Dairy Sci. Technol. 89 (2009) 83–98

DOI: 10.1051/dst/2008037

Available online at:
www.dairy-journal.org

Original article

Impact of ultrasound on dairy spoilage microbes and milk components

Michelle CAMERON, Lynn D. MCMASTER, Trevor J. BRITZ*

Department of Food Science, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa

Received 23 May 2007 – Accepted 28 November 2008

Abstract – Numerous reports in the literature suggest pasteurisation failures in the dairy industry as a possible cause for an end product with a poor quality. Ultrasonication offers the dairy industry a non-thermal alternative to pasteurisation. The aim of this study was to evaluate the use of ultrasonication as an alternative to heat pasteurisation. Ultrasound was found to eliminate spoilage and potential pathogens to zero or to levels acceptable by South African and British milk legislation, even when initial inoculum loads of 5× higher than permitted were present before treatment. Viable cell counts of *E. coli* were reduced by 100% after 10.0 min of ultrasonication. The data obtained also showed that viable counts of *Pseudomonas fluorescens* were reduced by 100% after 6.0 min and *Listeria monocytogenes* was reduced by 99% after 10.0 min. An infra-red based apparatus was used to analyse raw and pasteurised milk after an ultrasonic treatment. Ultrasonication did not lead to decreases in the protein or lactose content of both raw and pasteurised milk. Kjeldahl nitrogen determinations confirmed that ultrasonication had no detrimental effect on the total protein or casein content of pasteurised milk. This study indicated that ultrasonication lead to an increase in the fat concentration. This was explained by the larger surface area of the fat globules after ultrasonication, which led to an increase in light scattering as observed by the MilkoScan. Alkaline phosphatase and lactoperoxidase activities were also investigated as potential indicators of an effective ultrasonic treatment. Ultrasonication was, however, found to be ineffective in deactivating both enzymes used regularly by the dairy industry as indicators of effective thermal processes.

ultrasound / milk / microorganism / protein / *D*-value

摘要 – 超声波对乳制品腐败微生物和乳成分的影响。许多文献报道巴氏杀菌法在乳品工业中使用有可能导致最终产品的质量较差。超声波作为一种非热杀菌技术在乳品工业中有可能替代巴氏杀菌。本研究对超声波法替代巴氏杀菌方法的可能性进行了评价。超声波可以将乳中腐败菌和潜在病原菌的菌数降到零或者达到南非和英国乳品规定的标准, 甚至在原料乳 *E. coli* 高于规定菌数 5 倍的情况下,经过 10 min 处理后, 100% 的 *E. coli* 被致死。实验数据显示经过 6 min 的超声波处理后, 100% 的 *Pseudomonas fluorescens* 被致死; 而经过 10 min 的超声波处理后 99% 的 *Listeria monocytogenes* 被致死。采用红外光谱法测定经超声波处理的原奶和巴氏杀菌奶，超声波不能引起原奶和巴氏杀菌奶的乳糖和蛋白质的减少。根据凯氏定氮的检测结果证明超声波对巴氏杀菌奶的总蛋白和酪蛋白含量没有影响。研究表明, 超声波导致脂肪含量增加, 原因是超声波处理后脂肪球表面积较大而使得光散射增加, 因此用 MilkoScan 光谱仪测定结果偏高。同时研究了超声波处理对碱性磷酸酶和乳过氧化物酶活性的影响, 这两种酶在乳品工业中作为热处理效果的评价指标。然而, 超声波处理不能引起这两种酶的失活。

超声 / 乳 / 微生物 / 蛋白质 / *D*-值

* Corresponding author (通讯作者): tjbritz@sun.ac.za

Résumé – Impact des ultrasons sur les microbes d'altération du lait et sur ses composants. De nombreuses publications suggèrent que dans l'industrie laitière, la mauvaise qualité du produit fini peut provenir de défaillances de la pasteurisation. Le but de cette étude a été d'évaluer l'utilisation de l'ultrasonication comme alternative au traitement thermique. Les résultats ont montré que les ultrasons éliminaient totalement, ou à des niveaux acceptables par la législation sud-africaine et anglaise, la flore d'altération et les pathogènes potentiels, et ce même quand les charges d'inoculum initiales avant traitement étaient 5 fois supérieures à celles autorisées. La réduction du nombre de cellules viables après ultrasonication était de 100 % après 10.0 min pour *E. coli*, de 100 % après 6.0 min pour *Pseudomonas fluorescens* et de 99 % après 10.0 min pour *Listeria monocytogenes*. L'analyse par infrarouge du lait cru et du lait pasteurisé après traitement aux ultrasons ne montrait aucune diminution de la teneur en protéines et en lactose dans ces deux laits. La quantification de l'azote par Kjeldahl confirmait l'absence d'effet préjudiciable de l'ultrasonication sur les teneurs en protéines totales ou en caséines du lait pasteurisé. Cette étude montre que l'ultrasonication conduit à une augmentation de la concentration en matière grasse. Ceci s'explique par la plus grande surface des globules gras après ultrasonication qui conduit à une augmentation de la diffraction de la lumière observée par le MilkoScan. Les activités de la phosphatase alcaline et de la lactoperoxydase, habituellement utilisées dans l'industrie laitière comme indicateurs de traitement thermique efficace, ont aussi été étudiées. Cependant, l'ultrasonication s'est avérée inefficace pour désactiver ces deux enzymes.

ultrason / lait / microorganisme / protéine / réduction décimale

1. INTRODUCTION

Traditional thermal pasteurisation and sterilisation processes are the most common methods used by the food and dairy industry for the inactivation of microorganisms. Although *E. coli* is reported to be destroyed by pasteurisation, there are reports on its ability (including the pathogenic strain O157:H7) to form biofilms within pasteurisation equipment, leading to pasteurisation failures [8, 39]. *Listeria monocytogenes* [9] and *Pseudomonas* spp. [41] have also been reported to survive commercial pasteurisation.

The use of ultrasound to inactivate microbes was reported in the late 1920's [16], but its limited lethal effect on spoilage microbes prohibited it from being used as a sterilisation method. Improvements in ultrasound generation technology over the last decade have again stimulated interest in microbial inactivation by ultrasound [31].

Ultrasonic waves are generated by mechanical vibrations of frequencies above 20 kHz [17]. When these waves propagate into liquid media, alternating compressions and rarefactions are produced. If the amplitude of the ultrasonic wave is high enough, cavitation, which is the making and breaking of microscopic bubbles, will occur. When the bubbles reach a critical size, they collapse violently. This violent collapse is thought to be mechanical forces resulting in the breaking and shearing of cell walls leading to cell death. According to Ciccolini et al. [7], the effects of cavitation on microbial suspensions include: dispersion of microbial clumps; cell wall puncturing; modification of cellular activity; and increased sensitivity to heat. However, it must always be remembered that the effectiveness of ultrasonication is known to be influenced by the microbial strain tested, the suspending medium, the size of the cell [24] as well as electrical power input.

