ORIGINAL RESEARCH

Processing of extended shelf life milk using microfiltration

WOLFGANG HOFFMANN,* CHRISTIAN KIESNER, INGRID CLAWIN-RÄDECKER, DIERK MARTIN, KURT EINHOFF, PETER CHRISTIAN LORENZEN, HANS MEISEL, PHILIPP HAMMER, GERTRAUD SUHREN and PAUL TEUFEL

Federal Research Centre for Nutrition and Food, Location Kiel, Hermann-Weigmann-Strasse 1, D-24103 Kiel, Germany

Extended shelf life (ESL) milk was processed with integrated microfiltration (pore size 1.4 μm). The germ-enriched retentate was not used for the final whole milk. Microfiltration led only to a negligible change in the content of the main components of the ESL product compared with the source milk. The total protein was only slightly decreased (0.02–0.03%) and the ratio of the protein fractions was unchanged within the measurement accuracy. The furosine content of the isolated fat globuline membrane fraction could be used as a diagnostic to prove cream had been subjected to high-temperature treatment. The shelf life of the ESL milk was distinctly prolonged compared to HTST-pasteurized milk.

Keywords Acid-soluble whey proteins, ESL consumer milk, Furosine, Microfiltration, Protein content, Shelf life.

INTRODUCTION

Extended shelf life (ESL) milk fills the gap between high-temperature short-time (HTST) pasteurized milk with a shelf life of about 1 week at cold storage and ultra-high temperature (UHT) milk, which can be stored for a few months without cooling. Several processing alternatives for the production of ESL milk with sensory properties similar to that of pasteurized milk were propagated and established during the last years. One option is the integration of a microfiltration step. It reduces the bacterial load of milk by mechanical separation without heat-induced chemical alterations. Therefore, the necessary time/temperature combinations of a subsequent heat treatment to attain a certain shelf life may be milder.

This concept was introduced both for consumer milk and cheese milk more than 20 years ago (Fauquant *et al*. 1988; Hansen 1988; Malmberg and Holm 1988; Meersohn 1989; Olesen and Jensen 1989; Lindberg and Bredahl 1990; Eckner and Zottola 1991; Trouve *et al*. 1991; Madec *et al*. 1992; Pedersen 1992). Most experiences with a microfiltered ESL milk are based on the patented Bactocatch® system (Holm *et al*. 1986). This process and its variants comprise microfiltration of separated skim milk resulting in a permeate, which was added with or without subsequent HTST pasteurization to the highly heated (115–130°C, 4–6 s) mixture of microfiltration retentate and required amount of cream. Finally, the recombined and fat-adjusted milk is filled aseptically. Microfiltration is carried out with a ceramic membrane having an average pore size of 1.4 μm. In order to prevent rapid clogging and fouling of the membrane and to guarantee a high and constant flux, a special circulation system was developed (Larsen 1992). In this system, a small and uniform transmembrane pressure (UTP) on the entire filter was implemented. A spore reduction of about 3 $\log_{10}$ steps was achieved. However, new multilayered membranes with the same average pore size but a narrower size distribution enabled a spore reduction of 4–5 $\log_{10}$ steps. Investigations of Olesen and Jensen (1989) showed that the content of spores in the initial milk had a significant influence on the content of spores in the microfiltered milk, whereas processing parameters such as concentration pressure and ratio or pasteurization had only a minor effect. Larsen (1989) analysed a decrease of 0.02% total protein after microfiltration. This very slight alteration of protein content was confirmed by results of Hoffmann *et al*. (1996). In an IDF conference in 1996, the contemporary status of research activities was presented (Jelen *et al*. 1997).

During the last decade, the fractionation of milk proteins by microfiltration had become more important. The production of microfiltered ESL consumer milk seemed to arouse new interest again and additional knowledge was desirable. On request of the responsible German Federal Ministry, a modified process for manufacturing consumer milk using microfiltration was developed. In contrast

*Author for correspondence. E-mail: wolfgang.hoffmann@bfel.de

to the Bactocatch® process, the germ-enriched retentate of microfiltration was not used for the final product. Raw milk, all intermediate products and the resulting whole milk were analysed. The quantitative ratio of all products and their protein content, the composition of protein and different potential distinguishing features of heat load within the total process were of special interest. The sensory results during cold storage of the ESL milk should only be an indicator of prospective consumer acceptance and attainable shelf life.

