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Optimization of centrifugal separation of α-lactalbumin and β-lactoglobulin

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Whey proteins, which are mainly composed of β-lactoglobulin (β-lg) and α-lactalbumin (α-la), account for about 20% of the proteins of bovine milk. In this study we investigated the effect of pH, dry matter content, concentration factor, heat treatment and centrifugation on the separation of α-la from β-lg using clarified whey as raw material. α-La precipitation was highest, 23.3%, when the dry matter content ranged from 5.8% to 25.7%. The optimum pH of α-la precipitation depended on the dry matter content. The separation efficiency increased when the concentration factor and heat treatment time at 55°C increased. A longer centrifugation time and higher separation speed did not have a marked effect on the separation efficiency. Separation was more efficient with a higher centrifugation speed at concentration levels 30 X and 60 X. The separation efficiency did not improve when the temperature was raised from 55°C to 65°C but it was better at a concentration level 120 X than at 60 X and 30 X, and also at concentration level 60 X than with 30 X.

Key words: α-lactalbumin, β-lactoglobulin, separation fractionation, filtration, concentration

Introduction

Whey proteins, which are mainly composed of β-lactoglobulin (β-lg), ~3.3 g/kg whey, and α-lactalbumin (α-la), ~1.0 g/kg whey, account for about 20% of the protein content of bovine milk (Walstra and Jenness 1984). Many efforts have been made to separate these major components with a view to producing whey protein concentrates with improved and specific functional properties (Amundson et al. 1982, Pearce 1983, Pearce 1987, Maubois et al. 1987, Rosenberg 1995). These efforts have involved gentle heating of whey, followed by concentration of whey at acidic pH values to produce selective precipitation of α-la or β-lg.

Pearce (1983) reported that maximum pre-

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cipitation of α-la in whey and optimum separation of the proteins were obtained at about 65°C when the whey was kept at a pH range from 4.1 to 4.3 for several minutes. No clarifying pretreatment of whey was used. McDonough et al. (1974) and de Wit (1984) showed that the presence of fat impairs the whipping properties of a whey protein concentrate (WPC). Lipids are also susceptible to oxidation and thus may contribute to an off-flavour, which is best prevented by removal of lipids from whey (de Boer et al. 1977). In a clarification method introduced by Maubois et al. (1987) for the pretreatment method of whey, the residual fat and lipid components were removed with microfiltration. Tupasela et al. (1994) studied the effect of clarification of whey on whey ultrafiltration. We discuss here the effect of clarification of whey on the separation of α-la from β-lg. The effects of pH, concentration factor, dry matter content, heat treatment and centrifugation were studied. All experiments were conducted on pilot-plant or laboratory scale.

Material and methods

Whey

Fresh edam cheese whey was obtained from the Food Research Institute's dairy plant, and the cheese milk was pasteurized at 74°C for 15 s. After the cheese had been made the whey was centrifuged and cooled to 2°C in a process tank. $CaCl_2$ solution ($CaCl_2$ x $2H_2O$ 662.1 g/l a.d. H_2O), 720 ml per 100 l whey, was added to the whey and the pH of the whey was adjusted to 7.3 with 1 M NaOH solution using a process based on that of Fauquant et al. (1985). Immediately after these treatments, the whey was heated at 50°C for 8 min in a mixing tank provided with a heating/cooling jacket. After heat treatment the whey was cooled to 40°C with ice water.

Whey processing

Heat-treated whey was microfiltered with an APV CL 3/40 microfiltration (MF) unit equipped with 0.2 μm ceramic membranes (Ceraver, France) in two modules. Each module had a membrane area of 1.4 m^2. In the MF unit the whey circulation speed was 5 m/s and the temperature was kept at between 35 and 40°C with a heat exchange section in the circulation loop. The whey inlet pressure was 3 bar. The whey was microfiltered by recycling in a batch run. The microfiltration permeate was collected and the temperature was lowered to 20°C in a process tank by circulating ice water in the agitator. The MF unit was cleaned after each run with 1% NaOH solution.

