ISSN: 2347-467X, Vol. 11, No. (3) 2023, Pg. 1153-1165

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

Evaluation of High Hydrostatic Pressure as an Alternative Method for Camel Milk Preservation

FAHAD M ALJASASS[1]*, SIDDIG H HAMAD[2], SALAH M ALEID[2,3] and AHMED A EL NESHWEY[4]

[1]King Abdulaziz City for Science and Technology, Institute of Advanced Agricultural and Food Technologies, Riyadh, Saudi Arabia

[2]Dept. of Food and Nutrition Sciences, College of Agric. and Food Sciences, King Faisal University, Alahsa, Saudi Arabia

[3]Date Palm Research Centre of Excellence, King Faisal University, Alahsa, Saudi Arabia

[4]Dept. of Food Science, College of Agriculture, Zagazig University, Egypt

Abstract

In this work, high hydrostatic pressure was applied to preserve camel milk. It is used as an alternative method to heat treatment which usually damage some nutrient components of milk. Fresh samples were subjected to pressure treatments at 200-600 Megapascals (MPa) for 5 minutes and 40°C. Treatment at 200 MPa reduced microbial contamination up to 0.12 log cycles. The killing effect increased with increased pressure to exceed three log cycles at pressures up to 400 MPa. Gram negative bacteria were more affected by high pressure treatments than gram positive ones. Enterobacteriaceae can be controlled by pressure treatments up to 300 MPa. Pressure treatments up to 350 MPa can cause clotting of camel milk, a phenomenon not observed in cow or goat milk and also not observed in camel milk at heat treatment up to boiling. Camel milk treated at 300 MPa and stored at 3ºC showed no signs of microbial spoilage up to 15 days of storage, while the microbial load of untreated samples stored at the same temperature reached the spoilage level in about a week. High pressure treatment of camel milk resulted in a decrease in its proteolytic activity, but had no significant effect on other chemical attributes such as color, fat oxidation, pH value and the organoleptic characteristics. High hydrostatic pressure up to 300 MPa can be successfully used to preserve camel milk against microbial spoilage. The phenomenon of camel milk clotting at pressures above 300 MPa needs investigation.

Article History

Received: 13 November 2023

Accepted: 14 December 2023

Keywords

Camel Milk;
Chemical Composition;
Clotting;
High Hydrostatic Pressure;
Microbial Contamination.

CONTACT Fahad M Aljasass aljasass@kacst.edu.sa King Abdulaziz City for Science and Technology, Institute of Advanced Agricultural and Food Technologies, Riyadh, Saudi Arabia

Doi: https://dx.doi.org/10.12944/CRNFSJ.11.3.20

Introduction

Currently, high hydrostatic pressure processing (HHPP) is of increasing interest to biological and food systems, primarily because it permits microbial and enzyme inactivation at low or moderate temperatures (Aganovic, 2021, Huang *et al.,* 2020, Heinz and Buckow, 2010, Katsaros *et al.,* 2010, Linton *et al.,* 2004, Polydera *et al.,* 2004, Ritz *et al.,* 2002, Cheftel and Culioli, 1997). The interest in HHPP is due in part to consumers' ever increasing demands for processed foods that are similar to their respective raw materials in terms of color, flavor and texture (Deliza *et al.,* 2005, Ahvenainen, 2000, Gould, 2000, Mandava *et al.,* 1995). High hydrostatic pressure processed products were marketed for the first time in 1991 in Japan, and this technology is applied at present for the processing of various foods including jams, sauces, juices, cakes and desserts (Beresford and Lane, 1999). Many researchers studies the effects of pressure on water and lipid transitions, on the structure and function of proteins and on the activity of microorganisms. Because of its effect on the activity of microorganisms, high-pressure treatment can be used for their control in foods, especially in foods sensitive to other preservation treatments such as heating (Lado and Yousef, 2002, Carlez *et al.,* 1994). The effect of high pressure on the viability of the microorganisms is a combination of factors which cause changes in morphology, genetic makeup, enzyme-mediated cellular functions, cell membranes, cell wall, and spore coats (Campus, 2010, Zare, 2004). Detectable effects of high pressure treatment on microbial cells include an increase in the permeability of cell membranes, possible inhibition of enzymes vital for survival and reproduction of the bacterial cells and ribosome dissociation which has been shown to limit cell viability at high pressure (Campus, 2010 and Simonin *et al,* 2012). In most cases, the effect of high pressure treatment on gram-positive bacteria is less pronounced than on gram-negative species (Kadam *et al.,* 2012). Cells in the stationary phase of growth are more resistant to high pressure treatment than cells in the exponential phase and bacterial spores are always more resistant than vegetative cells (Trujillo *et al.,* 2002). The chemical composition and properties of camel milk have also been recently reviewed in greater details by Rahmeh *et al.* (2018).

