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ORIGINAL RESEARCH

A survey of the quality of extended shelf life (ESL) milk in relation to HTST and UHT milk

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Thirty milk samples [high-temperature short-time (HTST) milk, extended shelf life (ESL) milk (directly heated, indirectly heated, microfiltered), ultra-high temperature milk] from 17 German dairies were analysed. Total viable counts of directly or indirectly heated ESL milk were significantly lower than those in microfiltered ESL and HTST milk. Evaluation of indigenous enzyme activity revealed sufficient heat treatments in all milk samples. The manufacturing processes were differentiated by estimating furosine and acid soluble whey proteins. Sensory examinations revealed a preference for HTST heated and microfiltered ESL milk. However, a significant discrimination of drinking milk types was not possible. Vitamin losses were not detected, and concentrations of vitamins in different types of milk were comparable.

Keywords Drinking milk, Microbiological status, Head load evaluation, Sensory quality, Vitamin status.

INTRODUCTION

Extended shelf life (ESL) milk has a shelf life of about 3 weeks under chill chain conditions and fills the gap between high-temperature short-time (HTST)-heated milk, which typically is assigned a shelf life of 10 days and ultra-high temperature (UHT)-heated milk, which can be stored for a few months without cooling (Hoffmann *et al.* 2006). In Germany, ESL milk has a market share of 20–25%. For comparison, UHT milk has a share of approximately 70%, and HTST milk has a market share of 5–10% (BLE 2008). The term ESL milk as well as the manufacturing process is not legally defined in the European Union. A thermal treatment or combination of heat treatment and membrane filtration are involved in the production of ESL milk. The thermal process requires direct or indirect heating at 123–127°C with a holding time of 1–5 s. Traditional HTST pasteurisation is carried out at 72–75°C for 15–30 s, and UHT milk is heated at a minimum of 135°C for a few seconds (sterilisation value $F_0 \geq 3$ min) (Schwermann and Schwenzow 2008a,b; Kaufmann *et al.* 2009). The combined treatment process for ESL milk includes microfiltration of skim milk through ceramic membranes with an average pore diameter of 0.8–1.4 μm. As a result, a spore reduction of 3–5 $\log_{10}$ steps is achieved and most other forms of micro-organisms are also separated. The so enriched retentate and a specific amount of cream are heated at 123–127°C, homogenised, and mixed with the HTST heated skim milk permeate (Hoffmann *et al.* 2006; Schwermann and Schwenzow 2008c; Henke 2009).

Microbial spoilage of heat treated drinking milk is caused by heat-resistant or recontaminating micro-organisms. The former group is represented by spore forming micro-organisms, mainly *Bacillus* spp. and Enterococci. The latter group consists of Gram-positive nonspore forming bacteria and Gram-negative bacteria. These organisms gain significance if they are psychrophilic or psychrotrophic and able to multiply at refrigeration temperatures (Blake *et al.* 1995; Mayr *et al.* 2004; Kress *et al.* 2005; Kaufmann and Kulozik 2008).

Several parameters are suited for the evaluation of milk heating processes. Alkaline phosphatase (ALP, EC 3.1.3.1) is an appropriate indicator for the evaluation of pasteurisation or the addition of raw milk to pasteurised milk or milk products

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(Schlimme *et al.* 1998; Shakeel-Ur-Rehman *et al.* 2003; Harding and Garry 2005; Commission Regulation No 1664/2006 (EC, 2006). Lactoperoxidase (LPO, EC 1.11.1.7) is one of the most heat stable enzymes in bovine milk (Seifu *et al.* 2005). In addition, lipase (LIP, EC 3.1.1.3), a milk enzyme that is less stable than ALP and LPO, is used as an indicator for heat treatments (Martin *et al.* 2005).

The determination of the acid soluble whey proteins, including lactoferrin, serum albumin and immunoglobulin, has been recommended for the evaluation of heat treatments such as HTST heating (Mayer *et al.* 2009). In HTST-heated milk the degree of denaturation of the whey proteins α-lactalbumin and β-lactoglobulin is typically 10–20%, whereas immunoglobulins are approximately 50% denatured (Schlimme *et al.* 1996; Clawin-Rädecker *et al.* 2000). The determination of furosine can be used to evaluate the extent of the *Maillard* reaction in a wide range of milk processing conditions and allows a direct quantification of heat damage to food protein (Clawin-Rädecker *et al.* 1996; Pellegrino *et al.* 1996).

Kaufmann *et al.* (2009) found that ESL milk frequently possesses a cooked flavour, but is less pronounced than in UHT milk. Kaufmann and Kulozik (2007) noted that microfiltered ESL milk shows a foreign flavour at the end of minimum shelf life. This may be due to insufficient inactivation of indigenous milk enzymes and enzymes of microbial origin. Blake *et al.* (1995) noticed increasing cooked flavours and other off flavours with increasing heat input in ESL milk produced by direct steam injection. In addition, light-induced changes in the taste of microfiltered ESL milk during storage have been reported (Rysstad and Kolstad 2006; Kaufmann and Kulozik 2007). However, studies by our group have revealed that the sensory properties of ESL milk produced by microfiltration were comparable with those of HTST-heated milk over the entire length of storage (Hoffmann *et al.* 2006).

The nutritional quality of ESL milk is frequently questioned, independent of the manufacturing process. Although neither the analyses of individual ESL milk samples nor the results of systematic investigations of ultra-high-heated (UHT) milk justify this assumption, it is alleged that heating causes significant and relevant mineral (calcium) and vitamin losses in ESL milk. Health risks (digestive leukocytosis) following ESL milk consumption have also been suspected (Gallmann *et al.* 2001).

The aim of the present work was to evaluate and compare the microbiological status, composition, denaturation and inactivation of milk ingredients such as whey proteins, vitamins, and indigenous enzymes, formation of *Maillard* and lipolysis products and the assessment of the sensory properties of drinking milk types present on the German market in relation to the shelf life.

MATERIALS AND METHODS

Drinking milk samples

Thirty whole milk samples (at least five packs per lot) from 17 German dairies were taken directly from the respective production line and sent immediately to the Max Rubner-Institut. The cold chain conditions (max. 8°C) were maintained during transportation of the samples from the factory to the Institute. For chemical and sensory analysis, the samples were stored at 4–6°C. For microbiological testing the samples were stored at 8°C until the end of shelf life as declared by the producer. The sample material consisted of 16 different ESL milk products that were directly heated (n = 8), indirectly heated (n = 2) or microfiltered and heat treated (n = 6). For comparison, HTST heated (n = 6) and UHT heated (n = 8) drinking milk samples were also examined. One lot of the HTST milk was not used for microbiological testing because all packs were leaky.

Analysis of main ingredients

The amount of fat, protein and dry matter was determined by infrared measurement (MilkoScan 50; Foss, Rellingen, Germany). The MilkoScan was calibrated by standardised milk, delivered by the central milk laboratory of Schleswig-Holstein, Kiel, Germany. All measurements were performed in triplicate with maximum deviations of 0.01%.

