuged sediment, in the level of sedimenting plasma proteins not bound to the milk fat fraction, and in the solubility of calcium salts and phosphorus salts, as well as a decrease in their levels in the colloidal form, related to the fat content of milk. High-pressure homogenization led to slight changes in active acidity and conductivity (increase), as well as to a substantial decrease in heat stability (max. 42.4%) and a shorter rennet coagulation time (max. 34.5%), directly proportional to fat concentration in milk.

INTRODUCTION

Homogenization is a technological process commonly applied in the dairy industry to assure emulsion stability, which is achieved by making milk fat globules smaller and more uniform. Apart from increasing the degree of fat dispersion, the process causes also changes in the state of milk proteins. In the case of whole milk, these are changes in milk fat dispersion and the adsorption of milk plasma proteins, mainly casein, on the fat-plasma interface [Cano-Ruiz & Richter, 1997; Dalgleish & Robson, 1985]. The fat to protein ratio determines the size and surface areas of milk fat globules, as well as the degree of milk protein adsorption. The quantity of proteins adsorbed on the surfaces of milk fat globules increases with an increase in the fat content of milk, reaching 10 mg/m^2 milk fat at the fat to protein ratio below 4. When this value is exceeded, the protein surface coverage decreases [Tomas *et al.*, 1994].

In order to prevent fat formation during long-time storage of liquid dairy products, homogenization is carried out at a pressure around 20 MPa. High-pressure (100 MPa or above) homogenization enables achieving a high degree of milk fat dispersion, and improving the microbiological quality of milk [Hayes & Kelly, 2003; Thiebaud *et al.*, 2003; Hayes *et al.*, 2005]. In addition, the temperature rise caused by high pressure enables starting homogenization at room temperature (about 20°C) or directly after cold storage (4°C), omitting the stage of milk heating.

The aim of the present study was to determine the effects of high-pressure homogenization on changes in some physicochemical properties of milk, depending on fat content of milk.

MATERIALS AND METHODS

The experimental materials comprised samples of milk standardized to 2% fat or 4% fat, homogenized at 100 MPa using a PANDA SN-3439 homogenizer (Niro Soavi) at the Chair of Process Engineering and Equipment and Energy Management, University of Warmia and Mazury in Olsztyn. Non-homogenized milk samples containing 2% and 4% fat served as control. Milk samples were preserved by an addition of 1 mL 2% solution of sodium azide per 1 L milk.

The degree of milk fat dispersion was estimated by a microscopic method, on milk samples prepared according to the Polish Standard PN-A-86059:1975. The results of observations provided the basis for calculating the volume-surface average diameters (d_{vs}) and surface area of milk fat globules. The amount of milk plasma proteins bound to milk fat was calculated based on the determinations of the concentrations of protein and fat in milk and in milk serum after centrifugation (10 500 g, 30 min, 20°C) of the samples with 28.6 g saccharose/100 g milk [Cano-Ruiz & Richter, 1997]. Changes in the form of protein compounds not bound to the milk fat fraction were estimated based on the determinations of the protein compounds sedimenting during ultracentrifugation (68 000 g, 35min, 35°C) [Thompson *et al.*, 1969], and presented as their proportion in milk plasma proteins. The levels of soluble calcium [Satia & Raadsveld, 1969] and soluble phosphorus [Swartling &

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Mattson, 1954] were determined in the supernatant obtained by milk ultracentrifugation, and presented as their proportions in relation to total calcium and total phosphorus. The technological suitability of homogenized milk was estimated on the basis of: active acidity – determined using a pH-meter, type WTW inoLab pH Level 1; conductivity – determined using a conductometer, type WTW inoLab Cond Level 1; heat stability – determined by the method developed by White & Davies and modified by Kruk *et al.* [1979], and rennet coagulation time [Alais & Jolles, 1964].

The experiment was performed in seven replications.

RESULTS AND DISCUSSION

The results of studies on the effects of high-pressure homogenization on the degree of milk fat dispersion showed that the diameters of fat globules decreased over fourfold (Photo 1). Due to modifications of the emulsion phase and much greater surface area of milk fat globules observed after high-pressure homogenization in the present experiment, as well as an insufficient amount of membranous material from native fat globules, milk proteins participate in the reconstruction of membranes of the newly-formed interface. The amount of plasma proteins, bound to the milk fat fraction, in milk containing 2% or 4% fat was 21.1 g/100 g total protein and 39.8 g/100 g total protein, respectively (Table 1). Both the decrease in the diameters of milk fat globules and their greater uniformity, resulting from homogenization, as well as their higher density caused