MATERIALS AND METHODS

Processing of microfiltered ESL whole milk

Fresh bulk milk was obtained from the experimental farm of the Federal Research Centre for Nutrition Food and Food, location Kiel, Germany. Subsequent processing (trial A) is shown in Figure 1. It includes homogenization and high heating of a mixture (15.0% fat) of separated cream (31.5% fat) and part of microfiltration permeate. The remaining permeate was HTST pasteurized and added to the highly heated mixture before the final whole milk (3.5% fat) was filled, cooled and stored. In an independent second trial (trial B), the separated raw cream (33.5% fat) of the fresh bulk milk was mixed with the permeate of microfiltration to the fat-adjusted whole milk (3.5% fat), which was subsequently homogenized (200/50 bar) and HTST pasteurized before filling. The sample abbreviations of this process are specified in Table 1. Predominantly, the results of trial A are presented. Results of trial B were considered for those process steps that corresponded to the steps in trial A (i.e. separation of raw cream, and microfiltration of skim milk).

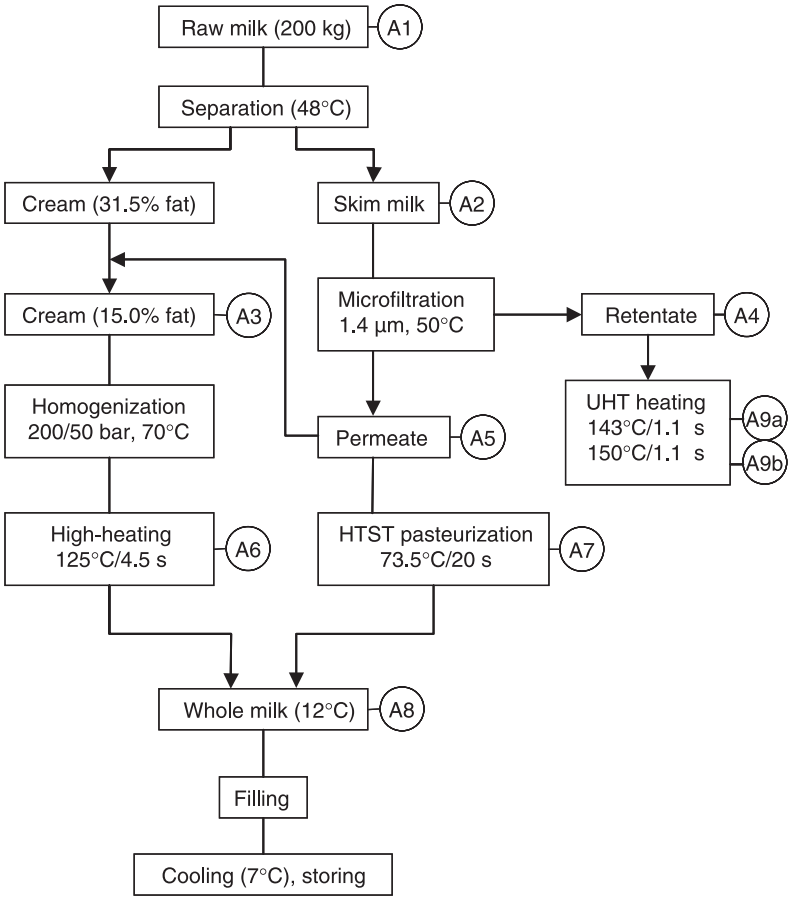

Figure 1 Processing of extended shelf life milk: Flowchart and sampling plan (A1-A9b) of trial A.

The cross-flow microfiltration unit (MFS-1, TetraPak Processing, Reinbek, Germany) had internal circuits for permeate and retentate and was equipped with a one-layer module (multichannel element, total filtering surface 0.2 m^2, Al_2O_3, Membralox® P19-40, SCT, France). In preceding water tests, the flow conditions were optimized in order to have a small uniform transmembrane pressure and a high wall shear stress (247 Pa). Homogenization and subsequent heat treatment (plate heat exchanger, 120 L/h) of the intermediate products were carried out in pilot plants (APV Deutschland, Unna, Germany).

Total processing of ESL milk could not be performed continuously, but was aimed at a minimum of bacterial recontamination. Cream and skim milk, permeate and retentate were collected in autoclaved cans before further processing. After heat treatment, the resulting whole milk was conveyed into an autoclaved can under a sterile bank before it was filled into autoclaved glass bottles with screw caps.