Characterisation of the microbes in milk samples

All samples were stored unopened at 8°C until the end of declared shelf life. This means that ESL milk samples were stored for 17–26 days, HTST milk samples for 7–10 days. UHT milk was not tested. For microbiological testing five packs of each lot of milk were used. Total viable count (TVC) at 30°C and 6.5°C were determined by application of ISO 4833:2003, a pour plate method utilising plate-count skim milk agar. Enumeration of presumptive *Enterobacteriaceae* at 30°C was performed according to ISO/DIS 21528-2, a pour plate method utilising violet red bile dextrose agar

(VRBD). Enumeration of presumptive Enterococci at 37°C was achieved by application of method M 7.8.2 of the Official Collection of Test Methods of the German State Agricultural Test and Research Institute (LUFA), a surface spatula method utilising kanamycin-esculin-azide agar. Enumeration of aerobic sporeformers was conducted at 37°C by spreading 0.1 mL of the sample or appropriate dilutions on the surface of blood agar. Counting was performed by experienced staff. Qualitative detection of *Listeria* spp. in 25 mL was performed according to ISO 11290-1:2005. Bacterial numbers are presented in colony-forming units per mL (cfu/mL).

Determination of indigenous enzyme activities

The activity of ALP (EC 3.1.3.1) was determined with the Fluorophos™ method (Advanced Instruments, Norwood, MA, USA) according to DIN EN ISO 11816 – 1:2006. The activities of LIP (EC 3.1.1.3) and LPO (EC 1.11.1.7) were measured with reflectometric test kits (Reflectoquant®; Merck, Darmstadt, Germany) (Martin *et al.* 2005). Each sample was determined in duplicate.

Evaluation of the furosine content

Furosine was analysed after acid hydrolysis by ion-pair reversed-phase liquid chromatography according to IDF 193 (Clawin-Rädecker and Schlimme 1998; Clawin-Rädecker *et al.* 2000). Milk samples were analysed in duplicate. The repeatability was 0.5 mg/100 g of protein for HTST milk or less than 10% of the arithmetic mean of the results.

Determination of denatured whey protein

A reversed-phase high-performance liquid chromatography (RP-HPLC) method was used for the determination of acid soluble whey proteins according to DIN EN ISO 10473. To determinate the repeatability one sample was measured in quintuplicate. The coefficient of variation was between 0.5% and 4.5% for individual whey proteins (Clawin-Rädecker *et al.* 2000; DIN EN ISO 10473; Resmini *et al.* 1989). Therefore, the analysis of the other milk samples was performed only once.

Characterisation of titratable acidity (TA)

The TA of milk samples was determined in duplicate in accordance with L01.00-7:2002-05 (BVL 2002) and is presented in SH units. This method implies a repeatability of 0.2 SH. Fresh samples were analysed shortly after receipt. Moreover, microfiltered ESL milk samples were analysed repeatedly after 8–16 days, just at their best-before date, using a sealed milk carton stored at 4°C (sample age between 18 and 24 days).

Assessment of the sensory properties

Sensory studies (quantitative descriptive analysis) were carried out according to DIN 10967. A panel of 10 assessors was trained for sensory evaluation of drinking milk. One half of the panel were assessors with experience in descriptive sensory analysis of dairy products and the other half were volunteers. The attributes used for quantitative descriptive analysis were taken from ISO 22935-2 – IDF 99-2 and Lee *et al.* (2003). An appropriate selection of key attributes was developed by preliminary tests on commercial drinking milk samples purchased from German food markets. In each case, five odour and taste descriptors (sweet, milky, cooked, foreign odour/taste, total intensity) were used. One session per day with a maximum of three samples was conducted. ESL milk and HTST milk was examined at the end of the minimum shelf life. UHT milk was evaluated after a storage period of 2 weeks. All samples were stored at 4–6°C. Samples of 20 mL each were examined at 20°C and rated according to a structured scale from 0 (not present) to 5 (very strong). The data were collected and evaluated using FIZZ version 2.40 (Biosystemes, Couternon, France). The statistics for box plots were calculated in Excel using the quintile function.

Estimation of the vitamin status

The contents of fat- and water-soluble vitamins were determined by HPLC immediately after sample arrival, at the end of shelf life and halfway between arrival and the end of shelf life. To evaluate the storage stability of vitamins in drinking milk, samples were stored at 4–6°C until the end of shelf life and at 20°C once the end of the shelf life was reached. After opening the packs, the samples were kept in the dark under nitrogen and an antioxidant was added to some samples.

Sample preparation

After denaturation of lipoproteins, alkaline (NaOH) saponification and extraction with n-hexane:toluene 1:1 (Farah *et al.* 1992), fat-soluble vitamin content of the sample (retinol, α-tocopherol) was determined. Extraction of water-soluble vitamins was carried out according to Albalá-Hurtado *et al.* (1997), whereby trichloroacetic acid was used to hydrolyse potential vitamin–protein and vitamin–phosphate complexes. All water-soluble vitamins were analysed according to Heudi

et al. (2005). In addition, recovery tests were conducted by spiking milk samples with vitamins before saponification. All handling was conduced in a darkened room and samples were kept in brown glass containers.

HPLC separation of fat- and water-soluble vitamins

The analytical HPLC system consisted of an Elite LaChrom chromatograph (VWR-Hitachi, Darmstadt, Germany) equipped with a diode array (DAD L-2455) and an L-2458 fluorescence detector (VWR Darmstadt, Germany). A Reprosil-pur 120 C18 AQ (3 µm, 125 × 4.6 mm) column (Trentec, Gerlingen, Germany) equipped with a guard column (10–4.6 mm) was used for the determination of fat-soluble vitamins. Water-soluble vitamins were analysed using Chromolith® Performance RP-18 columns (endcapped, 100 × 4.6 mm; Merck, Darmstadt, Germany). HPLC conditions for the determination of fat- and water-soluble vitamins are summarised in Table 1.

Statistical analysis

Vitamin concentrations are presented as medians, with box plots representing the 25th and 75th percentile and whiskers denoting the 5th and 95th percentile. Time courses of vitamin concentration during shelf life are presented as the mean ± SD. Effects of the manufacturing process (heat treatment) and the duration of storage were statistically analysed by two-factorial (repeated measurement) ANOVA. Kruskal–Wallis tests were performed to separately analyse the effects of manufacturing process (heat treatment) and the duration of storage for each sampling day (beginning, middle and end of shelf life).