Other advantageous effects of ultrasonic waves in milk include: fat may be homogenised [5, 45]; gases are removed [28]; and the antioxidant activity enhanced [40]. Villamiel and de Jong [45] reported that continuous-flow ultrasonic treatment could be a promising technique for milk processing.

Heat processing may lead to deterioration of the organoleptic properties and also

the nutritional value of milk [10, 13]. Protein is probably the most valuable constituent of milk, due to its high nutritional quality and unique physico-chemical and functional properties. These properties are fundamental to the production and characteristics of many dairy products, such as cheese or yogurt [19, 35].

Enzymes are another important component of milk, although not from a nutritional point of view. The two enzymes that are regularly utilised from a practical point of view by the dairy industry are alkaline phosphatase and lactoperoxidase. Alkaline phosphatase (ALP) has a thermal resistance greater than that of most non-endospore-forming microbes commonly found in milk. This enzyme is deactivated when heated to 71.6 °C for 15 s. Therefore, ALP is used universally as an indicator of successful implementation of high temperature short time (HTST) pasteurisation [25]. Lactoperoxidase in contrast, is used for assessing the effectiveness of an ultra high temperature (UHT) treatment of milk as this enzyme is inactivated by temperatures higher than 80 °C [4]. Thus, UHT milk after an effective heat treatment would test negatively for lactoperoxidase activity, whilst HTST pasteurised milk remains lactoperoxidase positive [44].

The importance of different milk components when processing milk to produce cheese, yogurt, etc., has lead to extensive studies on the effect of heat on the different milk components. Ultrasonication is a relatively new alternative to pasteurisation, and therefore, the need exists to further evaluate the impact of ultrasonication on dairy spoilage microbes as well as on the different milk components.

The aim of this study was to investigate the lethality of ultrasound in terms of eliminating a selection of microbes from milk. Furthermore, any possible detrimental effect of ultrasound on native milk proteins, fats and lactose was determined along with the impact of ultrasound on alkaline phosphatase and lactoperoxidase activity.

2. MATERIALS AND METHODS

2.1. Microbial cultures

Escherichia coli, *Listeria monocytogenes* and *Pseudomonas fluorescens* were evaluated in this study. Strain purity was regularly checked by microscopy and Gram stains, and the identity confirmed using the API system (bioMérieux SA, Marcy-l'Étoile, France).

A broth subculture of the appropriate microbe was prepared by inoculating 10 mL sterile nutrient broth (Merck) with a test microbe, and incubating for 24 h at 35 °C. A 100-mL sterile container, containing 90 mL broth was inoculated with 5 mL of the 24 h culture and incubated for a further 24 h prior to the ultrasonic treatments.

2.2. Ultrasonication of inoculated milk

Two mL of the appropriate culture was centrifuged for 10 min at 6000× *g* (Eppendorf 5415D centrifuge, Eppendorf, Hamburg, Germany). The pellet was suspended in sterile saline solution (0.85% w/v) and the data from standard curves were used to determine the desired cell concentration for inoculation of milk. Full cream (3.4% milk fat) UHT (ultra high temperature) milk was inoculated with an aliquot of culture to yield an approximate inoculum level of either 1×10^4 or 1×10^6 colony forming units per mL (cfu·mL^{-1}).

For ultrasonication, a 40-mL sample of the inoculated milk was put into a sterile, jacketed glass sample holder connected to an ice-waterbath to maintain a temperature of between 4 °C and 6 °C, to maintain a sample temperature of between 24 °C and 26 °C. The tip of the probe was placed 2 cm below the surface of the milk sample resulting in the probe being

1 cm above the bottom of the sample. A 750 W, 20 kHz Vibra-Cell High Intensity Ultrasonic Processor VCX 750 (Sonics & Materials, Inc., Newtown, CT, USA), fitted with an autoclavable 13 mm diameter probe with a replaceable tip, was used for ultrasonication. With this unit, feedback from the probe was continuously evaluated, and the frequency and power were automatically adjusted to ensure optimum ultrasonic delivery. The Vibra-Cell is also able to monitor the energy (in Joules) and the temperature of the sample being processed. Samples were treated using five different time regimes: 2.5, 5.0, 6.0, 7.5 and 10.0 min at 100% displacement amplitude (124 μm).

Two separate ultrasonic treatments were done in duplicate of each sample. Duplicate dilutions were made from each treated sample; the pour-plate technique and plate count agar (PCA) (Merck) were used for enumeration at 35 °C. UHT milk that had not been inoculated with a test organism, served as controls. These controls showed no microbial growth after 24 h incubation.

The efficacy of ultrasonication treatments in terms of eliminating microbes was measured by their decimal reduction time (D), which for this study was defined as the time (min) of a given treatment for the number of survivors to be reduced by one log cycle. D-values were calculated from the slope of the regression line plotted with the counts (cfu·mL^{-1}) of the straight portion of the survival curve. In this study, the D-value at 20 kHz/750 W was abbreviated as D_{US}.

2.3. Ultrasonication of raw and pasteurised uninoculated milk

Commercially pasteurised full cream milk, obtained from a local supermarket, and raw milk collected from the Welgevallen Experimental Farm of the University of Stellenbosch were used during this study.

Uninoculated milk was ultrasonicated as described for the inoculated milk.

2.4. Chemical analysis

All uninoculated milk samples were preserved with Bronopol Microtabs (D & F Control Systems, Inc.) and analysed for protein (%), fat (%), lactose (%) and somatic cell counts (SCC) (cells per mL) within 24 h of the applied ultrasonic treatment. Analyses were done at the Dairy Institute of the Agricultural Research Council (ARC) at Elsenburg using a MilkoScan FT 6000 (FOSS, Denmark) and a Fossomatic FC 6000 (FOSS, Denmark). Samples were subjected to ultrasonication for 0, 1, 5, 10 and 15 min and five samples were analysed for each treatment time.

2.5. Kjeldahl determinations

2.5.1. Total protein

Total protein determinations were done using the International Dairy Federation 20B (1993) standard method [21] with a few modifications. One gram of commercially pasteurised full cream milk was weighed into a Kjeldahl flask, and to this 18 mL H_2SO_4 (98.08% m/v) (Saarchem) and 1 Kjeldahl tablet (Saarchem) were added. A 1 g water sample served as the control. Digestion was carried out for 1.5 h using a Büchi Digestion Unit K-424 (Büchi, Flawil, Switzerland). After digestion was completed, the samples were allowed to cool to room temperature and 45 mL distilled water was added to each flask. The flasks were connected to a Büchi Distillation Unit K-350 (Büchi, Flawil, Switzerland) and 85 mL NaOH (32% m/v) (Merck) were automatically added followed by a 4 min distillation. The distillate was collected in a 20 mL H_3BO_3 (4% m/v) (BDH) solution containing 100 μL indicator. The indicator was a mixture of 0.59 g

methyl red (Merck) and 0.29 g methylene blue (Merck) in 500 mL 96% (v/v) ethanol (Merck). This was then titrated with 0.05 N H_2SO_4 to the first trace of pink. The burette reading was recorded and the nitrogen content was determined using the following formula [21]:

$$\text{Nitrogen} = \frac{1.4 \times \text{N} \times \text{TV}}{\text{sample weight (g)}} = \text{g nitrogen} \cdot 100\ \text{g}^{-1}\ \text{milk}$$

where 1.4 = 1.4 mg nitrogen neutralised by 1 mL 0.1 N H_2SO_4,
N = normality of H_2SO_4,
TV = titration value.