High hydrostatic pressure treatment of milk improved its microbiological quality and extended its shelf life (Garcia-Risco *et al.,* 1998, Lopez-Fandino *et al.,* 1996, Patterson *et al.,* 1995). However, this treatment induced changes in milk constituents (Huppertz *et al.,* 2006). Milk constituents affected by HHPP treatment are minerals, casein and whey proteins (Huppertz *et al.,* 2006). The mineral balance of milk changes as a result of HHPP treatment. The concentration of ionic calcium in milk increased after HHPP treatment (Zobrist *et al.,* 2005, Lopez-Fandino *et al.,* 1996). However, the extent of this increase was variable because HHPP- induced ionization of calcium was reversed rapidly on subsequent storage of milk (Zobrist *et al.,* 2005). The level of calcium and phosphate in the serum phase of milk increased after HHPP treatment, with the maximum increase occurring at 300 MPa (Lopez-Fandino *et al.,* 1996). Milk pH increased as a result of the increase of phosphate in the milk serum. Generally, all of these increases are irreversible when milk is subsequently stored at 5°C, but they disappear at a storage temperature of 20°C (Zobrist *et al.,* 2005). High hydrostatic pressure treatment also induced changes in casein micelles, including their size, their number per unit volume, and their content of casein (Huppertz *et al.,* 2006). High hydrostatic pressure processing causes the disruption of casein micelles and the subsequent dissociation of caseins from the micelle. This effect is attributed to a probable calcium phosphate solubilization in the micelle in addition to disruption of intramicellar hydrophobic and electrostatic interactions (Huppertz *et al.,* 2004a, Huppertz *et al.,* 2004b, Schrader *et al.,* 1997). Camels are by far the most important farm animals traditionally reared in Saudi Arabia, and their milk is very popular among Saudis. It is consumed as fresh milk or processed into different products including the famous traditionally fermented product “IGT”. Camel milk contains many compounds of nutritional and health values, most of which can be destroyed if heating is used for preservation. Therefore, the aim of this work was to use high hydrostatic pressure technology as an alternative method in the preservation of camel milk under the environmental conditions of the Kingdom of Saudi Arabia.

Materials and Methods

Sample Collection and Pressure Treatment of Camel Milk

Camel milk samples were collected in sterile bottles from farms at the periphery of Hofuf City and

pressure treated on the same day. Portions (15 ml) of four freshly milked samples from different animals at different lactation periods (1.5-3 months) were packed into polyethylene pouches (oxygen permeability 50 cm3/m2/24h at 1 bar, 23°C and 0% relative humidity, Somerville Packing, Lisburn, N. Ireland) and evacuated using a Tower-Vac machine (Tower Industry Co, Ltd, Korea). Pressure treatments were 200, 250, 300, 350, 400, 450, 500 and 600 MPa at 40°C for 5 minutes, which depended on values obtained from personal trails and from literature cited. Microbiological, chemical and physical analysis was conducted before and after treatment and then during storage at 3°C for several days.

Samples were HHPP treated in a Stansted 'FOOD-LAB' model S-FL-850-9-W high hydrostatic pressure research apparatus (Stansted Fluid Power Ltd., Stansted, UK) with maximum working pressure of 900 MPa (9000 bar, 130300 psi). Usable diameter and height inside product basket were 37 and 300 mm, respectively. Working fluid Cool Flow MPG (Hydratech, UK). Fixed and detachable thermocouples monitor temperature of working fluid and sample, respectively. Pressure come-up time was about 200 MPa/minute and pressure release time about 5 seconds/100 MPa. Temperature increase due to adiabatic heating was about 2.5°C/100 MPa. Temperature ranges were -20°C to +90°C.