RESULTS AND DISCUSSION

Analysis of main ingredients

Fat, protein and dry matter content of 29 whole milk samples are summarised in Table 2. Unfortunately, one HTST milk sample was fat-reduced and contained only 1.6% fat. Therefore, this sample

Table 1 HPLC conditions for the determination of fat- and water-soluble vitamins in milk

Vitamin	*Detection (nm)*	*RT (min)*	*Reproducibility*[a] *CV_r (%)*
Fat-soluble vitamins			
Injection volume 20 µL; flow: 1.0 mL/min			
Mobile phase: 2.5% i-propanol in n-hexane			
Retinol	FL (330/408 EX/EM)	4.40	4.45
α-tocopherol	FL (295/330 EX/EM)	4.70	4.05
Water-soluble vitamins			
Injection volume 50 µL; flow 0.8 mL/min			
ACN:TFA-gradient[b] 5′ 0:100; ↗6′ 25:75; 8′ ↗40:60; 1′ = 40:60; 1′ ↘0:100; 4′ = 0:100%			
Vitamin B1	UV 282; 0–10 min FL (290/410 EX/EM) 20–25 min FL (400/520 EX/EM)	2.39	3.1
Vitamin B2	UV 282; 0–10 min FL (290/410 EX/EM) 20–25 min FL (400/520 EX/EM)	13.61	3.8
Vitamin B5	UV 210	10.99	2.6
Vitamin B6	UV 282	4.01	6.2
Vitamin B8	UV 210	13.69	1.2
Vitamin B9	UV 282	12.76	5.3
Vitamin B12	UV 210 + 360	13.26	–
Niacin	UV 282	3.09	2.5
Vitamin C	UV 282	1.93	2.1

EM, emission; EX, extinction; FL, fluorescence detection; HPLC, high-performance liquid chromatography; UV, ultraviolet detection.

[a]According to Heudi *et al.* (2005).

[b]%Acetonitril:%Trifluoracetic acid (0.025%), pH 2.6.

Table 2 Composition of drinking milk samples

Heating process	*Samples (n)*	*Fat (%)*	*Protein (%)*	*Dry matter (%)*
HTST heated	Sum (n = 5)			
	Min–max (n = 4)	3.45–3.54	3.25–3.38	12.43–12.55
	Mean ± SD (n = 4)	3.50 ± 0.04	3.32 ± 0.05	12.48 ± 0.05
	Outlying (n = 1)	4.17	3.11	12.70
ESL, microfiltered	Sum (n = 6)			
	Min–max (n = 5)	3.50–3.56	3.24–3.41	12.30–12.56
	Mean ± SD (n = 5)	3.52 ± 0.03	3.34 ± 0.07	12.48 ± 0.11
	Outlyling (n = 1)	4.24	3.13	12.86
ESL, directly heated	Sum (n = 8)			
	Min–max (n = 8)	3.49–3.90	3.28–3.49	12.28–13.15
	Mean ± SD (n = 8)	3.65 ± 0.16	3.38 ± 0.07	12.70 ± 0.28
ESL, indirectly heated	Sum (n = 2)			
	Min–max (n = 2)	3.56	3.37–3.48	12.57–12.73
	Mean ± SD (n = 2)	3.56	3.43 ± 0.08	12.65 ± 0.11
UHT heating	Sum (n = 8)			
	Min–max (n = 8)	3.50–3.62	3.22–3.47	12.36–12.76
	Mean ± SD (n = 8)	3.57 ± 0.05	3.37 ± 0.08	12.57 ± 0.12

ESL, extended shelf life; HTST, high-temperature short-time; UHT, ultra-high temperature.

was not considered for inclusion in Table 2. On average, 25 of the samples had 3.55% fat (3.45–3.65%), 3.36% protein (3.22–3.48%) and 12.55% dry matter (12.28–12.76%). Two samples contained more than 4% fat and are listed separately. The wide range of fat and dry matter content of directly heated milk was attributed to two samples. These samples showed a high fat content (3.80% and 3.90%), as well as a high protein and dry matter content (13.15% and 13.03%). Obviously, less water was added to these samples during steam injection or infusion than was removed during subsequent vacuum cooling.

Microbiological status of drinking milk types

Microbiological testing was applied to gain an overview of the microbiological status of the different milk types at the end of shelf life, which can be seen as a worst case scenario at the latest moment of consumption. For this purpose the milk was stored at 8°C as declared by the producers whereas at the retail level temperatures are usually much lower (2–6°C). TVC of directly heated ESL milk did not show growth at 30°C or 6.5°C in six out of eight milk samples. Growth occurred in all samples (n = 6) of microfiltered ESL milk but did not occur in all of the respective cartons. Additionally, growth was observed in all samples (n = 5) and packs of HTST-heated milk. Two lots of HTST-heated milk (all five packs) and three packs of directly heated ESL milk showed growth indicative of spoilage. However, sample deterioration was not detected by visual and olfactory inspection. TVC ranges are displayed in Table 3. The colony counts detected in directly heated ESL milk are consistent with those reported by Blake *et al.* (1995) and Mayr *et al.* (2004). Analysis of variance showed that the TVC in directly heated ESL milk were significantly lower than in HTST-heated milk ($P < 0.01$). However, HTST-heated milk and microfiltered ESL milk did not show significant differences in TVC. Due to the low numbers of samples, these results should not be overestimated. A determination of growth curves was not aim of this study. Neither Enterococci, *Enterobacteriaceae* nor *Listeria* spp. were found in milk samples.

Bacilli, aerobic spore-formers, were detected in all five samples of HTST-heated milk tested. All five packs of four samples and four packs of the remaining samples showed growth. Ranges of colony counts are presented in Table 4. Bacilli were not detected in any type of ESL milk. In principle, spores of bacilli are able to survive HTST treatment and direct and indirect heat treatment of ESL milk. For example, *D*-values of 0.24 min at 129°C have been reported for *Bacillus cereus* (ICMSF 1996). Indeed, bacilli were detected in most packs of the HTST-heated milk samples, but were not observed in ESL milk. This can not be explained as no growth curves were determined. It can only be speculated that in the ESL milk samples psychrotrophic bacilli were not present in the

Table 3 Total viable count at 30°C and 6°C in HTST pasteurised milk and ESL milk (positive packs only, five packs per milk sample were examined)

	Range of colony counts (cfu/mL)		
Heating process	*30°C*	*6.5°C*	*Packs (n)*
HTST heated	2.1×10^4–8.4×10^4	3.6×10^4–9.5×10^4	5
	1.6×10^3–3.1×10^3	2.1×10^3–2.4×10^6	5
	2.1×10^3–3.8×10^3	2.4×10^2–2.0×10^3	5
	2.0×10^5–1.4×10^6	1.0×10^5–1.3×10^6	5
	6.4×10^5–1.5×10^6	7.7×10^5–>1×10^6	5
ESL, directly heated	7.6×10^6	4.2×10^6	1
	<10	1.5×10^1–6.5×10^1	5
	1.5×10^3–1.0×10^5	$>10^7$	2
ESL, microfiltered	3.0×10^1–3.8×10^2	<10	2
	1.0×10^2	<10	1
	5×10^0–8.0×10^1	<10	5
	1.0×10^1–3.0×10^1	<10	3
	5×10^0–7.0×10^2	<10	2
	2.0×10^1–2.4×10^3	<10	5

ESL, extended shelf life; HTST, high-temperature short-time.