The permeate was concentrated with a membrane ultrafiltration unit I (PCI Bro MK, UK; cut-off 9000) and unit II (Millipore, Pellicon Cassette, USA; cut-off 10 000 PTGC). The pH of the WPC was adjusted to the desired level with 2 M HCl, after which the WPC was heated in a water bath to 55°C or 65°C for 5, 15 or 30 min. This treatment caused precipitation of α-la. The precipitate was collected by centrifugation (Sorvall RC-5B Superspeed Centrifuge using a SS-34 rotor, Du Pont Instruments, Connecticut, USA), at 5000 rpm and 10 000 rpm (3020 g and 12 100 g, respectively).

Analysis

The total solids content of differently treated wheys was determined after drying the wheys for 16 h at 102°C using the modified method of IDF 4 (1958). The protein content was determined by the Kjeldahl method with N conversion of 6.38 (TECATOR 1975). The pH was measured with a Knick Portamess 752 pH meter (Berlin, Germany). α-La and β-lg were identified and quantified by a FPLC Mono Q HR 5/5 chromatography column (Pharmacia) connected to a UV detector (280 nm) using the modified method of Humbrey and Newsome (1984).

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Results and discussion

Effect of whey dry matter content and pH on separation

First we studied how α-la precipitated when the pH range was from 3.2 to 4.5 at a dry matter content 12.5%. The α-la precipitation level was obtained when the β-lg content in supernatant was determined. The results in Figure 1 show that the pH range from 3.4 to 3.8 is the most favourable for the precipitation of α-la. When the dry matter content of whey was 12.5% and the pH ranged from 3.2 to 4.5 the maximum α-la precipitation was achieved at a pH value of around 3.6. In the next trial we had ten different whey dry matter contents (from 5.8% to 25.7%) and α- la and β-lg were separated at seven different pH values (from 3.4 to 4.0). Higher pH values, 3.9 and 4.0, were still included in the experiment because the dry matter content of three samples were lower than earlier. Figure 2 shows that at the lowest dry matter content, 5.8%, α-la did not precipitate well enough. When the dry matter content was raised to 9.2%, more α-la was precipitated; maximum precipitation was achieved at a dry matter content of 23.3%. The pH optimum of α-la precipitation decreased as the dry matter content increased. Figure 3 suggests that, in the separation of β-lg from α-la, the dry matter content had a greater effect than the protein content, because the dry matter content line has a better positive correlation with the β-lg content line than has the protein content (%, protein in dry matter) line.

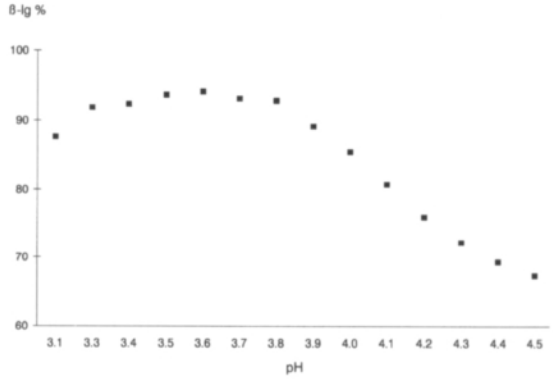

Fig. 1. β-lg content (%) of supernatant. Dry matter content of whey 12.5%, heat treatment 55°C/30 min and centrifugation 10 000 rpm/20 min.