Analytical Methods

Microbiological Analysis

One ml of camel milk sample was added to 9 ml sterile peptone water. Serial dilutions (if necessary) were prepared in test tubes containing 9 ml sterile peptone water and aliquots (1.0 or 0.1 ml) plated out in duplicate. Total bacterial count on Plate Count Agar dishes (PCA Oxoid, CM0325) using the pour plate method. The plates incubated at 30˚C for 2 to 3 days and the counts expressed as colony forming units per ml (cfu/ml) of the sample. The gram reaction of bacteria was examined using 3% KOH. A loopful from a colony was mixed in a drop of KOH on a slide. Formation of slimy threads means gram negative reaction. In doubtful cases a gram stain was performed. To determine the percentage of gram positive and gram negative bacteria in a population, all colonies in a dish containing about 30 colonies were tested.

Chemical Analysis

Thiobarbituric Acid Value

was determined according to the method described by Wrolstad *et al.* (2005).

Physical Analysis

Color

Color measurements were made with a Hunterlab Color MiniScanEZ /4500L (USA) color difference meter standardized with black and green tiles. The measured parameters were the degree of lightness (L*), redness (a*), and yellowness (b*).

Organoleptic Evaluation

The evaluation was done by eight untrained panelists. The milk sample was treated at 250, 300 and 350 MPa, 40°C for 5 minutes. Panelists were asked to tell whether there were differences in taste and color between samples and to determine which sample has typical camel's milk taste and color if differences were detected. The evaluation was done using the 9-point hedonic scale (Lim, 2011).

Statistical Analysis

Analysis of variance (Steel and Torrie1980) of the data collected performed in a randomized complete block design (RCBD). Significant differences was considered when p-value is (P>0.05) for the tabulated mean values using Duncan's Multiple Range test.

Results and Discussions

High Hydrostatic Pressure Treatment of Camel Milk

Results obtained from preliminary experiments showed that the main concerns regarding the microbial contamination of camel milk are the total bacterial counts. The levels of contamination with other microbial groups such as *Enterobacteriaceae,* molds and yeasts were relatively low. Hence, optimization experiments were mainly concentrated on the total bacterial counts. Another concern was the effect of HP treatment on milk clotting.

Effect of HP on the total Bacterial Count

The results of the effect of HP on the total bacterial count are shown in Tables 1 and 2. In general, the total bacterial count in these raw camel milk samples was low compared to values reported for

raw cow milk, with an average of 105 cfu/ml, (Griffins, 2000). The first sample was found contaminated with $2.3x10^3$ cfu/ml. Treatment at 200, 250 and 300 MPa reduced this load by 0.06, 0.28 and 0.46 log cycles, respectively. However, clotting appeared starting from 350 MPa treatment, and the level of contamination continued to decrease by 0.5, 0.61 and 0.8 log cycles for the treatments 350, 400 and 450 MPa, respectively (Table 1). The second sample was initially contaminated with 9.9x102 cfu/ml. Treatment at 200 MPa reduced this level by 0.12 log cycles and reduction continued to reach 1.46 log cycles at 350 MPa with a remaining load of only 35 cfu/ml. The treatments at 400 to 600 MPa reduced contamination to non-detectable levels representing about three log cycles but the milk clotted. The third and fourth samples were treated at 300, 350 and 400 MPa, and clotting in both of them occurred at the treatment of 400 MPa. The third sample was initially contaminated with $1.4x10^3$ cfu/ml and the 300 MPa treatment reduced this load by 0.63 log cycles. In case of the 350 and 400 MPa treatments the load was reduced by 0.97 and 1.15 log cycles, respectively and $1.0x10^2$ cfu/ml remained unaffected. Treatment of the fourth sample at 300 MPa reduced the load of mesophilic aerobic bacteria from $1.7x10^3$ cfu/ml before treatment by 1.23 log cycles. The 350 and 400 treatments further reduced the load by 1.35 and 1.43 log cycles, respectively and only 62 cfu/ml remained unaffected.