Analytical methods

Fat content was determined according to Gerber or Köhler, and total protein content ($N \times 6.38$) and casein ($6.38 \times$ (total-N–noncasein-N)) were performed according to Kjeldahl (Methodenbuch 2003). A komplexometrical method was used for quantification of calcium (Methodenbuch 2003). These analyses ($n = 4$) had coefficients of variation between 0.08 and 0.19%. The whey protein content in total protein was determined ($n = 5$) by derivative ultraviolet (UV) spectroscopy according to Meisel (1995) using a modified formula to calculate the second derivative results expressed as Q_{D2} values: $Q_{D2} = d_1/d_2$ (d_1 = second derivative maximum at 294 nm–minimum at 290 nm; d_2 = second derivative maximum at 287 nm–minimum at 283 nm). A reversed-phase high-pressure liquid chromatography (HPLC) method was used for the determination of acid-soluble whey proteins. These analyses were carried out in duplicate with a coefficient of variation between 2.8 and 6.0% (Clawin-Rädecker *et al*. 2000a; German DIN 10473; Resmini *et al*. 1989). Lactulose was determined enzymatically (according to the German official method §35 LMBG L-01.00 31) and furosine was analysed after acid hydrolysis by ion-pair reversed-phase liquid chromatography (Clawin-Rädecker and Schlimme 1995; Clawin-Rädecker *et al*. 2000b). Lactulose and furosine

Table 1 Quantitative ratio of all products and its protein content during the processing of extended shelf life (ESL) milk (trial B)

Product (sample abbreviation)	*Weight assessment (kg)*	*Fat (%)*	*Protein (% N × 6.38)*	*Quantitative protein ratio (kg protein)*
Raw milk (B1)	100.0	4.19	3.38	100.0
Skim milk (B2)	87.6	0.04	3.52	91.2
Cream (B3)	12.4	33.5	2.40	8.8
Retentate (B4)	3.6	0.04	3.95	4.2
Permeate (B5)	84.0	0.03	3.50	87.0
Fat-adjusted whole milk (B6)	93.8	3.54	3.38	93.8
ESL whole milk (B7)	93.8	3.54	3.38	93.8

were determined in duplicate and the coefficients of variation were 2.5% and 1.9%, respectively. The activity of adenosine deaminase (ADA, EC 3.5.4.4) was measured after incubation at 37°C by duplicate determination of the enzymatic product inosine using a dual-column HPLC analyser (detection limit: 13 mU/L at 37°C) (Martin *et al*. 1998).

Total bacterial content and content of thermodurics were determined according to standard methods. The standard deviation of repeatability s_r of samples analysed in duplicate were better than indicated in ISO 4833 ($s_r = 0.086$ $\log_{10}$ colony-forming units (cfu)/mL) (EN ISO 4833 2003; Richardson 1985). A germ-proof sample was defined by a detection limit of 10 cfu/mL. Somatic cells were analysed in triplicate by Fossomatic 360 (Foss, Germany).