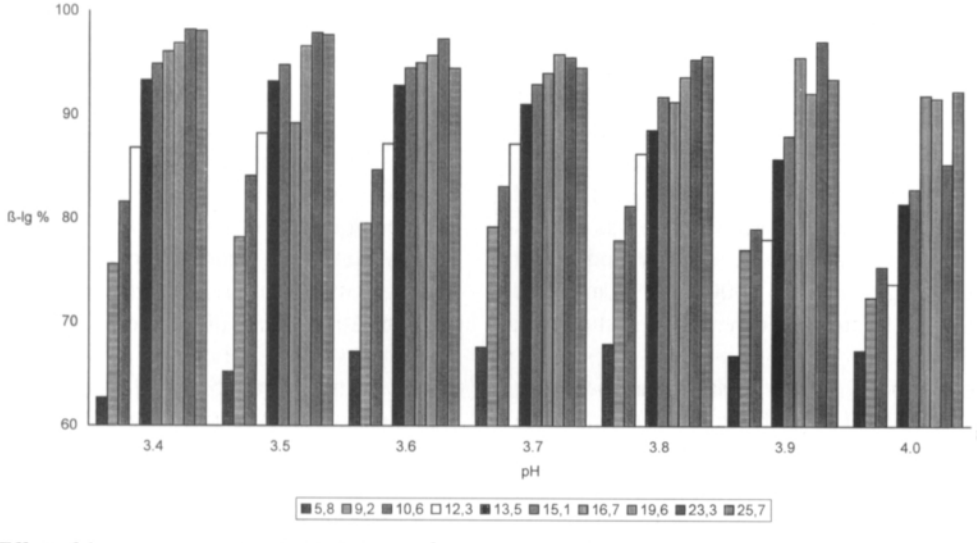

Fig. 2. Effect of dry matter content and pH of whey on β-lg contents (%) in supernatants. Dry matter contents are presented below the figure.

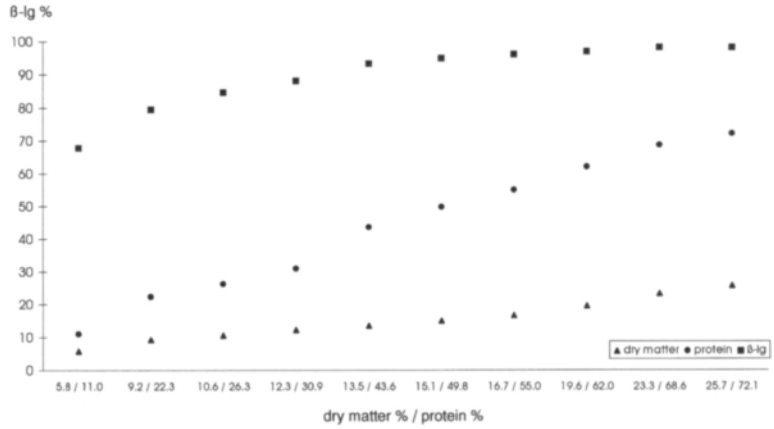

Fig. 3. β-lg contents (%) in supernatant versus whey dry matter content (%) and protein content (%, protein in dry matter).

Effect of concentration factor, heat treatment and centrifugation speed and time on α-la and β-lg separation

In this trial we used three different concentration factors, 30 X, 60 X and 120 X. The dry matter contents (%) were 11.6, 14.5 and 19.0, respectively. The whey was processed as described above. The pH before treatments was adjusted to 3.6 with 2 M HCl. In trial 1 the concentration factor was 30 X and the temperature treatments were 55°C/5 min, 55°C/15 min, 55°C/30 min and 65°C/30 min. Each heat-treated sample was centrifuged at 5000 rpm/20 min and 10 000 rpm/20 min. In trial 2 the concentration factor was 60 X and the treatment was as in trial 1. In trial 3 the concentration factor was 120 X. The sample was treated as in trial 1 and 2, but was centrifuged also at 5000 rpm/10 min and 10 000 rpm/10 min. The results are shown in Table 1.

From Table 1 it can be seen that the ratios (α-la/β-lg X 100) range from 7 to 20. The lower the value, the purer was the supernatant with regard to β-lg. Note that when the concentration factor and heat treatment time at 55°C increased, the separation efficiency also increased. A longer centrifugation time and greater separation speed did not have a marked effect on separation efficiency. Separation efficiency improved with the higher centrifugation speed at concentration levels 30 X and 60 X but not when the temperature was raised from 55°C to 65°C. Separation efficiency was better at a concentration level 120 X than at 60 X and 30 X, and also at concentration level 60 X than at 30 X.