The crude protein content, expressed as a percentage by mass, was obtained by multiplying the nitrogen content by 6.38 which is the reciprocate of the % nitrogen in protein for dairy products [21]. Four samples were analysed for each treatment time (0, 1, 5, 10 and 15 min).

2.5.2. Casein

The casein fraction of the total protein content was obtained by determining the portion of non-casein nitrogen and subtracting this value from the total nitrogen [36]. For the non-casein nitrogen determination, the samples received a pretreatment before Kjeldahl nitrogen determinations were done. A 10 g milk sample was weighed into a volumetric flask and 70–80 mL distilled water (40 °C) and 1 mL of a 10% (v/v) acetic acid (Saarchem) solution added and mixed. After 10 min, 1 mL of a 1 N sodium acetate (Saarchem) solution was added. The sample was allowed to cool to room temperature before the volume was adjusted to 100 mL with distilled water. The mixture was filtered (Whatman no. 40) and 20 mL of the filtrate was poured into a Kjeldahl flask, and a nitrogen determination was done. A 20 mL water sample served as the control.

The non-casein nitrogen (NCN) was determined using the following formula [36]:

$$\text{NCN} = \frac{1.4 \times \text{N} \times \text{TV}}{{}^{1}/_{5}\text{ of sample weighed (g milk)}} = \text{g nitrogen} \cdot 100\ \text{g}^{-1}\ \text{milk}$$

where 1.4 = 1.4 mg nitrogen neutralised by 1 mL 0.1 N H_2SO_4,
N = normality of H_2SO_4,
TV = titration value.

The crude protein content, expressed as a percentage by mass, was obtained by multiplying the nitrogen content by 6.38 which is the reciprocate of the % nitrogen in protein for dairy products [21]. Four samples were analysed for each treatment time (0, 1, 5, 10 and 15 min).

2.6. Alkaline phosphatase

Alkaline phosphatase activity was determined according to the standard method of the International Dairy Federation [22]. Five mL of a buffered 4-nitrophenyl disodium orthophosphate solution (BDH) was added to 1 mL milk, and incubated in a waterbath at 37 °C for 2 h. After 2 h the samples were visually compared with the control. Commercially pasteurised milk was used as a negative control. All determinations were done in triplicate.

2.7. Lactoperoxidase

Lactoperoxidase activity was determined by adding 1 mL of a 0.5% (v/v) guaiacol solution (BDH) to 5 mL milk. One drop of hydrogen peroxide (ACE Chemicals) was added and the mixture left to stand at room temperature for 3 min, after which the samples were visually inspected for colour changes. UHT milk served as a negative control. Triplicate determinations were done for each sample.

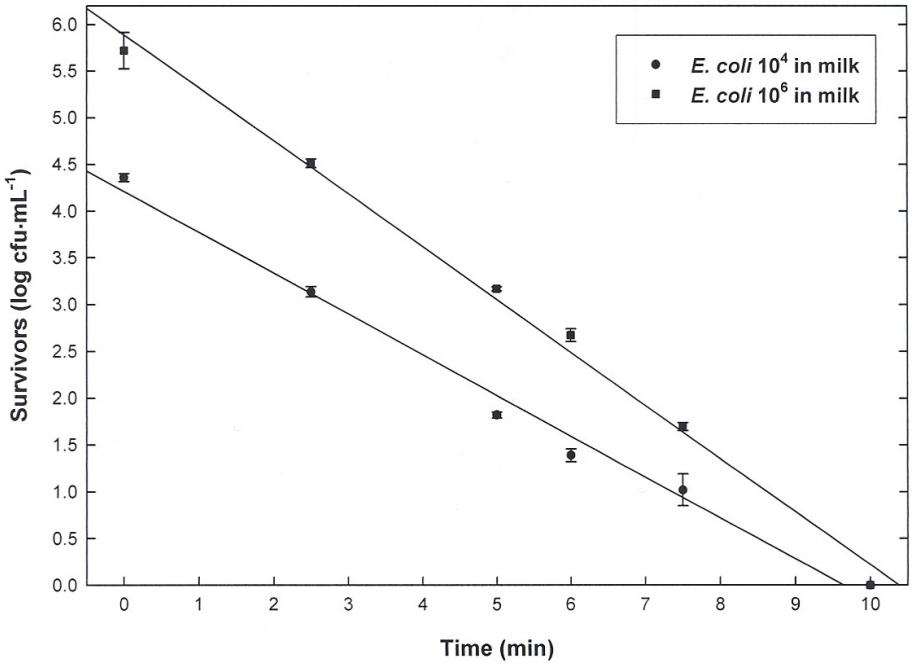

Figure 1. Regression (95% confidence level) of the data showing the impact of ultrasonication at 20 kHz on *Escherichia coli* at different starting concentrations in UHT milk. (Each data point represents quadruple values. The standard deviation was used as the error-bar.)

2.8. Statistical analysis

Statistical analysis (using Statistica 7.1 software) was done on the data obtained from the MilkoScan for both the raw and pasteurised milk. One-way ANOVA was used to determine if there were significant differences between average measurements for the different time treatments. The Bonferroni post-hoc test was used to compare pairwise treatments. In cases where violations from the ANOVA assumptions were suspect, non-parametric bootstrap was performed. In all cases however, the non-parametric results were the same as the ANOVA results, and therefore only the ANOVA results was reported. Every point on the graphs for the MilkoScan results indicates the average value calculated from 5 repetitions. The error-bars represent the 95% confidence interval.

3. RESULTS

3.1. Impact of ultrasound on dairy microbes

3.1.1. Escherichia coli

A 100% elimination of *E. coli* was achieved after 10.0 min of ultrasonication. Both the 1×10^4 and 1×10^6 cfu·mL^{-1} inocula in milk were reduced to zero with a 4.32 log reduction and a 5.34 log reduction, respectively (Fig. 1). The D_{US} values for *E. coli* were 2.3 min (1×10^4 cfu·mL^{-1}) and 1.7 min (1×10^6 cfu·mL^{-1}) in milk.