Table 4 Colony counts of *Bacillus* spp. in HTST-heated milk (positive packs only, five packs per milk sample were examined)

Heating process	*Range of colony counts (cfu/mL)*	*Packs (n)*
HTST heated	3.0–1.8×10^1	5
	8.0×10^1–2.4×10^2	5
	1.1×10^1–1.0×10^3	5
	1.4×10^2–2.2×10^5	4
	1.3×10^3–5.8×10^4	5

HTST, high-temperature short-time.

contaminating flora or were overgrown by other contaminants. Possibly it is not a result of processing technology as Kress *et al.* (2005) have shown that bacilli may survive a direct or indirect heat treatment.

Activity of indigenous milk enzymes

The determination of ALP activity is often used for the evaluation of treatment efficiency for HTST heated and microfiltered ESL milk. The results of ALP activity tests (Table 5) show that all HTST and microfiltered ESL samples had received an adequate heat treatment, i.e. in the referred samples, the activities measured are far below the threshold value of 350 mU/L for pasteurised milk. Generally, ALP activities of 0.02–0.07 U/L are found in HTST-heated milk (Lorenzen *et al.* 2010). LPO is appropriate for assessing the heating efficiency in directly and indirectly heated ESL milk. The results in Table 5 proved that a sufficient heat load was applied. The indigenous milk enzyme LIP is relatively thermolabile compared with ALP and LPO. In previous heating experiments (Martin *et al.* 2005) with bulk raw milk (LIP activity: 236 U/L), at 70°C/9.5 s, a LIP activity of 16 U/L was observed, corresponding to approximately 8% residual activity. However, at 75°C/9.5 s no quantifiable activity could be determined. The present examination shows that LIP activities were below the measurement limit of <6 U/L, irrespective of the manufacturing process applied. Literature data (Kaufmann *et al.* 2009) reveals that microfiltered ESL milk at the end of shelf life contains higher LIP activities (residual activity: 64 ± 4%) than directly or indirectly heated ESL milk (40 ± 3%); however, this could not be confirmed by the present investigation. Higher LIP activities in microfiltered ESL milk are not surprising considering the fact that LIP occurs predominantly in cream which is subjected to high-temperature heating during the manufacturing of ESL milk. Typically, total bacterial counts in raw milk are low, thus low LIP activities of microbial origin are to be expected. In addition, LPO activities in microfiltered ESL milk are similar to those in HTST-heated milk (Table 5). These measured LPO data confirms the studies of Kaufmann *et al.* (2009).

The eight UHT-heated milk samples were stored at 6°C and 20°C until the end of shelf life expiry date. Table 6 shows ALP activities after a storage period of 2 weeks and at the end of shelf life expiry date. At a storage temperature of 6°C, only negligible changes in activity were found, however, at a storage temperature of 20°C a significant increase in ALP activity was detected at the end of shelf life expiry date. LPO reactivation could not be determined in UHT milk until the end of shelf life. The detected enzyme activities were below the measurement level of <5 U/L.

Table 5 Activities of alkaline phosphatase, lactoperoxidase and lipase in drinking milk (U/L), expressed as mean values of duplicate determination

Heating process	*ALP*	*LPO*	*LIP*
HTST heated	<0.01	300	<6
	0.06	1420	<6
	0.09	1250	<6
	0.03	1060	<6
	0.03	2100	<6
	0.05	950	<6
ESL, microfiltered	<0.01	500	<6
	0.03	1480	<6
	<0.01	570	<6
	0.03	1690	<6
	0.02	130	<6
	<0.01	83	<6
ESL, directly heated	0.05	<5	<6
	0.06	<5	<6
	0.10	<5	<6
	0.11	<5	<6
	0.40	<5	<6
	<0.01	<5	<6
	0.04	<5	<6
	0.04	<5	<6
ESL, indirectly heated	0.06	<5	<6
	0.04	<5	<6
UHT heated	0.40	<5	<6
	0.02	<5	<6
	0.06	<5	<6
	0.12	<5	<6
	0.50	<5	<6
	<0.01	<5	<6
	0.20	<5	<6
	<0.01	<5	<6

ALP, alkaline phosphatase; ESL, extended shelf life; HTST, high-temperature short-time; LIP, lipase; LPO, lactoperoxidase; UHT, ultra-high temperature.

Contents of acid-soluble whey proteins and furosine

High-temperature short-time heated milk samples (Table 7) showed significantly lower contents of lactoferrin, serum albumin and immunoglobulin (approximately 50% on average) in comparison with raw milk data (Clawin-Rädecker *et al.* 2000). The average content of the main whey proteins α-lactalbumin and β-lactoglobulin did not differ significantly from raw milk data. A few milk samples showed lower levels of β-lactoglobulin and immunoglobulin, indicating a higher heat load during HTST heating. However, the evaluation of the heat load is difficult due to a possible wide variations range of the individual whey proteins in raw milk.

Depending on the manufacturing process, ESL milk samples showed different levels of acid soluble whey proteins. After direct or indirect heating, the acid soluble content of lactoferrin and immunoglobulin is below the detection limit of 0.2 and 0.5 mg/100 mL. In general the acid soluble content of β-lactoglobulin and serum albumin of directly heated ESL milk was approximately 50% and 40%, respectively, of the mean value of HTST-heated milk samples. The most heat-resistant whey protein α-lactalbumin was mostly native after treatment (about 90%). Due to the differences in heating processes applied to the samples, great variation in the amount of acid soluble α-lactalbumin and β-lactoglobulin was observed. Indirectly heated ESL milk samples showed the lowest whey protein concentrations and were similar to UHT milk treated with low heat input. The amount of acid soluble whey protein in microfiltered ESL milk samples were not significantly different compared with the results of HTST-heated milk. Lactoferrin and immunoglobulin were not significantly affected by high heating of cream and UF-retentate. According to the results regarding the denaturation of whey proteins, microfiltered ESL milk is more similar to traditional HTST-heated milk, while directly and indirectly high heated ESL milk should be classified between HTST milk and UHT milk.

Relating the acid soluble β-lactoglobulin content to the whole protein content of milk may eliminate seasonal and regional variations of protein composition (Table 7). A minimum of 10% acid-soluble β-lactoglobulin has been recommended for HTST-heated milk, which is traditionally LPO positive (Mayer *et al.* 2009). The present study reveals that HTST-heated milk samples and microfiltered ESL milk samples contained more than 10% acid soluble β-lactoglobulin. This result confirms the studies of Pellegrino *et al.* (1996) and Villamiel *et al.* (1999).