Table 1: Effect of different pressure treatments on the total bacterial count and on clotting of samples of fresh camel milk treated at 40°C for 5 minutes

Pressure (MPa)	Mesophilic aerobic bacteria (cfu/ml)	Reduction (log cycles)	Note
Sample 1			
0	$2.3x10^3$		No clotting
200	$2.0x10^3$	0.06	No clotting
250	$1.2x10^3$	0.28	No clotting
300	$8.0x10^2$	0.46	No Clotting
350	$7.2x10^2$	0.5	Clotting
400	$5.6x10^2$	0.61	Clotting
450	$3.6x10^2$	0.8	Clotting
Sample 2			
0	$9.9x10^2$		No Clotting
200	$7.5x10^2$	0.12	No Clotting
250	$3.6x10^2$	0.44	No Clotting
300	90	1.05	No Clotting
350	35	1.46	No Clotting
400-600	n.d.	>3.0	Clotting
Sample 3			
0	$1.4x10^3$		No Clotting
300	$3.3x10^2$	0.63	No Clotting
350	$1.5x10^2$	0.97	No Clotting
400	$1.0x10^2$	1.15	Clotting
Sample 4			
0	$1.7x10^3$		No Clotting
300	$1.0x10^2$	1.23	No Clotting
350	75	1.35	No Clotting
400	62	1.43	Clotting

Table 2: Reduction in total bacterial count in 4 fresh camel milk samples treated at different levels of pressure at 40°c for 5 minutes (initial loads were $9.9x10^2$, $1.4x10^3$, $1.7x10^3$ and $2.3x10^3$ cfu/ml)

Pressure (MPa)	Reduction (log cycles)
200	0.06-0.12
250	0.28-0.44
300	0.46-1.23
350	0.50-1.46
400	0.61- >3.0
450	0.80- >3.0
500	>3.0
600	>3.0

Reduction in loads of mesophilic aerobic bacteria in camel milk resulting from high pressure treatment varied for each pressure level (Table 2). It seems that differences in effect depend mainly on the types of contaminating bacteria. It was observed that high pressure treatment kills mainly gram negative bacteria while gram positive ones are less affected, this is in agreement with reports made by many researchers (Naik *et al.,* 2013; Kadam *et al.,* 2012, Chawla *et al.,* 2011). Examination of the bacterial flora of sample 1 before pressure treatment showed that it was made of about 10% gram negative and 90% gram positive bacteria. Pressure treatment up to 450 MPa reduced the load from $2.3x10^3$ to $3.6x10^2$ cfu/ml, i.e. by 0.8 log cycles only, and the remaining flora was about 100% gram positive bacteria (results not shown). It is obvious that this treatment killed all population of gram negative bacteria but only part of the population of the gram positive ones. On the other hand, the flora of sample 2 was made of about 80% gram negative and 20% gram positive bacteria before treatment. Pressure treatment at 300 MPa reduced the load from $9.9x10^2$ to 90 cfu/ml, and the remaining flora was 100% gram positive bacteria. An increase of pressure treatment to 400 MPa reduced the load to non-detectable levels (Table 1), unlike the case of the bacteria of sample 1 which resisted pressures up to 450 MPa. This indicates that gram positive bacteria have varying degrees of resistance to high pressure treatment. This observation is supported by reports from other researchers. For example, Pásztor-Huszár (2008) reported that a treatment at 600 MPa for 8 minutes reduced contamination of milk with the gram positive bacterium *Listeria monocytogenes* by 7 log cycles, whereas a treatment of 600 MPa for 10 minutes reduced contamination of milk with the gram positive bacterium *Staphylococcus aureus* by only 1.5 log cycles. Similar effects were noticed in samples 3 and 4, which were mainly contaminated with gram positive bacteria and pressure treatment up to 400 MPa didn't result in a complete removal of bacterial contamination. The maximum level of reduction reached by a 200 MPa treatment was 0.12 log cycles. The level of reduction increased up to 0.44 log cycles at 250 MPa and reached up to 1.23 and 1.46 log cycles at 300 and 350 MPa, respectively (Table 2). Treatments up to 400 MPa resulted in reductions up to 3.0 log cycles. From these results, it can be concluded that treatments at 300 to 350 MPa will be enough to reduce contamination with mesophilic aerobic bacteria in camel milk to the levels of 10^2 cfu/ml or less. Trujillo *et al.* (1999) and Buffa *et al.* (2001) reported that HHP treatment of 500 MPa at 20°C for 15 minutes produced an effect equivalent to HTST pasteurization at 72°C for 15 seconds in the reduction of microbial contamination of goat's milk. According to Drake *et al.* (1997) pasteurization and high pressure treatments produced comparable results in the control of microbial contamination of milks and in cheeses made from these milks. Lado and Yousef (2002) reported a 5.9 log cycle reduction in contamination with E. coli in milk after high pressure treatment at 500 MPa and 25°C for 5 minutes.