3.1.2. Listeria monocytogenes

In this study, ultrasonication of *L. monocytogenes* for 10.0 min in milk resulted

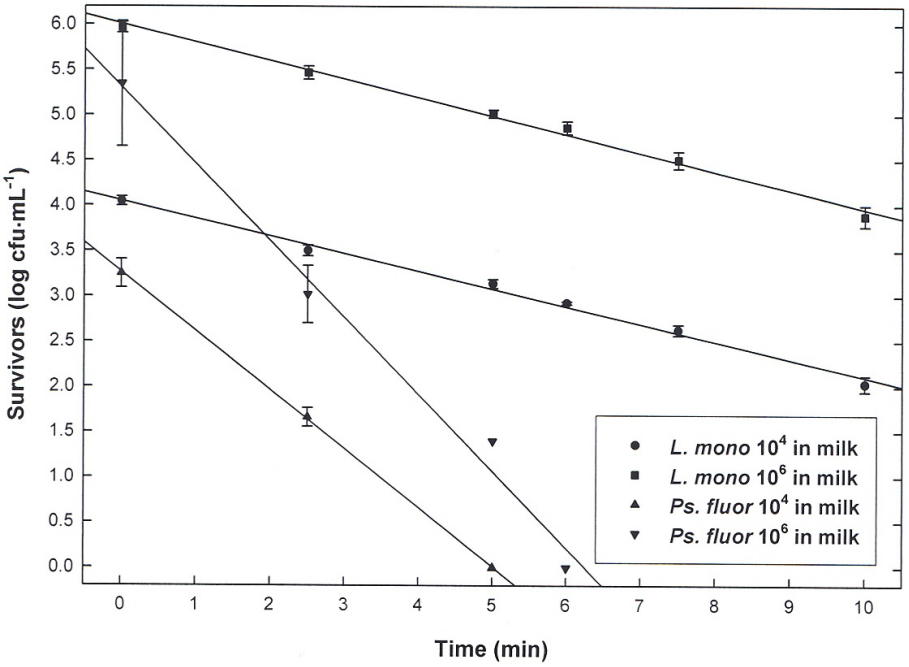

Figure 2. Regression (95% confidence level) of the data showing the impact of ultrasonication at 20 kHz on *Listeria monocytogenes* and *Pseudomonas fluorescens* at different starting concentrations in UHT milk. (Each data point represents quadruple values. The standard deviation was used as the error-bar.)

in an elimination of 99% of an initial load of 1×10^4 cfu·mL^{-1} with a 2.00 log reduction, and a 99.14% reduction for a 1×10^6 cfu·mL^{-1} inoculum with a 2.07 log reduction (Fig. 2). The D_{US} for *L. monocytogenes* in milk was 5.1 min (1×10^4 cfu·mL^{-1}) and 4.9 min (1×10^6 cfu·mL^{-1}).

3.1.3. Pseudomonas fluorescens

Ultrasonication of *Ps. fluorescens* resulted in a 100% elimination of all viable cells (Fig. 2). In milk, all viable cells of a 1×10^4 cfu·mL^{-1} inoculum were eliminated after only a 5.0 min ultrasonic treatment. This is equivalent to a 3.26 log reduction. When the initial inoculum in milk was increased to 1×10^6 cfu·mL^{-1}, a treatment time of 6.0 min was required to eliminate all viable cells with a 5.64 log reduction. The D_{US} was calculated to be 1.6 min (1×10^4 cfu·mL^{-1}) and 1.1 min (1×10^6 cfu·mL^{-1}) in milk.

3.2. Impact of ultrasound on milk components

The dairy industry routinely uses an infra-red based apparatus (MilkoScan) to analyse and evaluate the quality of each supplier's milk. The MilkoScan was therefore used in this study to determine whether possible changes to the composition of both raw and pasteurised milk after ultrasonication could be detected. Data obtained from the MilkoScan for raw milk after an ultrasonic treatment are summarised in Table I, and the data from

Table I. MilkoScan results of the different milk components after an ultrasonication treatment of raw milk.

	Treatment time				
Fraction	**0 min**	**1 min**	**5 min**	**10 min**	**15 min**
Protein (%)	3.03^{b}	3.22^{a}	3.25^{a}	3.25^{a}	3.24^{a}
	(SD ± 0.000)	(SD ± 0.030)	(SD ± 0.030)	(SD ± 0.030)	(SD ± 0.025)
Fat (%)	2.54^{c}	2.62^{a}	2.67^{b}	2.67^{b}	2.66^{ab}
	(SD ± 0.015)	(SD ± 0.030)	(SD ± 0.010)	(SD ± 0.030)	(SD ± 0.030)
Lactose (%)	4.80^{c}	4.81^{a}	4.82^{ab}	4.83^{b}	4.83^{b}
	(SD ± 0.005)	(SD ± 0.015)	(SD ± 0.005)	(SD ± 0.005)	(SD ± 0.005)
SCC (cells·mL^{-1})	$229\,400^{b}$	$12\,800^{a}$	7000^{a}	6800^{a}	8000^{a}
	(SD ± 12 000)	(SD ± 3000)	(SD ± 4000)	(SD ± 1000)	(SD ± 5500)

The values given are means (n = 5); values in parentheses are the standard deviation.
SCC = somatic cell count.
Values with different superscripts in a row differs significantly ($P < 0.05$).

Table II. MilkoScan results of the different milk components after an ultrasonication treatment of pasteurised milk.

	Treatment time				
Fraction	**0 min**	**1 min**	**5 min**	**10 min**	**15 min**
Protein (%)	3.12^{ab}	3.12^{a}	3.11^{ab}	3.11^{b}	3.11^{ab}
	(SD ± 0.005)	(SD ± 0.005)	(SD ± 0.000)	(SD ± 0.005)	(SD ± 0.000)
Fat (%)	3.48^{a}	3.48^{a}	3.52^{b}	3.52^{b}	3.52^{b}
	(SD ± 0.005)	(SD ± 0.005)	(SD ± 0.005)	(SD ± 0.005)	(SD ± 0.000)
Lactose (%)	4.80^{a}	4.81^{a}	4.82^{a}	4.82^{a}	4.81^{a}
	(SD ± 0.010)	(SD ± 0.010)	(SD ± 0.015)	(SD ± 0.010)	(SD ± 0.010)
SCC (cells·mL^{-1})	$71\,200^{b}$	$27\,000^{c}$	9400^{a}	5800^{a}	4600^{a}
	(SD ± 5500)	(SD ± 4000)	(SD ± 3000)	(SD ± 2000)	(SD ± 1000)

The values given are means (n = 5); values in parentheses are the standard deviation.
SCC = somatic cell count.
Values with different superscripts in a row differs significantly ($P < 0.05$).

the MilkoScan for pasteurised milk that had been ultrasonicated are summarised in Table II.

3.2.1. Protein

The data for raw milk showed a statistically significant increase ($P \leq 0.01$) in the protein content from 0 min (3.03%) to 1 min (3.22%) of the ultrasonic treatment, after which there were no further significant changes noted for the protein content for the remainder of the ultrasonic treatment (Tab. I).

A significant decrease ($P = 0.01$) in the protein content of pasteurised milk after ultrasonication was observed from a 1 min (3.12%) to a 10 min (3.11%) treatment (Tab. II).