Table 6 Alkaline phosphatase activity (U/L) in ultra-high temperature milk after a storage of 14 days and at the end of shelf life, expressed as mean values of duplicate determination

Storage temperature	*ALP after 14 days*	*ALP at the end of shelf life*
6°C	370.3	370.3
20°C	n.d.	591.0
6°C	370.3	381.8
20°C	n.d.	599.5
6°C	24.3	32.2
20°C	n.d.	462.9
6°C	62.1	69.4
20°C	n.d.	293.1
6°C	116.1	107.6
20°C	n.d.	310.5
6°C	502.2	517.6
20°C	n.d.	1154.1
6°C	<10	<10
20°C	n.d.	95.7
6°C	22.5	25.3
20°C	n.d.	321.3
6°C	<10	<10
20°C	n.d.	376.1

ALP, alkaline phosphatase; n.d., not determined.

The furosine content of HTST-heated milk samples (Figure 1) was 5.5–7.2 mg/100 g of protein and did not exceed the limit of 8.5 mg/100 g (Schlimme *et al.* 1996, Clawin-Rädecker *et al.* 2006). Directly and indirectly heated ESL milk samples showed significantly higher amounts of furosine, ranging from 11.1 to 22.6 mg/100 g of protein. Furosine concentrations above 20 mg/100 g of protein indicate a higher head load

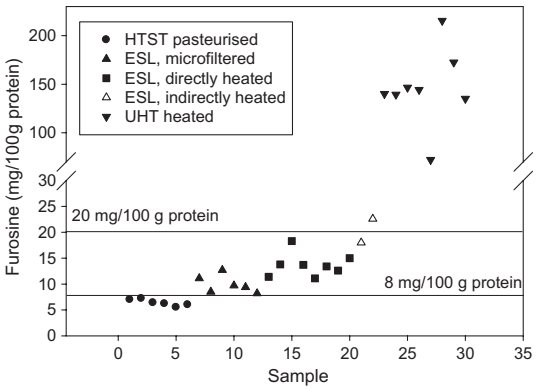

Figure 1 Furosine content of extended shelf life (ESL) milk in relation to high-temperature short-time (HTST) and ultra-high temperature (UHT) milk.

typical of an indirect heating process. Nevertheless, the furosine level of ESL milk is much lower than the level observed in UHT milk samples, where the mean was 145.7 mg/100 g of protein. The furosine content of microfiltered ESL milk was in between the value of HTST heated and directly heated ESL milk (8.2–12.7 mg/100 g protein). The furosine content of microfiltered ESL milk was dependent on furosine formation in high-heated cream and UF-retentate. Microfiltered ESL milk exceeded the amount of furosine in HTST-heated milk with one exception (8.2 mg/100 g protein). Microfiltered ESL milk, produced by our group, showed no increased furosine levels. This milk was produced in a pilot plant with HTST heated cream and the retentate was not added to the milk (Hoffmann *et al.* 2006; Clawin-Rädecker *et al.* 2006). However, a differentiation of microfiltered and directly heated ESL milk was possible

Table 7 Acid soluble whey protein content of drinking milk samples

	α-lactalbumin (mg/100 mL)		*β-lactoglobulin (mg/100 mL)*		*Serum albumin (mg/100 mL)*		*Lactoferrin (mg/100 mL)*		*Immunoglobulin (mg/100 mL)*		*β-LG/protein (%)*	
Heating process	*Mean*	*Min–max*	*Mean*	*Min–max*	*Mean*	*Min–max*	*Mean*	*Min–max*	*Mean*	*Min–max*	*Mean*	*Min–max*
Raw milk[a] (n = 9)	118.1	89.6–150.0	411.1	299.8–577.1	33.0	19.8–65.8	14.8[b]	11.7–17.8	67.1	40.3–103.4	11.3	8.9–13.3
HTST heated (n = 6)	111.8	102.0–115.2	410.0	330.2–474.5	17.2	13.5–20.5	7.1	1.8–12.3	28.5	13.8–38.2	12.0	10.3–14.2
ESL, microfiltered (n = 6)	106.1	99.8–111.5	381.7	357.4–414.8	16.1	13.7–18.4	6.8	5.5–8.4	21.9	14.1–29.6	11.2	10.9–11.8
ESL, directly heated (n = 8)	100.6	96.6–106.6	208.4	158.9–296.8	6.3	4.6–8.8	n.d.		n.d.		6.0	4.5–8.4
ESL, indirectly heated (n = 2)	84.1	81.5–86.7	50.7	34.1–67.3	n.d.		n.d.		n.d.		1.4	1.0–1.9
UHT (n = 8)	31.4	12.5–57.4	15.3	6.3–29.9	n.d.		n.d.		n.d.		0.4	0.2–0.9

Detection limit below 0.2–0.5 mg/100 mL.

β-LG, β-lactoglobulin; ESL, extended shelf life; HTST, high-temperature short-time; n. d., not detectable; UHT, ultra-high temperature.

[a]Clawin-Rädecker and Schlimme (1998).

[b]Ingrid Clawin-Rädecker, unpublished data.

due to their different acid soluble immunoglobulin level.

Titratable acidity

Titratable acidity records the buffering capacity of milk and indicates any change in the concentration of acidic components in milk even if the pH does not change. The TA of milk is often increased by heat treatment. Thus, TA increases with increasing temperature and duration of treatment. Simultaneously, reversibility of enhanced TA decreases and the time required for reconstitution increases. These effects were confirmed by the present study. The quantification of free fatty acids (FFA) in milk fat was not practicable, because a loss particularly of short chain fatty acids occurs during fat extraction leading to inaccurate results.

The TA of the investigated UHT milk samples (n = 8) was 7.0 ± 0.2 SH on average, whereas the HTST-heated milk samples (n = 5) had a mean TA of 6.6 ± 0.3 SH. ESL milk samples (n = 17) also yielded an average of 6.6 ± 0.3 SH. However, the indirectly heated or microfiltered milk samples (n = 8) had a mean TA of 6.8 ± 0.1 SH, indicating a slightly increased heat load in comparison with HTST-heated milk. In contrast, directly heated

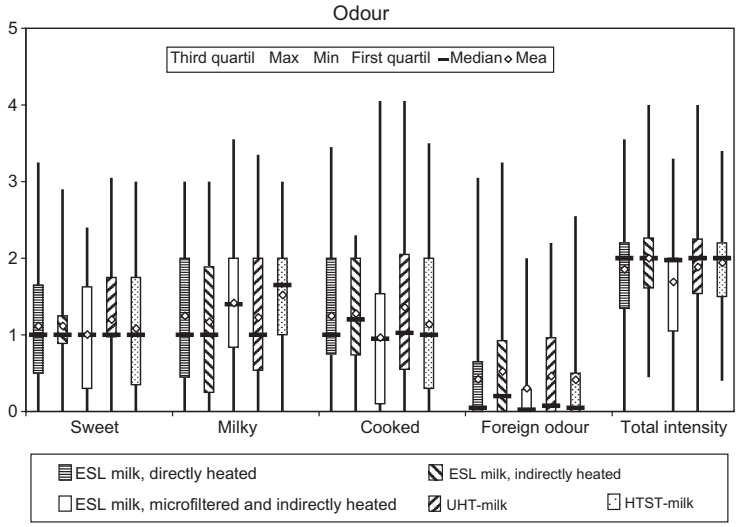

Figure 2 Box plots for odour properties of drinking milk (box gives median values, arithmetic means and 25th to 75th percentiles; bars illustrate highest/lowest observed values).