Effect of HP on Milk Clotting

Clotting in three out of the four camel milk samples tested occurred at 400 MPa, while it occurred at 350 MPa in one sample, which had the highest initial microbial load. The phenomenon of clotting after high pressure treatment was not found in milks of cows or goats. The possible explanation for this phenomenon could be linked to the proteins and minerals of camel milk. It is known that HP treatment of bovine milk destabilizes the casein micelles resulting in a reduction in the average casein micelles diameter (Huppertz *et al.,* 2006, Huppertz *et al.,* 2004a, b; Anema *et al.,* 2008; Orlien *et al.,* 2006). Abo-Tarboush (1994) indicated that the protein content was relatively lower and the ash content was substantially higher in camel milk than

cow milk. The mean casein content of camel milk (1.9 to 2.04%) is lower than that of cow milk (2.58 to 2.68%) as reported by Mehaia and Al-kanhal (1992) and Mehaia *et al.* (1995). The casein fraction constituted about 61% to 71% of the crude protein in camel milk compared to 78% in cow milk (Mehaia and Al-kanhal,1992; Mehaia *et al.,* 1995). Moreover, Larsson-Raznikiewicz and Mohamed (1986) found that each of the four main casein fractions in cow milk appeared to have their counterparts in camel milk; however, they showed obvious differences in the two milks. Therefore, these differences between the two milks could be responsible for their behavior regarding clotting as a result of HP treatment. To clarify this point, camel milk samples from different animals were tested at different lactation stages and in the hot summer months (45-50ºC) and the cold winter months (below 10 ºC) and no clear trends were found, although clotting occurred more frequently in summer. Camel milk was also heated to boiling point in this study and no clotting occurred as a result of this treatment, which means that this phenomenon is linked only to HP. Moreover, cow and goat milk were also treated with HP up to 600 MPa and no clotting was observed indicating that the phenomenon is seen in camel milk only. Huppertz *et al.* (2005) reported that application of 100 or 250 MPa treatments to buffalo milk caused no denaturation of α-lg, while application of 400, 600 or 800 MPa treatments caused denaturation of about 6%, more than 50% and more than 90% of α-lg, respectively. β-lg of buffalo milk is not denatured at a treatment of 100 MPa, more than 85% denatured at 250 MPa and practically all of it is denatured at 400-800 MPa. According to Gaucheron *et al.* (1997) treatment of milk at 400 MPa resulted in the denaturation of up to 90% of total β-lg.

Microbial Load of Camel Milk Stored At 3°C After HP Treatment

Portions of the four samples treated at 300 MPs together with untreated portions were stored at 3°C. Total bacterial count (incubation at 7, 15 and 30°C for 2 to 7 days) and counts of Enterobacteriaceae (incubation at 37°C for 24 hours) were determined during storage. In all samples the bacteria gave higher counts at incubation temperatures of 15 and 30°C than at 7°C, indicating that they were either mesophilic or psychrotrophic rather than psychrophilic.