Data obtained for Kjeldahl protein determinations on pasteurised milk are

Table III. Kjeldahl protein results for pasteurised milk after an ultrasonication treatment.

	Treatment time				
Fraction	**0 min**	**1 min**	**5 min**	**10 min**	**15 min**
Total protein (%)	3.67[a] (SD ± 0.020)	3.68[a] (SD ± 0.015)	3.67[a] (SD ± 0.020)	3.67[a] (SD ± 0.025)	3.67[a] (SD ± 0.010)
Casein (%) of total protein	80.60[a] (SD ± 0.20)	80.55[a] (SD ± 0.25)	80.45[a] (SD ± 0.15)	80.53[a] (SD ± 0.20)	80.55[a] (SD ± 0.15)

The values given are means (n = 4); values in parentheses are the standard deviation.
Values with different superscripts in a row differs significantly ($P < 0.05$).

summarised in Table III. The pasteurised milk used in the MilkoScan and Kjeldahl experiments were from different batches, which explains the differences in the determined protein concentrations obtained from the two methods. The results indicated that there was no statistically significant changes in either the total protein content ($P = 0.93$) or the casein fraction of the total protein content ($P = 0.82$) after ultrasonication of pasteurised milk. It was decided not to do Kjeldahl protein determinations on raw milk, due to possible interference by the large fat globules of the unhomogenised milk.

3.2.2. Fat

The fat content of raw milk showed a significant increase ($P \leq 0.01$) from 0 min (2.54%) to a 1 min (2.62%) ultrasonic treatment and also from 1 min (2.62%) to 5 min (2.67%) of ultrasonication (Tab. I). The total increase in fat content from 0 min to 5 min of ultrasonication was 5.11%. After 5 min of ultrasonication no further statistical changes in the fat content were observed for the remainder of the treatment time.

The data obtained indicated a significant increase ($P \leq 0.01$) in fat content for pasteurised milk from a 1 min (3.48%) to a 5 min (3.52%) ultrasonic treatment, after which no significant changes were observed for the remainder of the ultrasonic treatment (Tab. II).

3.2.3. Lactose

The results obtained for the lactose content of raw milk (Tab. I) indicated a significant increase ($P \leq 0.01$) in lactose from 0 min (4.80%) to 1 min (4.81%) of ultrasonication, and also from 1 min (4.81%) to 5 min (4.82%) of the ultrasonic treatment. No further significant increase was observed after 5 min of ultrasonication of the raw milk.

The data obtained showed no significant changes for the lactose content ($P = 0.06$) of pasteurised milk after the ultrasonic treatment (Tab. II).

3.2.4. Somatic cell count

A significant decrease ($P \leq 0.01$) in SCC was observed when raw milk was given an ultrasonic treatment. Cell counts decreased from 229 400 cells·mL^{-1} (0 min) to 12 800 cells·mL^{-1} after 1 min of ultrasonication (a 94.42% reduction), after which no further significant decreases were observed. The SCC of the raw milk was found to be 8000 cells·mL^{-1} after a 15 min ultrasonic treatment (Tab. I).

The data obtained for the SCC of pasteurised milk after the ultrasonic treatment showed a significant decrease ($P \leq 0.01$) in SCC from 0 min (71 200 cells·mL^{-1}) to 1 min (27 000 cells·mL^{-1}) and also from 1 min (27 000 cells·mL^{-1}) to 5 min (9400 cells·mL^{-1}) of treatment. The SCC of the pasteurised milk was

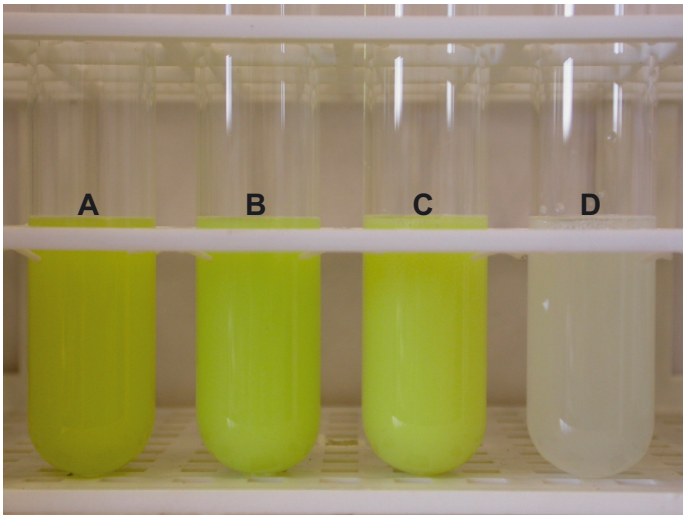

Figure 3. Impact of ultrasonication on the alkaline phosphatase activity in milk (A = raw milk; B = raw milk + 5 min ultrasonication; C = raw milk + 10 min ultrasonication; D = negative control – pasteurised milk).

4600 cells·mL^{-1} after 15 min of ultrasonication (Tab. II).

3.3. Impact of ultrasound on milk enzyme activity

3.3.1. Alkaline phosphatase

The results for alkaline phosphatase activity of ultrasonicated and non-ultrasonicated milk showed that ultrasonication of raw milk do not decrease ALP activity. Untreated raw milk, raw milk that had been ultrasonicated for 5 min and raw milk that had been ultrasonicated for 10 min all remained positive for ALP activity (Fig. 3).

3.3.2. Lactoperoxidase

The results obtained for the peroxidase test of ultrasonicated and non-ultrasonicated milk showed that ultrasonicating raw milk for either 5 min or 10 min reduces peroxidase activity to a degree/extent comparable with that found in pasteurised milk. However, total inactivation of peroxidase, as was found when UHT milk was tested, could not be achieved with an ultrasonic treatment time of 10 min (Fig. 4).

4. DISCUSSION

4.1. Impact of ultrasound on dairy microbes

4.1.1. Escherichia coli

The dairy industry generally considers the presence of *E. coli* in dairy products as an indication of faecal and post-pasteurisation contamination. The South African "milk law" states that when the VRB MUG agar method is used, no *E. coli* may be present in 1.0 mL of pasteurised milk [3]. Gram-negative microbes have been reported to be very sensitive to ultrasonication [2]; however, small microbes tend to be more resistant despite their

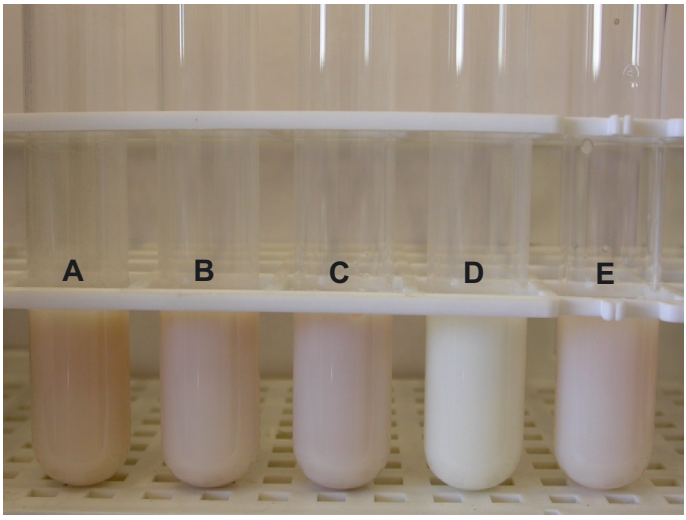

Figure 4. Impact of ultrasonication on the lactoperoxidase activity in milk (A = raw milk; B = raw milk + 5 min ultrasonication; C = raw milk + 10 min ultrasonication; D = negative control – UHT milk; E = pasteurised milk).