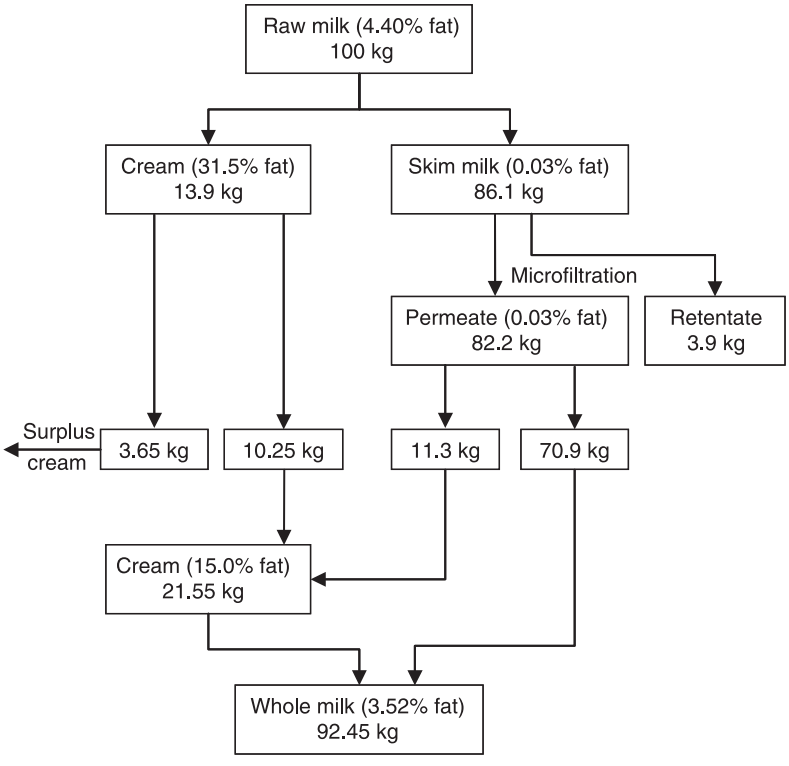

Figure 2 Quantitative ratio and analysed fat content of all products in trial A.

Sensory profile analysis was carried out by 8–10 trained panellists. The whole milk samples were slowly warmed to 20°C and filled into plastic cups with lids 30 min before testing. A test form comprising a sensory vocabulary of several appearance, odour and taste attributes was given to the panel. The intensity of the attributes was quantified in a six-point scale (from 0 = not perceptible to 5 = very strong). Analysis considered only attributes that were recognized by a minimum of half the panellists. These dates were averaged and presented in spider webs.

RESULTS AND DISCUSSION

Quantitative ratio of all products and its protein content

Figure 2 shows the quantity and the analysed fat content of each product during the processing of ESL milk in trial A. To simplify matters, the raw milk quantity was set to 100 kg. As the retentate amounted to 3.9 kg and surplus cream was not used in the subsequent processing, the yield of whole milk was 92.45 kg. 21.55 kg (cream with 15.0% fat) of the ESL milk was highly heated, the remaining part was HTST pasteurized. In trial B, 3.6 kg retentate and 2.6 kg surplus cream decreased the yield of the completely HTST-pasteurized whole milk to 93.8 kg (Table 1).

The analysed protein content of the products in trials A and B and the corresponding quantitative protein ratio are shown in Figure 3 and Table 1 in which the protein quantity of raw milk was set to 100 kg. According to this setting, the retentate A4 with 3.88% protein contained 4.6 kg (= 3.88 × 3.9/3.30) of the protein input, but only 3.9 kg of the total milk quantity used (see above). The 3.6 kg retentate B4 contained 4.2 kg of the available protein. After microfiltration, the protein content of the skim milk was reduced from 3.45 to 3.42% in trial A, and from 3.52 to 3.50% in trial B. This very low decrease, which was already found by Larsen (1989) and Hoffmann *et al*. (1996), is in the Kjeldahl method's repeatability range (0.03%), although it could still be analytically recorded. The

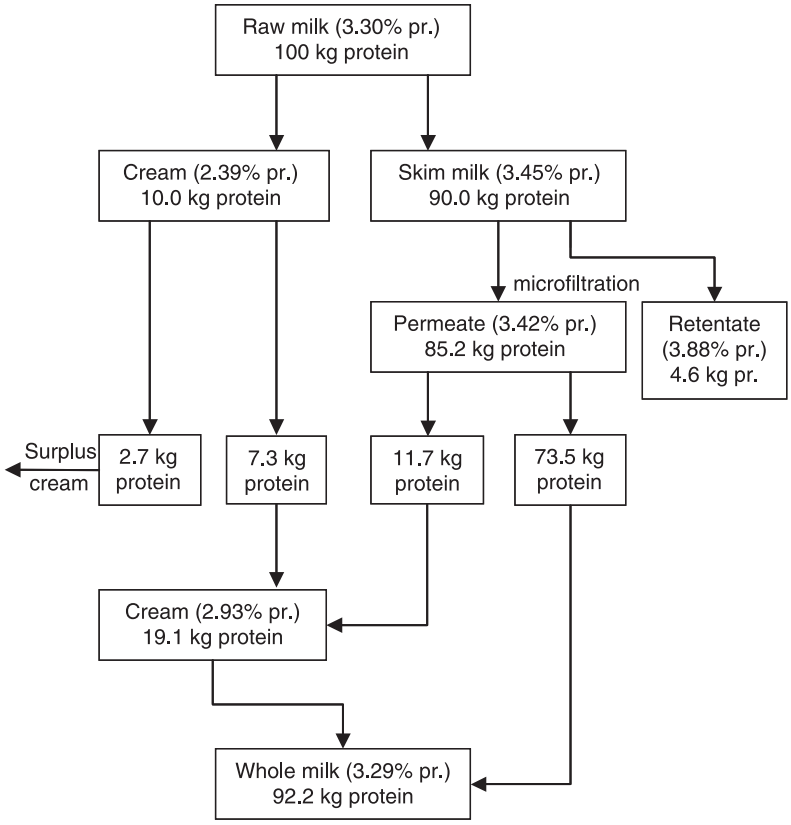

Figure 3 Analysed protein content (% pr.) and calculated quantitative protein ratio of all products in trial A.

difference in the analysed protein content of raw and ESL milk amounted to only 0.01%.