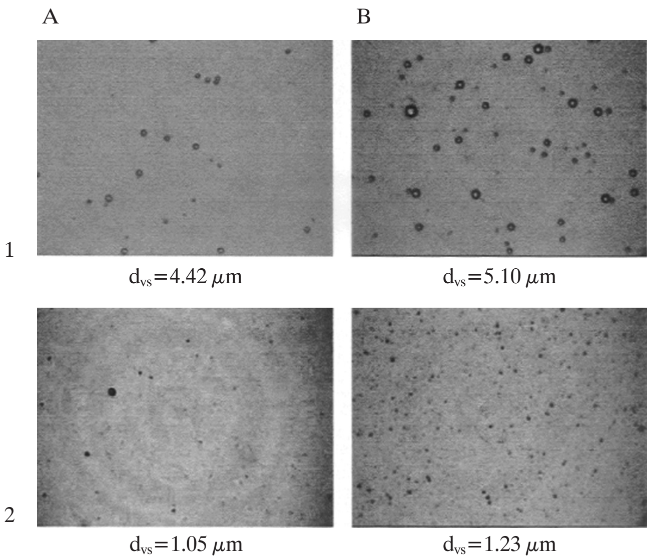

PHOTO 1. Microscopic image of fat globules in milk containing 2% fat (A) and 4% (B) (1 cm=28 μm). 1 – non-homogenized milk, 2 – homogenized milk.

TABLE. 1 Effect of high-pressure homogenization on some properties of milk fat.

Milk	Fat content (%)	Surface area of fat globules (m^2/mL fat)	Proportion of protein bound to the fat (g/100 g total protein)	Amount of protein bound to the fat (mg/g fat)
Non-homogenized	24	1.40	1.9	31
	4	1.20	3.8	28
Homogenized	2	5.71	21.1	534
	4	4.88	39.8	345

by the adsorption of milk plasma proteins on their surface, contribute to preventing cream separation during homogenized milk storage [Hayes *et al.*, 2005].

The amount of plasma proteins bound to 1 g milk fat decreases with an increase in the fat content of homogenized milk. Milk proteins, mostly casein, are spread across the increasing fat-plasma interface as a result of the surface forces in the course of adsorption, or that during homogenization casein micelles are broken into subunits which easily undergo adsorption on the surfaces of fat globules. Sharma *et al.* [1996], who studied the composition of proteins participating in the formation of fat globule membranes in recombined milk, demonstrated the presence of whole casein micelles or their fragments and lower amounts of whey proteins. Dalgleish *et al.* [1996] stressed the effects of milk microfluidization on casein micelle damage and adsorption of casein, present rather in the form of submicelles than in the native state, on the fat-plasma interface. It seems that micelle disintegration to subunits and their adsorption on the surfaces of milk fat globules increase the solubility of calcium salts and phosphorus salts, and decrease their levels in the colloidal form in consequence of high-pressure homogenization of milk containing 4% fat, as observed in this experiment (Table 2). It was also found that the process affected an increase in the amount of ultracentrifuged sediment and in the level of sedimenting plasma proteins not bound to the milk fat fraction (Table 2), which may suggest reaggregation of submicelles in homogenized milk. Sandra & Dalgleish [2005], who studied the influence of high-pressure homogenization on proteins in skim reconstituted milk, reported that casein micelles became smaller in size and that the process was accompanied by an increase in the solubility of κ- and α_s-fractions, most probably in consequence of casein micelle disintegration.

The specific character and range of changes in protein and mineral salts, dependent upon the fat content of milk subjected to high-pressure homogenization, decide about the technological suitability of milk (Table 3). As a result of

TABLE 2. Effect of high-pressure homogenization on changes in the forms of protein compounds and mineral salts in milk.

Milk	Fat content (%)	Dry sediment (g/100 g milk)	Sedimenting proteins (g/100 g plasma proteins)	Colloidal calcium (g/100 g total Ca)	Colloidal phosphorus (g/100 g total P)
Non-homogenized	2	2.67	66.4	66.9	69.1
	4	2.69	65.7	67.1	68.1
Homogenized	2	2.74	75.8	66.9	68.7
	4	2.71	76.5	61.1	59.9

TABLE 3. Effect of high-pressure homogenization on changes in some physicochemical properties of milk.

Milk	Fat content (%)	Active acidity pH	Conductivity (mS/cm)	Heat stability (min)	Rennet coagulation time (min)
Non-homogenized	2	6.74	5.24	10.55	3.44
	4	6.72	5.15	10.46	3.56
Homogenized	2	6.72	5.30	8.00	2.76
	4	6.71	5.20	6.02	2.33

homogenization, the pH of the samples decreased slightly, whereas electrical conductivity increased to some extent. Electrical conductivity of milk is predominantly determined by mineral salts, dissociated in the aqueous phase. Conductivity is also affected by the amount of fat contained in milk, due to its low electrical conductivity [Mabrook & Petty, 2003]. The effect of high-pressure homogenization on milk conductivity is related to modifications of the colloidal phase, *i.e.* a disturbance in the equilibrium between soluble and colloidal forms of calcium salts and phosphorus salts, as well as to changes in the dispersion and number of milk fat globules.