The initial total bacterial count of sample one was $2.3x10^3$ cfu/ml. This load increased in the untreated samples to $3.7x10^4$ cfu/ml at day 5 of storage and reached the spoilage level of $5.7x10^5$ cfu/ml (Jay *et al,* 2005) at day 11 to increase further to $6.3x10^7$ cfu/ml at day 15 (Fig. 1). This indicates that the contaminating bacteria were mostly psychrotrophic, which could grow fast and cause milk spoilage.

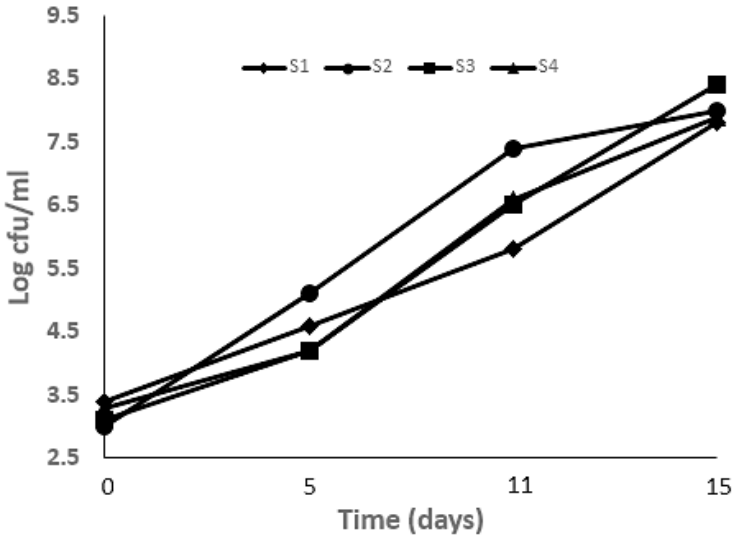

Fig.1: Total bacterial count in untreated camel mlk stored at 3°C. (S1 to S4 = Sample 1 to 4

Pressure treatment reduced the microbial load from the initial 2.3x103 to 8.0x102 cfu/ml. This load decreased continuously to $5.8x10^2$, $3.3x10^2$ and $2.6x10^2$ cfu/ml at days 5, 11 and 15, respectively (Fig. 2). It is apparent that these psychrotrophic bacteria could not grow after pressure treatment. This indicates that the bacteria, though not completely killed by the pressure treatment, were so strongly damaged that they could not grow at cold storage.

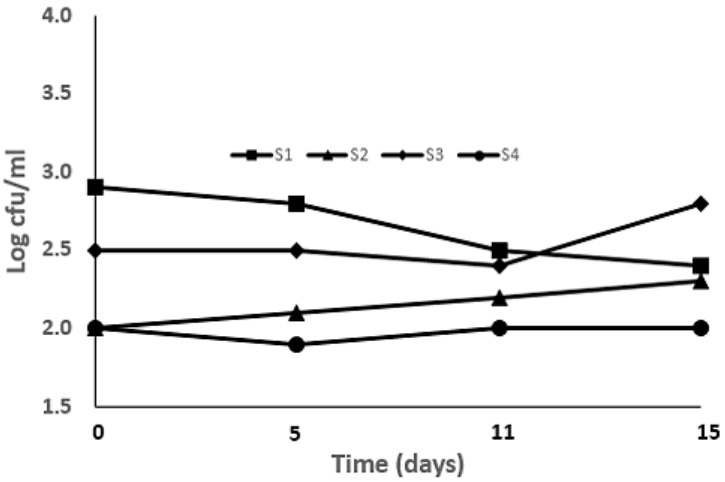

Fig. 2: Total bacterial count in camel mlk treated at 300 MPa and stored at 3°C. (S1 to S4 = Sample 1 to 4

The sample was initially contaminated with Enterobacteriaceae at a load of $3.5x10^3$ cfu/ml (Fig. 3). The load increased slightly to $1.0x10^4$ cfu/ml during the first week of storage then decreased till it reached $1.0x10^2$ cfu/ml at the end of the 15 days storage period. This indicates that Enterobacteriaceae are not able to grow in camel milk stored in the cold. The pressure treatments reduced contamination with Enterobacteriaceae to non-detectable levels and no increase in this load was observed during storage (results not shown). It can, therefore, be concluded that a pressure treatment of 300 MPa was enough to control contamination of camel milk with Enterobacteriaceae.