Further milk components

It was of special interest to examine whether microfiltration influenced the percentage of the main protein fractions in total milk protein. The results showed that only the retentates A4 and B4 (sample abbreviations see Figure 1 and Table 1) had an increased casein content (> 80%, Table 2) and a decreased content of whey protein in total protein (about 15%, Figure 4). All other casein results were ≤ 79.5% and the whey protein results were above 16%. Therefore, the manufactured ESL whole milk did not differ from the primary raw milk with regard to this criterion. The content of calcium, which is bound predominantly to casein, was not significantly influenced by microfiltration either. Skim milk and permeate of trials A and B contained 0.133 ± 0.005% Ca.

Analysis of the acid-soluble whey proteins served both for the assessment of the filtration effect and for the characterization of the thermal load of the intermediate products and the final whole milk. In both trials, the content of all analysed soluble whey proteins in permeate and retentate did not differ significantly.

The most heat-resistant α-lactalbumin was not denatured by HTST pasteurization (Table 2, see

Table 2 Selected results from products during the processing of ESL milk (trials A and B); *: see Fig. 1 and Table 1

	Casein/ protein ratio		*α-Lactalbumin (mg/100 mL)*		*Immunoglobulin G (mg/100 mL)*		*Adenosine deaminase (U/L 37°C)*		*Total bact. count (cfu/mL)*		*Thermodurics (cfu/mL)*		*Somatic cells/mL*	
Product (sample abbreviation)*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
Raw milk (A1/B1)	0.794	0.791	111.4	109.2	59.9	57.0	0.27	0.31	3 600	25 700	49	113		204 000
Skim milk (A2/B2)	0.792	0.792	109.3	114.8	61.8	62.5	0.32	0.34	9 500	29 000	160	168		50 000
Adjusted cream (A3)			109.7		57.5		0.61		1 500		118			
Retentate (A4/B4)	0.807	0.805	106.1	114.8	54.4	64.2	0.83	0.61	58 000	214 000	2550	1830		220 000
Permeate (A5/B5)	0.791	0.790	109.5	114.0	59.5	58.8	0.56	0.53	59	30	51	10		0
Adjusted cream HH (A6)			73.2		0		< 0.013		0		0			
Permeate HTST (A7)			109.7		45.4		0.71		40		44			
ESL whole milk (A8)	0.795		108.8		35.0		0.52		24		28			
Retentate UHT (A9a)			63.1		0		< 0.013		0		0			
Retentate UHT (A9b)			66.4		0		< 0.013		0		0			

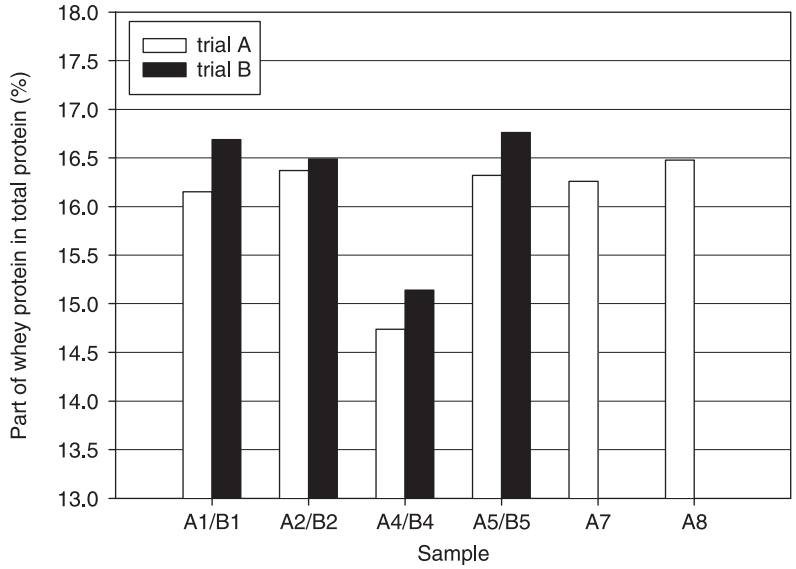

Figure 4 Analysed part of whey protein in total protein during the processing of extended shelf life milk (sample abbreviations see Figure 1 and Table 1).