Gram-status [23]. Although the use of ultrasound as a "sterilisation" technique, with reports dating back to before 1954, is not new, recent advances in acoustic technology have enabled researchers to construct equipment that is able to deliver more power than a decade ago. This increase in available power ultimately results in better cavitation, increasing the lethality of this technique.

In 1979, Utsunomiya and Kosaka [42] reported a 0.83% survival of *E. coli* (99.17% reduction) in saline after 10 min when treated at 700 kHz, but surprisingly, they reported no inactivation of *E. coli* in milk. In their article [42] they did not mention which type of milk was used, which would also influence the efficacy of ultrasonication. The results obtained in this study compares well with the findings of Utsunomiya and Kosaka [42] in terms of the % reduction achieved after a 10 min treatment in saline. However, the initial inoculation concentration was not mentioned, and a very high initial concentration or unavailability of enough power at a frequency of 700 kHz might be the reason why 100% elimination was not achieved.

4.1.2. Listeria monocytogenes

Listeria monocytogenes is considered to be an important Gram-positive dairy pathogen with the ability to grow at refrigeration temperatures [33].

Pagán et al. [30] reported a *D*-value of 4.3 min for *L. monocytogenes* ultrasonicated (20 kHz and an amplitude of 117 μm) at ambient temperature. It was not clear what the initial cell concentration (cfu·mL^{-1}) used by Pagán et al. [30] had been, and that could explain the slight difference between the *D*-values obtained in this study and those obtained by Pagán et al. [30]. There are a number of factors that influence the efficiency of ultrasonication (strain of microbe, initial concentration, treatment medium, amplitude of sound waves, growth phase, etc.), and omitting or neglecting to mention them

makes comparisons between the results of different research groups difficult. The statement that Gram-positive bacteria are more resistant to the detrimental effect of ultrasound [18, 45] certainly holds true for the three strains tested in this study. *Listeria monocytogenes* showed more resistance, and longer D_{US}-values compared to the two Gram-negative microbes tested.

4.1.3. Pseudomonas fluorescens

Pseudomonas is frequently present in raw milk [12], however, this Gram-negative microbe is not reported to survive pasteurisation, and its presence in pasteurised milk is usually ascribed to post-pasteurisation contamination [1].

The results obtained from this study compare well with some of the results reported by Villamiel and de Jong [45]. They reported log reductions of between 0.6 and 4.2 for *Ps. fluorescens* in Trypticase Soy Broth with an initial concentration of 6.9–7.7 log cfu·mL^{-1}. The ultrasonication apparatus they used had a fixed frequency of 20 kHz, and a maximum power output of 150 W. They used a continuous system with flow rates of 50 and 33 mL·min^{-1}. In addition to this, they used ultrasound in combination with a heat treatment. The differences in treatment parameters used in this study compared with those used by Villamiel and de Jong [45] make it difficult to explain why they obtained such a very low log reduction (0.6) in some cases.

4.2. Impact of ultrasound on milk components

4.2.1. Protein

Milk protein is an important milk component in the production of a variety of dairy products as it is linked to total yield of the final product [38]. An increase in the protein content of milk leads to a higher yield when, for instance, cheese is manufactured. The protein content of milk is dependant on the breed of cow, individual cows of the same breed, lactation stage as well as the season. The protein content of milk is known to vary between 2.9–5.0% [4].

The authors have no explanation for the slight (6.48%) increase in protein content observed for raw milk after ultrasonication. This increase would, however, not have any negative impact on total cheese yield if the milk was intended for the manufacturing of cheese, as an increase in protein content is generally accepted to increase the cheese yield.

The protein component of milk is one of the main contributors to total cheese yield [34], with an increase in protein content resulting in an increase in total cheese yield. The decrease (0.32%) in the protein content noted for pasteurised milk was not significant (Tab. III).

Kjeldahl nitrogen analysis was used to determine the crude protein as well as the casein fraction of the total protein of pasteurised milk. Total milk protein contains about 80% casein [4], and casein is the dominant factor affecting curd firmness, syneresis rate, moisture retention, and ultimately the cheese quality and yield [15, 47]. The results obtained from this study indicate that, based on the fact that there was no decrease in the crude protein or casein content, the use of ultrasonicated milk for the production of cheese would have no negative effect on cheese yield.

4.2.2. Fat

Milk fat is another component of milk that is correlated to cheese yield [38]. A higher milk fat content ultimately leads to a higher yield of the final product. The fat content of milk typically varies between 2.5% and 6.0% depending on the breed of cow, stage of lactation and season [4].

This increase in fat content was an artefact due to the MilkoScan. The homogenisation of fats caused by ultrasonication leads to a decrease in the fat globule size, with a subsequent increase in the surface area of fat globules. The MFGM (milk fat globule membrane) is disrupted during ultrasonication [26]. The MilkoScan uses an infra-red light-based method and the increase in the surface area leads to higher fat content readings. The same trend is observed when raw and pasteurised/homogenised milk is analysed by the MilkoScan [43]. Homogenisation is employed by the dairy industry to reduce the size of the fat globules, thereby preventing creaming and coalescence during storage [20]. The homogenisation effect of ultrasonication is therefore an added benefit, as it might be possible to eliminate the homogenisation step altogether during fresh milk processing. Replacing both thermal pasteurisation and homogenisation with one process, i.e. ultrasonication could probably be cost effective in terms of initial equipment expenses as well as maintenance of the equipment.

Although a statistically significant increase was found when pasteurised milk was ultrasonicated, the measurements fall within the acceptable 0.05% fluctuation for replicates analysed with the MilkoScan (FOSS Integrator IMT software e-manual). The slight increase in the fat content of pasteurised milk after ultrasonication would thus not negatively impact the yield of any processed milk product.

4.2.3. Lactose

Lactose is a carbohydrate found exclusively in milk and is utilised as a carbon source during fermentation processes for the production of yogurt, cheese, etc. [6, 46]. The lactose content of milk varies between 3.6 and 5.5% [4].

Although a significant increase was found after ultrasonication of raw milk, the measurements fall within the acceptable 0.05% fluctuation for replicates analysed with the MilkoScan (FOSS Integrator IMT software e-manual). No statistically significant changes were observed for the lactose content of pasteurised milk after the ultrasonic treatment.