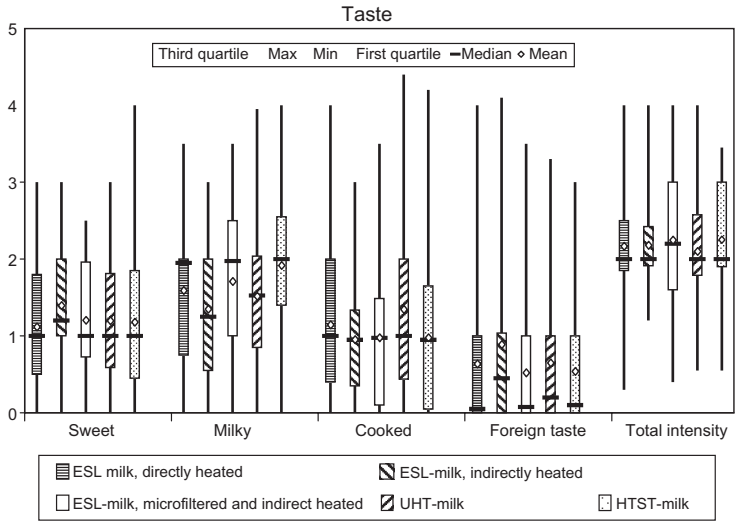

Figure 3 Box plots for taste properties of drinking milk (box gives median values, arithmetic means and 25th to 75th percentiles; bars illustrate highest/lowest observed values).

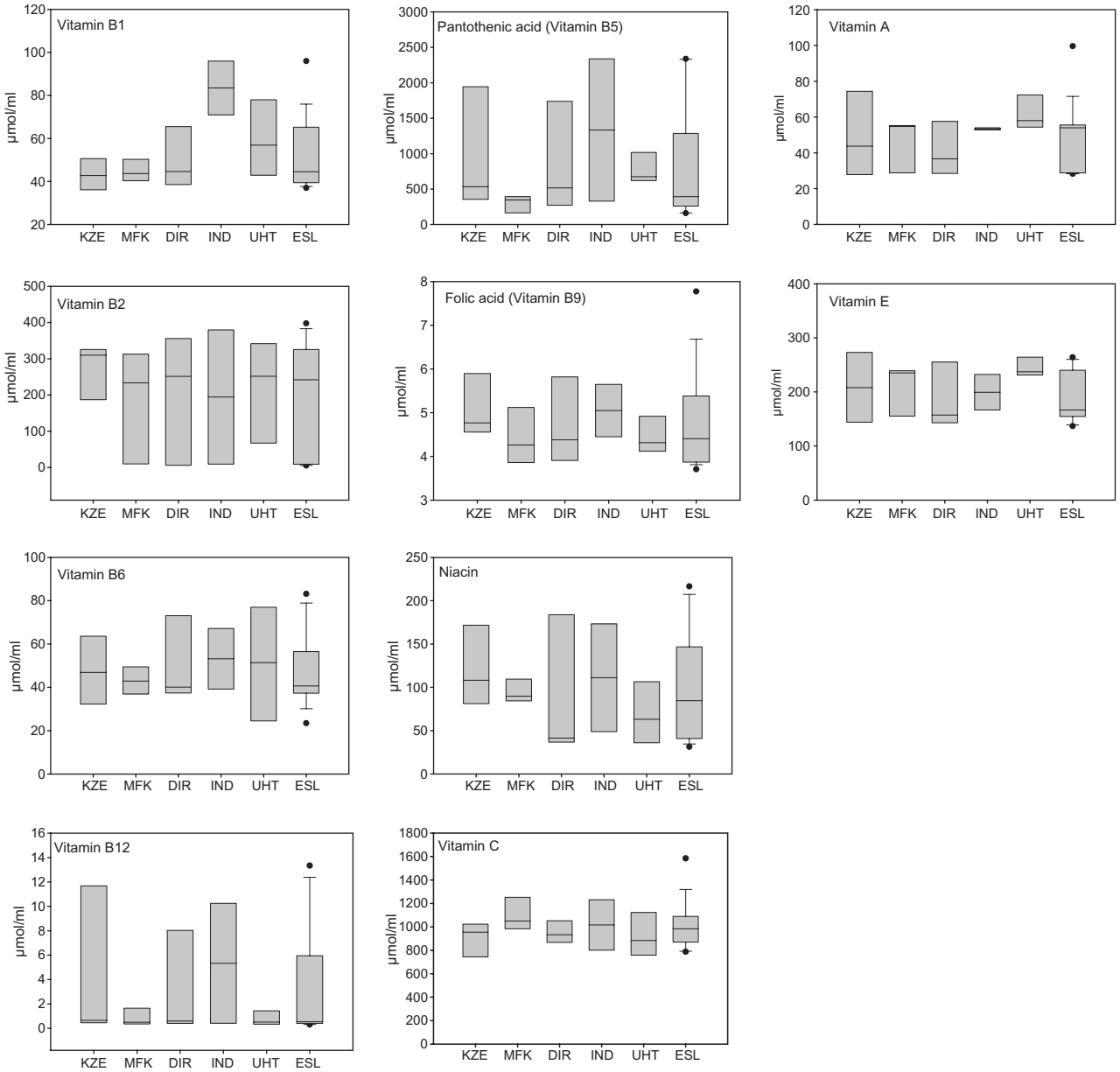

Figure 4 Contents of water- and fat-soluble vitamins in high-temperature short-time (HTST) heated (KZE), microfiltered (MFK), directly heated (DIR), indirectly heated (IND) extended shelf life (ESL) milk in relation to ultra-high temperature (UHT) milk (ESL reflects the median of all ESL milks).

ESL milk samples (n = 9) showed lower SH values of 6.4 ± 0.2. Presumably, this result is related to a decrease in volatile acids due to the manufacturing process. Microfiltered ESL milk exhibits a lower heat load than high temperature ESL milk, thus an increased residual activity of lipases can cause an accelerated formation of FFA and sensory defects during prolonged storage. An increase in the FFA content, as described by Kaufmann *et al.* (2009) by approximately 1.3 µmol/mL during a storage period of 21 days would analytically correspond to an increase in TA by 0.5 SH.