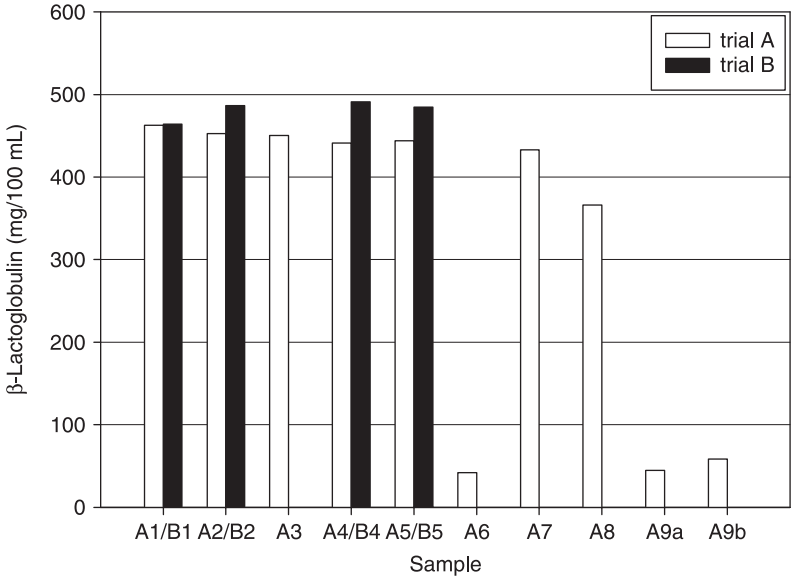

Figure 5 Analysed content of β-lactoglobulin during the processing of extended shelf life milk (sample abbreviations see Figure 1 and Table 1).

results of A5 and A7). However, high heating of cream and UHT heating of the retentate resulted in denaturation of 30–40% (see A6, A9a and A9b). With mixed highly heated cream and permeate, subsequent pasteurization ended in a not significantly different content compared to the raw milk (see A1 and A8).

β-Lactoglobulin was not affected by HTST pasteurization either (Figure 5, see results of A5 and A7), but denatured to about 90% by high heating or UHT heating (see A6, A9a, A9b). Therefore, the final whole milk of trial A (A8) contained about 20% less acid-soluble β-lactoglobulin. For the characterization of pasteurized peroxidase-positive milk a limit of ≥ 10% acid-soluble β-lactoglobulin in total protein is required. This limit was exceeded in the whole milk (11.1%) despite the high heating of cream.

The heat-labile immunoglobulins denatured to 24% by HTST pasteurization (Table 2, A5 and A7) and were not detected after high or UHT heating (A6, A9a, A9b). Hence, about 40% of immunoglobulins in the ESL milk (A8) were denatured.

The analysis of lactulose was restricted to the heated cream and the final ESL milk of trial A. 55 mg/kg lactulose in cream (A6) were distinctly above the limit of detection (10–30 mg/kg), whereas the 18 mg/kg in the ESL milk (A8) were within this limit. Only if the heat treatment of cream induced a lactulose formation clearly above 140 mg/kg would the final whole milk contain more than the detection limit (30 mg/kg).