During yogurt processing, lactose is fermented by the lactic acid bacteria (LAB), to produce lactic acid, resulting in a lowering of the pH. As no significant difference was observed for the lactose content of both pasteurised and raw milk after ultrasonication, it is suggested that it would be safe to use ultrasonicated milk for the manufacturing of yogurt. The availability of carbohydrates for fermentation by the LAB remains unchanged, therefore, the same tempo of lactic acid production during yogurt processing should be achieved.

4.2.4. Somatic cell count

The somatic cell count (SCC) of milk is commonly used as an indicator of mastitis in dairy cows, and results in reduced milk quality and milk yield [37].

The reduction in SCC after ultrasonication of raw and pasteurised milk was observed. It is well known that milk with a high SCC has a reduced sensory quality [29] and shelf-life [27]. Although the SCC was lowered by ultrasonication, this would not improve the sensory quality of the milk. It is therefore of utmost importance that the quality of raw milk be considered before accepting milk, as no processing method can compensate for milk of a poor quality.

4.3. Impact of ultrasound on milk enzyme activity

Alkaline phosphatase (ALP) is an indigenous enzyme that is always present in raw milk, with 30–40% of the enzyme bound to the milk fat globule membranes. The rest of the enzyme is dispersed

throughout the skimmed milk fraction, and probably associated with the lipoproteins [32]. This enzyme splits certain phosphoric acid-esters into phosphoric acid and the corresponding alcohols [4]. ALP is destroyed by pasteurisation at 72 °C for 15 s [4], therefore, the ALP test is commonly used for assessing the effectiveness of pasteurisation and also the safety of dairy products.

Lactoperoxidase is an enzyme found mainly in the whey fraction of milk and catalyses the transfer of oxygen from hydrogen peroxide to other substrates [4, 11]. Lactoperoxidase enzymes are used as an indicator of successful UHT treatments as these enzymes are inactivated by heat treatments above 80 °C [4, 14]. Therefore HTST pasteurised milk remains peroxidase positive. UHT milk tests as peroxidase negative [44] as UHT milk is heated to temperatures of above 100 °C.

The findings of Villamiel and de Jong [45] were confirmed by the results obtained in this investigation which showed that ultrasonication of milk, without the addition of heat, results in a positive ALP test. As would be expected, the commercially pasteurised milk tested negative for phosphatase activity. It can therefore be concluded that the ALP test cannot be used for assessing the effectiveness of ultrasonication as APL enzymes are not inactivated during ultrasonication. The lactoperoxidase test was also found to be an ineffective indicator of a sufficient ultrasonic treatment. An enzymatic indicator might not be a suitable option for the indication of a successful treatment, however, it is important that a quick and efficient method be identified before this technique will be considered as a viable alternative to traditional pasteurisation.

5. CONCLUSION

The South African "milk law" [3] states that raw milk with contamination levels of 200 000 cfu·mL^{-1} or less must be reduced to less than 50 000 cfu·mL^{-1} prior to selling as pasteurised milk, and may not contain any *E. coli* per 1 mL of milk. This is equivalent to a 75% reduction in viable counts. This study indicated that the number of viable cells for *P. fluorescens* (100% elimination) and *Listeria monocytogenes* (99% elimination) were reduced by more than 75%. Furthermore, all viable *E. coli* cells were eliminated. According to the SA "milk law", no *E. coli* may be present in either raw or pasteurised milk. It is thus evident that a final product that complies with legal requirements can be produced using ultrasound as an alternative for traditional thermal pasteurisation.

This study furthermore showed that ultrasound does not have a negative impact on the total protein content, fat content or the lactose content of milk. It is therefore suggested that ultrasonication may be employed effectively as a means of "pasteurisation" with no adverse effects on e.g. cheese yield.

Unfortunately, ultrasound does not inactivate alkaline phosphatase or lactoperoxidase enzymes. These enzymes can thus not be used to indicate a successful ultrasonic treatment. If ultrasonication is to be used as an alternative to thermal pasteurisation, a need exists to find a quick and efficient method to indicate whether ultrasonication was sufficient in terms of ensuring a microbiologically safe product. With regard to simplicity and accuracy, such a method must be comparable with the phosphatase and peroxidase tests.

If ultrasonication was to be used in combination with a mild heat treatment to target the heat-resistant microbes found in milk, the heat would allow the phosphatase enzymes to be inactivated. In this case, the phosphatase test could still be able to be employed as a quick and efficient method to indicate the elimination of spoilage and possible pathogenic bacteria, and therefore, a successful treatment.

Acknowledgements: The authors acknowledge the National Research Foundation of South Africa for funding.

REFERENCES

[1] Aaku E.N., Collison E.K., Gashe B.A., Mpuchane S., Microbiological quality of milk from two processing plants in Gaborone Botswana, Food Control 15 (2004) 181–186.

[2] Ahmed F.I.K., Russell C., Synergism between ultrasonic waves and hydrogen peroxide in the killing of micro-organisms, J. Appl. Bacteriol. 39 (1975) 31–40.

[3] Anonymous, Regulations relating to milk and dairy products. Foodstuffs, Cosmetics and Disinfectant Act, 1972, Act no. 54 of 1972, G.N.R. 1555/1997, Lex Patria Publishers, Johannesburg, South Africa.

[4] Anonymous, Dairy Processing Handbook, Tetra Pak Processing Systems AB, Lund, 2003.

[5] Burger H., Winder W.C., Homogenization and deaeration of milk by ultrasonic waves, J. Dairy Sci. 37 (1954) 645.

[6] Chammas G.I., Saliba R., Corrieu G., Béal C., Characterisation of lactic acid bacteria isolated from fermented milk "laban", Int. J. Food Microbiol. 110 (2006) 52–61.

[7] Ciccolini L., Taillandier P., Wilhem A.M., Delmas H., Strehaiano P., Low frequency thermo-ultrasonication of *Saccharomyces cerevisiae* suspensions: effect of temperature and of ultrasonic power, Chem. Eng. J. 65 (1997) 145–149.

[8] Dewanti R., Wong A.C.L., Influence of culture conditions on biofilm formation by *Escherichia coli* O157:H7, Int. J. Food Microbiol. 26 (1995) 147–164.

[9] Doyle M.P., Glass K.A., Beery J.T., García G.A., Pollard D.J., Schultz R.D., Survival of *Listeria monocytogenes* in milk during high-temperature, short-time pasteurization, Appl. Environ. Microbiol. 53 (1987) 1433–1438.

[10] Efigênia M., Povoa B., Moraes-Santos T., Effect of heat treatment on the nutritional quality of milk proteins, Int. Dairy J. 7 (1997) 609–612.

[11] Fox P.F., Kelly A.L., Indigenous enzymes in milk: overview and historical aspects – Part 1, Int. Dairy J. 16 (2006) 500–516.

[12] Frank J.F., Christen G.L., Bullerman L.B., Tests for groups of microorganisms, in: Marshall R.T. (Ed.), Standard Methods for the Examination of Dairy Products, APHA, Washington DC, 1993, pp. 271–286.