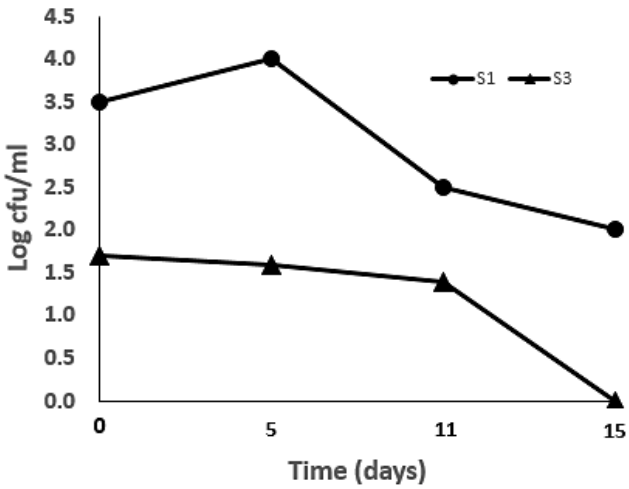

Fig. 3: Enterobacteriaceae in untreated camel mlk stored at 3°C. (S1 to S3 = Sample 1 to 3

Sample 2 was contaminated with $9.9x10^2$ cfu/ml. This load increased steadily in the untreated sample to $1.4x10^5$ cfu/ml at day 5 and reached the spoilage levels of $2.3x10^7$ and $1.1x10^8$ cfu/ml at days 11 and 15, respectively (Fig. 1). It appears that the population contaminating this sample was mostly psychrotrophic, fast growing bacteria. Pressure treatment reduced microbial contamination to approximately $2.0x10^2$ cfu/ml, which then increased slightly to $1.2x10^2$, $1.7x10^2$, and $2.2x10^2$ cfu/ml at days 5, 11 and 15, respectively (Fig. 2). The microbial contamination of this sample was initially low, and the pressure treatment reduced it to very low levels. Again, the population that remained after treatment practically did not show growth indicating that it suffered strong sub-lethal injury by the treatment. The sample was free of detectable contamination with Enterobacteriaceae.

Sample 3 was contaminated with $1.4x10^3$ cfu/ml (Fig. 1). The amount of this contamination increased steadily in storage to reach $1.5x10^4$ cfu/ml after 5 days and then the spoilage levels of $3.0x10^6$ and $2.8x10^8$ cfu/ml at days 9 and 14, respectively (Fig. 1). The population was therefore mostly psychrotrophic bacteria. Pressure treatment reduced the load to $3.3x10^2$ cfu/ml (Fig. 1). This population remained almost constant until day 9 to increase after that slightly to $8.1x10^2$ at day 14 (Fig. 2). It can be concluded that the sample was contaminated with bacterial population mostly made of psychrotrophic bacteria which showed some resistance to pressure treatment. Still, the population that remained after treatment was not able to grow and caused spoilage because it was probably strongly damaged by the treatment. The sample was also found to be contaminated with Enterobacteriaceae at 55 cfu/ml. This population decreased steadily in the untreated sample during storage to reach a non-detectable level at day 14 (Fig. 3). This is another proof that Enterobacteriaceae do not grow in camel milk stored in the cold. The pressure treatments reduced the population of Enterobacteriaceae in this sample to non-detectable levels, which is another confirmation that this group of bacteria, like most gram negative bacteria, is very sensitive to high pressure treatments.

Sample 4 was contaminated with $1.9x10^3$ cfu/ml. This load increased in the untreated sample to $1.6x10^4$ cfu/ml at day 4 and reached the spoilage levels of $3.9x10^6$ and $9.0x10^7$ cfu/ml at days 9 and 14, respectively (Fig. 1). This is a trend similar to cases of other samples discussed above, i.e. the sample was contaminated with mostly psychrotrophic bacteria which could grow fast and caused quick milk spoilage. Pressure treatment reduced the load to $1.0x10^2$ cfu/ml. This remaining population was not able to grow and caused milk spoilage during the cold storage of 14 days (Fig. 2). The sample was free of detectable contamination with Enterobacteriaceae.