The determination of furosine should both estimate the degree of the thermal load of the different products and reflect the effect of microfiltration on the Maillard reaction. The raw milk used contained 5.3 (A1) and 5.6 mg/100 g protein (B1), which was in the normal range of 5–7 mg/100 g protein (Figure 6). With increasing time for separation and microfiltration, a slight increase in the furosine content of the intermediate products was measured. This alteration may be explained by the moderate but continuous thermal load: both steps took about 2 h (trial A) or 1 h (trial B) at 48–50°C without intermediate cooling. A significant effect of microfiltration could not be verified; the marginally lower furosine content in the retentate (A4, B4) may be deduced from the higher casein content. Previous investigations had shown that in bovine raw milk the furosine content of casein is lower than that of the whey protein fraction. The high furosine content in highly heated cream (about 28 mg/100 g protein, A6) and the slight increase in permeate after pasteurization (A7) resulted in an ESL milk that exceeded the discussed limit for peroxidase-positive pasteurized milk of 8.5 mg/100 g clearly (about 12 mg/100 g protein, A8). UHT heating of retentate A4 raised furosine to more than 80 mg/100 g (A9a, A9b). An increased furosine content in pasteurized consumer milk may not only be explained by thermal load but also by added milk powder. Therefore, it was of analytical interest if a more specific analysis could distinguish between both possible reasons. The determination of furosine in the protein fractions of fat globule membranes seemed to be such a method, and was applied. After separation of the ESL milk (A8), the resulting cream was washed with demineralized water, again centrifuged, frozen (–20°C) overnight, warmed to 40°C and separated again. The analysis of the aqueous fraction that contained the desired protein fraction resulted in an increased furosine

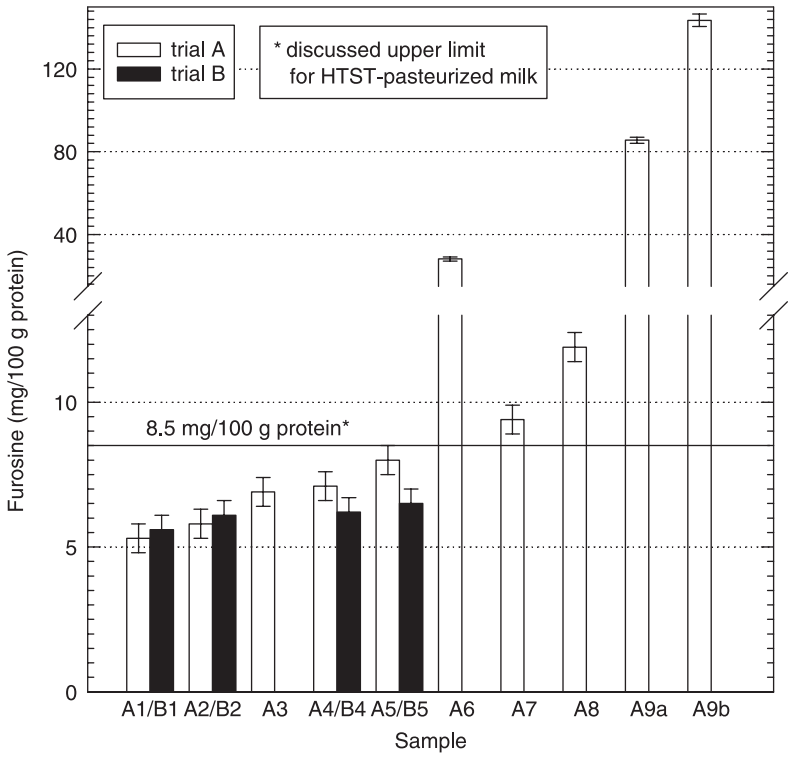

Figure 6 Analysed content of furosine during the processing of extended shelf life milk (sample abbreviations see Figure 1 and Table 1).

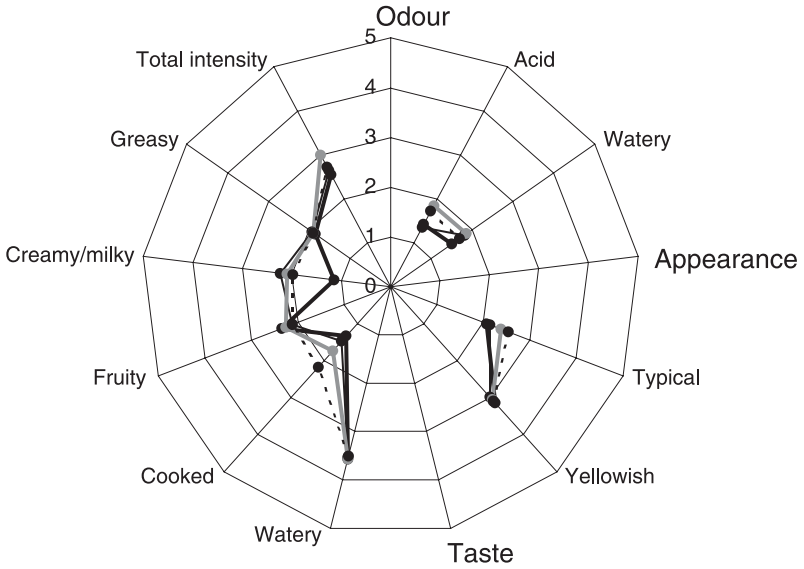

Figure 7 Mean values of often identified sensory attributes (clockwise arranged) at the profile analysis of stored whole milk samples; ——— ESL milk of trial A, 42 d old; ——— ESL milk of trial B, 22 d old; ——— HTST-pasteurized consumer milk; - - - - - highly heated consumer milk.