High-pressure homogenization reduced milk resistance to coagulating factors and this effect was directly proportional to the increase in the fat content of homogenized milk. The disturbance in the dynamic equilibrium of milk proteins and mineral salts manifested itself by a shorter rennet coagulation time (max. 34.5%), corresponding to the increase in the fat content of homogenized milk. Hayes & Kelly [2003] recorded a shorter rennet coagulation time as a result of high-pressure homogenization, additionally affected by the pressure applied.

Heat-pressure homogenization led to a decrease in heat stability (max. 42.4%), depended on the fat concentration in milk. Greater surface areas of milk fat globules, obtained in consequence of homogenization, and modifications of the colloidal fraction, resulting from the interactions between fat and plasma proteins adsorbed on the interface, decrease stability of milk proteins. Heat stability of homogenized milk is also connected with the presence of plasma proteins adsorbed on the surfaces of fat globules, and their interactions [McCrae *et al.*, 1994]. Moreover, the occurrence of casein on the surface of homogenized fat globules suggests that milk fat globules with adsorbed newly-formed protein materials behave like large casein micelles [Sharma & Singh, 1999]. This, in turn, contributes to a reduction in the heat stability of whole homogenized milk.

The influence of high-pressure homogenization on an increase in the susceptibility of whole milk to coagulating factors is of primary importance during high-temperature preservation of dairy products, and decides about their storage stability. Due to the simultaneous effects of two preserving factors, *i.e.* pressure and temperature, high-pressure homogenization enables modifying milk technology. Under optimum conditions, it offers the possibility to alter the physicochemical properties of milk, which is of great practical significance.

CONCLUSIONS

1. High-pressure homogenization caused modifications of the emulsion phase, *i.e.* an increase in milk fat dispersion, and of the colloidal phase, related to the adsorption of milk serum proteins on the surface of homogenized fat globules.

2. High-pressure homogenization is followed by an increase in the solubility of calcium salts and phosphorus salts and a decrease in their levels in the colloidal form, probably due to micelle disintegration to subunits and their adsorption on the surface of milk fat globules.

3. The increase in the amount of ultracentrifuged sediment and in the level of sedimenting plasma proteins not bound to the milk fat fraction suggests reaggregation of submicelles in homogenized milk.

4. Modifications of the colloidal phase caused by high-pressure homogenization contribute to changes in the technological suitability of milk, especially to an increase in the susceptibility of milk protein to coagulating factors. The process manifests itself by a reduction in the thermal stability of milk and a shorter rennet coagulation time, corresponding to the increase in the fat content of homogenized milk.

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HOMOGENIZACJA WYSOKOCIŚNIENIOWA MLEKA O ZRÓŻNICOWANEJ ZAWARTOŚCI TŁUSZCZU A JEGO CECHY FIZYKOCHEMICZNE

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W pracy określono wpływ homogenizacji wysokociśnieniowej, przeprowadzonej przy ciśnieniu 100 MPa na wybrane cechy fazy emulsyjnej i koloidalnej mleka znormalizowanego do zawartości tłuszczu 2% i 4%. Homogenizacja wysokociśnieniowa spowodowała zmniejszenie średnicy kuleczek tłuszczowych (fot. 1) i wzrost ich powierzchni (zmiany ponad 4-krotne), a także wzrost poziomu związków białkowych związanych z tłuszczem mlekowych, które stanowiły 21.1–39.8% białek serum mleka (tab. 1), przy czym powyższe zmiany uzależnione były od zawartości tłuszczu w mleku. W wyniku procesu stwierdzono wzrost ilości ultrawirowanego osadu i poziomu sedymentujących białek plazmy nie związanych z tłuszczem mlekowym, a ponadto wzrost rozpuszczalności soli wapnia i fosforu mleka i obniżenie ich poziomu w formie koloidalnej (tab. 2). Homogenizacja wysokociśnieniowa powodowała nieznaczne zmiany kwasowości czynnej (obniżenie pH) i przewodności elektrolitycznej mleka (wzrost) oraz wyraźne, pogłębiające się wraz ze wzrostem zawartości tłuszczu, obniżenie stabilności cieplnej (max. 42.4%) i skrócenie czasu krzepnięcia podpuszczkowego (max. 34.5%) mleka (tab. 3).