Conclusion

In this study α-la precipitation was highest at 23.3%. The optimum pH of α-la precipitation depended on the dry matter content. Separation efficiency increased with an increase in concentration factor and heat treatment time at 55°C, and also with a higher centrifugation speed at concentration levels 30 X and 60 X. There was no improvement when the temperature was raised from 55°C to 65°C. Separation efficiency was better at concentration level 120 X than at 60 X and 30 X, and concentration level 60 X than at 30 X.

In the light of the above results, and the industrial potential for separating these major whey protein components from whey, in order to pro-

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Table 1. Effect of concentration factor, heat treatment and centrifugation speed and time on α-la and β-lg separation. Concentration factor = 30 X, 60 X and 120 X; heat treatment = 55°C/5 min, 55°C/15 min, 55°C/30 min and 65°C/30 min; centrifugation speed and time = 5000 rpm/10 min, 5000 rpm/20 min, 10 000 rpm/10 min and 10 000 rpm/20 min.

Supernatant α-la/β-lg X 100 ratio				
Heat treatment	Concentration factor			Centrifugation speed and time
	30 X	60 X	120 X	
55°C/5 min	–	–	11	5000 rpm/10 min
	18	12	10	5000 rpm/20 min
	–	–	10	10 000 rpm/10 min
	20	12	10	10 000 rpm/20 min
55°C/15 min	–	–	8	5000 rpm/10 min
	16	11	8	5000 rpm/20 min
	–	–	8	10 000 rpm/10 min
	12	10	8	10 000 rpm/20 min
55°C/30 min	–	–	7	5000 rpm/10 min
	14	9	7	5000 rpm/20 min
	–	–	7	10 000 rpm/10 min
	13	8	7	10 000 rpm/20 min
65°C/30 min	–	–	9	5000 rpm/10 min
	13	11	8	5000 rpm/20 min
	–	–	9	10 000 rpm/10 min
	13	8	8	10 000 rpm/20 min

– = not determined

duce whey protein isolates with specific functional properties, the most suitable combination would be a concentration factor of 30 X to 60 X (dry matter content e. 13%) and temperature treatment at 55°C/30 min. This combination should be tested on industrial scale.

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SELOSTUS

α-lactalbumiinin ja β-lactoglobuliinin sentrifugointierotuksen optimointi

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Heraproteiinit käsittävät noin 20 % maidon proteiineista. Heraproteiinit koostuvat pääasiassa β-laktoglobuliinista (β-lg) ja α-laktalbumiinista (α-la). Tehdyissä kokeissa tutkimme pH:n, kuiva-ainepitoisuuden, väkevyyden, lämpökäsittelyn ja sentrifugoinnin vaikutusta α-la:n ja β-lg:n erottumiseen kirkastetusta juustoherasta.

α-laktalbumiinin saostuminen onnistui parhaiten, kun kuiva-ainepitoisuus oli 23,3 %, kuiva-ainepitoisuuden vaihdellessa 5,8 ja 25,7 %:n välillä. Kuiva-ainepitoisuus vaikutti α-la:n saostumiseen optimi pH-tasolla. Sentrifugointitulos parani, kun väkevyyttä ja aikaa nostettiin 55°C:n lämpökäsittelyssä. Pidempi sentrifugointiaika ja suurempi sentrifugointinopeus eivät vaikuttaneet paljon sentrifugointitulokseen. Konsentrointikertoimilla 30 X ja 60 X saatiin parempi sentrifugointitulos kun sentrifugointinopeutta kasvatettiin. Sentrifugointitulos ei parantunut, kun käsittelylämpötilaa nostettiin 55°C:sta 60°C:een. Konsentrointikertoimella 120 X saatiin parempi sentrifugointitulos kuin konsentrointikertoimilla 30 X ja 60 X, ja konsentrointikertoimella 60 X saatiiin parempi sentrifugointitulos kuin konsentrointikertoimella 30 X.