content of 20.5 mg/100 g protein in comparison to the ESL milk. This was a distinguishing feature of the cream which was highly heated during processing of the ESL milk.

Adenosine deaminase shows a thermal-induced activation/inactivation during heat treatment of milk. Hence, ADA is activated by HTST pasteurization (Table 2, A5 and A7) and inactivated by high heating (A6). The separated cream had, even after addition of permeate, an increased activation of ADA compared with that of raw milk (Table 2, see A3 and A1). This resulted from the temperature during separation (48°C) and from the high fat content (Martin *et al*. 1999; Martin and Schlimme 2002). After microfiltration (at 50°C) the retentate (A4, B4) had higher ADA activity than the permeate (A5, B5) despite the same thermal load. As expected, the activity in the highly heated cream (A6) was below the limit of detection (13 mU/L at 37°C) and, consistently, the final ESL milk showed a lower ADA activity (0.52 U/L, A8) than usual commercial pasteurized milk (0.7–0.9 U/L) (Martin *et al*. 1998) or the ESL milk of trial B (0.71 U/L). Therefore, an analysis of ADA activity seems to be suitable in order to prove a partial high level of heating during the processing of microfiltered consumer milk.

The raw milk obtained from the experimental farm was of good microbiological quality (Table 2, A1, B1), which was rather unfavourable for testing the bacterial retention of the microfiltration membrane. Depending on the original load the bacterial content of the permeate (A5, B5) decreased by 2–3 $\log_{10}$. After high heating (A6), no bacteria above the detection limit were measured. The final ESL milk A8 contained 24 cfu/mL.

The difference of somatic cells in raw milk (B1) and skim milk (B2) of trial B (Table 2) may be explained by separation of cells into the centrifuge slime and disintegration by the mechanical input. After microfiltration, the permeate (B5) was free of somatic cells.

Sensory profile analysis of ESL milks A8 and B7 during its shelf life showed no relevant deviations compared with that of HTST-pasteurized and directly highly heated consumer milk. However, after 42 days at 7°C, the taste of the ESL milk A8 was perceived as less 'creamy/milky' (Figure 7). The stored milk bottles of A8 had a pH value > 6.70 for 7 weeks at 7°C, the samples of B7 (no high heating of cream) for only 3 weeks. One week later, the pH value had decreased. These results suggest that on the one hand, the raw milk was of good microbiological quality, and on the other hand, the processing of the ESL milk was aimed at a minimum of bacterial recontamination, but could not be performed aseptically. Therefore, the results of the two trials should only be an indicator of prospective consumer acceptance and attainable shelf life.

CONCLUSIONS

Microfiltration led only to a negligible change in the content of the main components of the ESL product when compared with the source milk. The minimum fat content is prescribed by law anyhow, and the total protein was only slightly decreased

by microfiltration (0.02–0.03%); and the ratio of the protein fractions was unchanged within the accuracy of measurement. The same was valid for lactose (results not presented) and calcium.

A distinguishing mark of HTST-pasteurized milk was the decreased number of somatic cells, which could not pass through the microfiltration membrane with an average pore size of 1.4 μm. The microfiltered part (permeate) of the final whole milk was more than 85%.

The furosine content of the isolated fat globuline membrane fraction could be used as a diagnostic to prove cream had been subjected to high temperature treatment. Other potential distinguishing features of thermal load, e.g. the determination of lactulose formation or of acid-soluble whey proteins, were not affected and showed values typical of HTST-pasteurized milk. In contrast, the activity of adenosine deaminase, which was decreased in the ESL milk seemed to give indication of a higher heat load.

In spite of the non-aseptic manufacture, the shelf life of the ESL milk was distinctly prolonged compared with that of HTST-pasteurized milk. Sensory profile analysis of the two ESL milks during its shelf life showed no appreciable difference from the pasteurized milk, but further work will be needed to confirm these results.

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