[13] Frölich P.W., Processing of milk and the influence on milk components, New Food 5 (2002) 77–80.

[14] Griffiths M.W., Use of milk enzymes as indices of heat treatment, J. Food Protect. 49 (1986) 696–705.

[15] Guo M.R., Dixon P.H., Park Y.W., Gilmore J.A., Kingstedt P.S., Seasonal changes in the chemical composition of commingled goat milk, J. Dairy Sci. 84 (2001) E79–E83.

[16] Harvey E.N., Loomis A.L., The destruction of luminous bacteria by high frequency sound waves, J. Bacteriol. 17 (1929) 373–376.

[17] Hoover D.G., Minimally processed fruits and vegetables: reducing microbial load by nonthermal physical treatments, Food Technol. 51 (1997) 66–71.

[18] Hülsen U., Alternative heat treatment processes, Eur. Dairy Mag. 3 (1999) 20–24.

[19] Huppertz T., Fox P.F., de Kruif K.G., Kelly A.L., High pressure-induced changes in bovine milk proteins: a review, Biochim. Biophys. Acta 1764 (2006) 593–598.

[20] Huppertz T., Fox P.F., Kelly A.L., High pressure-induced changes in the creaming properties of bovine milk, Innov. Food Sci. Emerg. Technol. 4 (2003) 349–359.

[21] IDF 20B, Milk – determination of nitrogen content, in: Bulletin of the International Dairy Federation, Brussels, Belgium, 1993, pp. 1–4.

[22] IDF 82A, Milk and dried milk, buttermilk and buttermilk powder, whey and whey powder – detection of phosphatase activity, in: Bulletin of the International Dairy Federation, Brussels, Belgium, 1987, pp. 1–3.

[23] Jacobs S.E., Thornley M.J., The lethal action of ultrasonic waves on bacteria suspended in milk and other liquids, J. Appl. Bacteriol. 17 (1954) 38–56.

[24] Lee B.H., Kermasha S., Baker B.E., Thermal, ultrasonic and ultraviolet inactivation of *Salmonella* in thin films of aqueous media and chocolate, Food Microbiol. 6 (1989) 143–152.

[25] Lombardi P., Avallone L., D'angelo A., Mor T., Bogin E., Buffalo-milk enzyme levels, their sensitivity to heat inactivation, and their possible use as markers for pasteurization, J. Food Protect. 63 (2000) 970–973.

[26] Lopez C., Focus on the supramolecular structure of milk fat in dairy products, Reprod. Nutr. Dev. 45 (2005) 497–511.

[27] Ma Y., Ryan C., Barbano D.M., Galton D.M., Rudan M., Boor K., Effects of somatic cell count on quality and shelf-life of pasteurized fluid milk, J. Dairy Sci. 83 (2000) 264–274.

[28] Mason T.J., Power ultrasound in food processing – the way forward, in: Povey M.J.W., Mason T.J. (Eds.), Ultrasound in Food Processing, Blackie Academic & Professional, London, 1998, pp. 105–126.

[29] Munro G.L., Grieve P.A., Kitchen B.J., Effects of mastitis on milk yield, milk composition, processing properties, and yield and quality of milk products, Aust. J. Dairy Technol. 39 (1984) 7–16.

[30] Pagán R., Mañas P., Alvarez I., Condón S., Resistance of *Listeria monocytogenes* to ultrasonic waves under pressure at sublethal (manosonication) and lethal (manothermosonication) temperatures, Food Microbiol. 16 (1999) 139–148.

[31] Pagán R., Mañas P., Raso J., Condón S., Bacterial resistance to ultrasonic waves under pressure at nonlethal (manosonication) and lethal (manothermosonication) temperatures, Appl. Environ. Microbiolol. 65 (1999) 297–300.

[32] Painter C.J., Bradley R.L. Jr., Residual alkaline phosphatase activity in milks subjected to various time/temperature treatments, J. Food Protect. 60 (1997) 525–530.

[33] Pearson L.J., Marth E.H., *Listeria monocytogenes* – threat to a safe food supply: a review, J. Dairy Sci. 73 (1990) 912–928.

[34] Pulina G., Nudda A., Battacone G., Cannas A., Effects of nutrition on the contents of fat, protein, somatic cells, aromatic compounds, and undesirable substances in sheep milk, Anim. Feed Sci. Technol. 131 (2006) 255–291.

[35] Rattray W., Jelen P., Protein standardization of milk and dairy products, Trends Food Sci. Technol. 7 (1996) 227–234.

[36] Robertson N.H., Melk, Dairy Institute, ARC-Elsenburg, Stellenbosch, South Africa, 1999, pp. 1–5.

[37] Santos M.V., Ma Y., Barbano D.M., Effect of somatic cell count on proteolysis and lipolysis in pasteurized fluid milk during shelf-life storage, J. Dairy Sci. 86 (2003) 2491–2503.

[38] Soryal K.A., Zeng S.S., Min B.R., Hart S.P., Beyene F.A., Effect of feeding systems on composition of goat milk and yield of Domiati cheese, Small Ruminant Res. 54 (2004) 121–129.

[39] Stopforth J.D., Samelis J., Sofos J.N., Kendall P.A., Smith G.C., Influence of organic acid concentration on survival of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in beef carcass wash water and on model equipment surfaces, Food Microbiol. 20 (2003) 651–660.

[40] Taylor M.J., Richardson T., Antioxidant activity of skim milk: effect of sonication, J. Dairy Sci. 63 (1980) 1938–1942.

[41] Ternström A., Lindberg A.M., Molin G., Classification of the spoilage flora of raw and pasteurized bovine milk, with special reference to *Pseudomonas* and *Bacillus,* J. Appl. Bacteriol. 75 (1993) 25–34.

[42] Utsunomiya M., Kosaka Y., Application of supersonic waves to foods, J. Facul. Appl. Biol. Sci. Hiroshima University 18 (1979) 225–231.

[43] Van der Westhuizen L., Dairy Institute, ARC-Elsenburg, Stellenbosch, South Africa, 2006, personal communication.

[44] Villamiel M., Arias M., Corzo N., Olano A., Use of different thermal indices to assess the quality of pasteurized milks, Z. Lebensm. Unters. F. A. 208 (1999) 169–171.

[45] Villamiel M., de Jong P., Inactivation of *Pseudomonas fluorescens* and *Streptococcus thermophilus* in trypticase soy broth and total bacteria in milk by continuous-flow ultrasonic treatment and conventional heating, J. Food Eng. 45 (2000) 171–179.

[46] Williams A.G., Withers S.E., Banks J.M., Energy sources of non-starter lactic acid bacteria isolated from Cheddar cheese, Int. Dairy J. 10 (2000) 17–23.

[47] Zeng S.S., Soryal K., Fekadu B., Bah B., Popham T., Predictive formulae for goat cheese yield based on milk composition, Small Ruminant Res. 69 (2007) 180–186.