In the present study, a decrease in the average TA from 6.8 ± 0.1 SH to 6.6 ± 0.2 SH was observed in microfiltered ESL milk stored for approximately 17 days. The decline in TA may be attributed to the reversibility of the initial heat induced increase in TA. However, a release of FFA as described by Kaufmann *et al.* (2009) should have resulted in higher SH values at the best-before date. Thus, the extent of enzymatic lipolysis during storage of commercial ESL milk appears to be negligible. Acidification by microbial activity can also be ruled out, at least in the packs analysed for TA having been stored at 4°C.

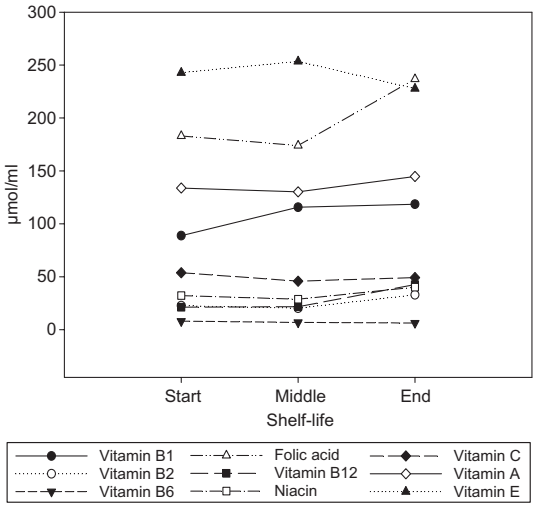

Figure 5 Changes in water- and fat-soluble vitamin concentrations (expressed as area under the curve) during storage of directly and indirectly heated extended shelf life milk. (Data in the figure are normalised, i.e. vitamin concentrations are expressed as percentage of mean concentration of the respective vitamin in all milk samples).

Sensory properties of drinking milk types

Box plots reflecting sensory properties of drinking milk are outlined in Figures 2 and 3. The plots revealed that discrimination of drinking milk produced by different processes was not possible although there seems to be a tendency for HTST-heated milk and microfiltered ESL milk to possess a 'milky' flavour (odour and taste). A pronounced foreign flavour in microfiltered ESL milk, described by Kaufmann and Kulozik (2007), was not found in the present study. This result is supported by low LIP activity and lipolytic changes in microfiltered milk and the results of TA (see above). The present results confirm the studies of Hoffmann *et al.* (2006) and show that the sensory properties of ESL milk produced by microfiltration are comparable with those of HTST-heated milk. It was not possible to differentiate from descriptive sensory analysis profiles between ESL milk produced by different manufacturing techniques. Additionally, a relationship between flavour profile of the milk samples and storage time was not observed. The large differences in the percentiles and the wide range of values observed may also be due to the heterogeneity of the panel (see Materials and Methods).

Vitamin status of drinking milk types

The amounts of fat- and water-soluble vitamins in HTST, ESL and UHT milk are presented in Figure 4. Figure 5 shows changes in fat- and water-soluble vitamin concentrations (derived from the area under the curve) during storage of directly and indirectly heated ESL milk. The data in the figures have been normalised, thus, vitamin concentrations are expressed as a percent of average concentration of the respective vitamin in all milk samples. The present study does not provide evidence for relevant or significant vitamin losses due to heat treatment and storage time or for lower concentrations of water- and fat-soluble vitamins in ESL milk than HTST-heated milk.

The storage stability of vitamins in ESL milk (Figure 5) was comparable with HTST-heated milk. A significant, but not relevant loss of vitamin B6 was observed during storage of ESL milk and the concentrations of vitamins B1, B6, B12, A and E increased slightly with increasing storage time while the residual vitamin content remained constant. The observed increase in vitamin concentrations during storage of heated milk is contrary to published data (Gallmann *et al.* 2001), thus, a bias may be present in this study, due to the relatively low number of samples examined. There have been occasional studies, describing increased folate concentrations during storage, but were observed exclusively in fermented milk products. Increased folate concentrations were attributed to the activity of starter cultures.

Overall, the results are in accordance with those of Eberhard *et al.* (2003), who analysed high-heated ESL milk after a 4 week storage period. With the exception of vitamin B6, concentrations of vitamins and folic acid did not differ between indirectly and directly heated ESL milk and HTST-heated milk. However, the concentration of vitamin B1 was reduced by ≤15 or 5% in directly or indirectly heated ESL milk.

CONCLUSIONS

Microbial analysis of whole milk samples at the end of declared shelf life showed that TVCs at 30°C or 6.5°C from directly or indirectly heated ESL milk were significantly lower than those from microfiltered ESL milk and HTST-heated milk. However, this result should not be overestimated due to the low number of samples used in this study. Evaluation of the indigenous enzyme activity (ALP, LPO) revealed that consumer milk products were heated adequately during treatment. The manufacturing processes used for the production of consumer milk can be differentiated by estimating furosine and acid soluble whey protein content.

The determination of TA demonstrated that the extent of lipolysis during storage of commercial ESL milk appears to be negligible. On the basis of descriptive sensory analysis, the sensory panel had a slight preference for HTST heated and microfiltered ESL milk, but a discrimination of drinking milk types produced by different production processes or duration of storage was not apparent. Altogether, ESL milk is a valuable food and its nutritional quality is comparable with HTST heated and UHT milk. This conclusion is evidenced in the unchanged protein and calcium concentration and the largely unchanged concentration of nutritionally important vitamins.

REFERENCES

Albalá-Hurtado S, Veciana-Nogués M T, Izquierdo-Pulido M and Mariné-Font A (1997) Determination of water-soluble vitamins in infant milk by high-performance liquid chromatography. *Journal of Chromatography A* **778** 247–253.

Blake M R, Weimer B C, McMahon D J and Savello P A (1995) Sensory and microbial quality of milk processed for extended shelf life by direct steam injection. *Journal of Food Protection* **58** 1007–1013.

BLE (2008). *Annual report of the Bundesanstalt für Landwirtschaft und Ernährung*. [Internet document] URL http://www.ble.de. Bonn, Germany: BLE.

BVL (2002) *Federal Office of Consumer Protection and Food Safety (2002) Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB, L 01.00-7:2002-05: Untersuchung von Lebensmitteln - Bestimmung des Säuregrades von Milch und flüssigen Milchprodukten*. Berlin: Beuth Verlag.

Clawin-Rädecker I and Schlimme E (1998) Nachweis nichtenzymatischer glycosylierter Proteine in boviner Kolostralmilch und reifer Frauenmilch durch die Bestimmung des Furosingehaltes. *Kieler Milchwirtschaftliche Forschungsberichte* **50** 133–146.

Clawin-Rädecker I, Koehn A and Schlimme E (1996) Furosin: Hitzeindikator in Milch und Frischkäse. *Deutsche Milchwirtschaft* **47** 902–905.

Clawin-Rädecker I, Kiesner C and Schlimme E (2000) Analyse der säurelöslichen Gehalte von α-Lactalbumin, β-Lactoglobulin, Serumalbumin und Immunglobulinfraktion in pasteurisierter Milch. *Kieler Milchwirtschaftliche Forschungsberichte* **52** 323–334.

Clawin-Rädecker I, Martin M, Meisel H, Lorenzen P C (2006) Auswirkungen der Mikrofiltration von Magermilch auf chemische Prameter. *Deutsche Molkerei Zeitung* **14** 20–15.

Eberhard P, Bütikofer U and Sieber R (2003) Vitamins in stored high heated milk. *Agrar-Forschung* **10** 62–65.

EC (2006) Commission Regulation No 1664/2006 (2006). *Official Journal of the European Union* L 320/13.

Farah Z, Rettenmaier R and Atkins D (1992) Vitamin content of camel milk. *International Journal of Vitamin and Nutrition Research* **62** 30–33.

Gallmann P U, Eberhard P and Sieber R (2001) Vor- und Nachteile länger haltbarer Past-Milch (ESL Milch). *Agrar-Forschung* **8** 112–117.

Harding F and Garry E (2005) Collaborative evaluation of a fluorometric method for measuring alkaline phosphatase activity in cow's, sheep's, and goat's milk. *Journal of Food Protection* **68** 1047–1053.

Henke J R (2009) Verfahrenstechniken zur Behandlung von ESL-Milch. *Food Technology Magazine* **20** 4–5.

Heudi O, Kilinç T and Fontannaz P (2005) Separation of water-soluble vitamins by reversed-phase high performance liquid chromatography with ultra-violet detection: application to polyvitaminated premixes. *Journal of Chromatography A* **1070** 49–56.

Hoffmann W, Kiesner C, Clawin-Rädecker I, Martin D, Einhoff K, Lorenzen P C, Meisel H, Hammer P, Suhren G and Teufel P (2006) Processing of extended shelf life milk using microfiltration. *International Journal of Dairy Technology* **59** 229–235.

ICMSF (1996) *Microorganisms in Food 5 – Microbiological Specifications of Food Pathogens*, pp. 27. London, Weinheim, New York, Tokyo, Melbourne, Madras: Blackie Academic and Professional.

Kaufmann V and Kulozik U (2007) Verfahrenskonzepte zur Herstellung von ESL-Milch – Stand der Technik und neue Optionen. *Deutsche Milchwirtschaft* **58** 268–271.

Kaufmann V and Kulozik U (2008) Verfahrenstechnische Einflussfaktoren auf die Qualität und Stabilität von ESL-Milch. *dmz-Deutsche Molkerei Zeitung* **129**, 28–30.

Kaufmann V, Scherer S and Kulozik U (2009) Stoffliche Veränderungen in Konsummilch durch haltbarkeitsverlängernde Verfahren. *Deutsche Milchwirtschaft* **60** 262–266.

Kress C, Schneider E and Usleber E (2005) Untersuchungen zum Keimgehalt pasteurisierter Konsummilch. *Archiv für Lebensmittelhygiene* **56** 76–79.

Lee G-H, Lee J-S and Shin M-G (2003) Sensory attribute comparison of consumer milk using descriptive analysis. *Food Science and Biotechnology* **12** 480–484.

Lorenzen P C, Martin D, Clawin-Rädecker I, Barth K and Knappstein K (2010) Activities of alkaline phosphatase, γ-glutamyltransferase and lactoperoxidase in cow, sheep and goat's milk in relation to heat treatment. *Small Ruminant Research* **89** 18–23.

Martin D, Linxweiler W, Tanzer D, Vormbrock R, Olt R, Kiesner C and Meisel H (2005) Use of the Reflectoquant® rapid tests for determination of thermal inactivation of the indigenous milk enzymes lipase, alkaline phosphatase and lactoperoxidase. *Deutsche Lebensmittel-Rundschau* **101** 281–286.

Mayer H, Raba B, Meier J and Schmid A (2009) Extended shelf life (ESL) milk – milk with excessive heat load? *European Dairy Magazine* **21** 18–22.

Mayr R, Gutser K, Busse M and Seiler H (2004) Gram positive non-sporeforming recontaminants are frequent spoilage organisms of German retail ESL (extended shelf life) milk. *Milchwissenschaft* **59** 262–266.

Pellegrino L, Tirelli F, Masotti F and Resmini P (1996) Significance of the main chemical indicators of heat load for characterizing raw, thermized, pasteurized and high temperature pasteurized milk. In *Heat Treatments and Alternative Methods*, pp 373–388. International Dairy Federation (ed) Brussels: ref. S.I.9602.

Resmini P, Pellegrino L, Hogenboom J A and Andreini R (1989) Evaluation of whey proteins by reverse-phase HPLC. *Scienza e Tecnica Lattiero-Casearia* **40** 7–23.

Rysstad G and Kolstad J (2006) Extended shelf life milk – advances in technology. *International Journal of Dairy Technology* **59** 85–96.

Schlimme E, Clawin-Rädecker I, Einhoff K, Kiesner C, Lorenzen P C, Martin D, Meisel H, Molkentin J and Precht D (1996) Unterscheidungsmerkmale zur Bewertung der Wärmebehandlung von Milch. *Kieler Milchwirtschaftliche Forschungsberichte* **48** 5–36.

Schlimme E, Kiesner C, Lorenzen P and Martin D (1998) Influence of heat treatment of milk on the activities of the indigenous milk enzymes alkaline phosphatase and adenosine deaminase. *Bulletin of the International Dairy Federation* **332** 25–31.

Schwermann S and Schwenzow U (2008a) Verfahrenskonzepte zur Herstellung von ESL-Milch. Teil 1: Grundsätzliches und direktes Erhitzungsverfahren. *Deutsche Milchwirtschaft* **59** 384–391.

Schwermann S and Schwenzow U (2008b) Verfahrenskonzepte zur Herstellung von ESL-Milch. Teil 2: Indirekte Erhitzung mit einer UHT-Anlage. *Deutsche Milchwirtschaft* **59** 428–432.

Schwermann S and Schwenzow U (2008c) Verfahrenskonzepte zur Herstellung von ESL-Milch. Teil 3: Mikrofiltration. *Deutsche Milchwirtschaft* **59** 462–467.

Seifu E, Buys E M and Donkin E F (2005) Significance of the lactoperoxidase system in the dairy industry and its potential applications: a review. *Trends in Food Science and Technology* **16** 137–154.

Shakeel-Ur-Rehman, Fleming C M, Farkye N Y and Fox P F (2003) Indigenous phosphatases in milk. In *Advanced Dairy Chemistry, Volume 1 Proteins*, pp 523–543. Fox P F, McSweeney P L H eds. London: Elsevier Applied Science.

Villamiel M, Arias M, Corzo N and Olano A (1999) Use of different thermal indices to assess the quality of pasteurized milks. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung* **208** 169–171.