These results indicate that camel milk stored in the refrigerator at around 3°C can be spoiled by psychrotrophic bacteria which frequently contaminate it. In most of the samples examined the initial population of contaminating bacteria was relatively low, lying in the range of 10^3 cfu/ml. High pressure treatment up at 300 for 5 minutes was enough to substantially reduce this level of contamination. In addition, the remaining population was probably strongly injured that none could grow, and cause milk spoilage stored at 3°C. Eszter (2009) reported that high pressure treatment up to 400 MPa reduced the mesophilic aerobic count of milk by several log cycles, and that the shelf-life of the high pressure treated milk was longer than that of heat treated milk. Initial levels of 5–6 log of non-starter lactic acid bacteria in raw milk were reduced by 3.66 logs after high pressure treatment at 600 MPa (Voigt *et al.* 2012).

Effect Of HP Treatment on the Chemical Properties of Camel Milk

Proteolytic Activity

HP treatment caused a reduction in the proteolytic activity of camel milk. The untreated milk sample in this study contained 4.23 µM/ml proteolytic enzymes. Treatments at 250 and 350 MPa significantly reduced this content to 3.61 and 2.98 µM/ml, respectively, whereas the treatment at 300 MPa reduced the content to 3.90 µM/ml, which was not significantly different from the content of the untreated sample (Table 3). This means that HP treatment can prevent or delay milk spoilage due to proteolysis caused either by microbial enzymes or enzymes naturally found in milk. The treatment can destroy heat-sable proteinases such as the alkaline milk proteinases and proteinases produced by psychrotrophic microorganisms such as Pseudomonas sp. Scollard *et al.* (2000) observed that treatments of milk at 300

MPa and above reduced the proteolytic activity, and that a treatment of 600 MPa for 30 minutes reduced plasmin activity to 40% of its value before treatment. According to Hayes and Kelly (2003) HP treatment of milk destroys plasmin and plasminogen-derived activity. Moreover, Juan *et al.* (2007) stated that treatment of milk at 500 MPa decelerate the proteolysis of cheeses due to a reduction of microbial population and inactivation of enzymes.

Table 3: Effect of HP treatment on the proteolytic activity, thiobarbituric acid (TBA) value and pH of camel milk.

Pressure (MPa)	Protease content (µM/ml)	TBA value (mgMA/kg)	pH
u	4.23^{a}±0.26	0.86^{b}±0.04	6.63^{b}±0.02
250	3.61^{b}±0.22	0.86^{b}±0.06	6.70^{a}±0.03
300	3.90^{ab}±0.28	1.25^{a}±0.15	6.68^{ab}±0.03
350	2.98^{c}±0.22	1.33^{a}±0.10	6.70^{a}±0.03

U = untreated, TBA = thiobarbituric acid, MA = malonaldehyde. Means in a column followed by different letters are significantly different ($p<0.05$)

Thiobarbituric Acid (TBA)

Treatment of camel milk at pressures above 250 MPa resulted in the oxidation of its fat. The TBA value of the milk samples before pressure treatments was 0.86 mg malonaldehyde/kg milk. Treatment at 250 MPa caused no significant change in this content, but treatments at 300 and 350 MPa resulted in significant increases to 1.25 and 1.33 mg/kg, respectively (Table 2). However, the level in all treatments was always lower than the 3.0 mg/kg considered acceptable in food products (Zare 2004).

pH

A camel milk sample of pH 6.63 was exposed to HP treatments. The treatments at 250 and 350 MPa increased this pH significantly to 6.70, while the treatment at 300 MPa caused an insignificant increase (Table 3). Altuner *et al* (2006) reported similar results for cow milk. The pH of untreated cow milk reported by these authors was 6.38, which increased to 6.41, 6.46 and 6.45 after treatments at 220, 330 and 440 MPa, respectively.

Effect Of HP Treatment on Color Properties of Camel Milk

HP treatment caused some changes in the color of camel milk as reflected in changes in the Hunter color scale (L*, a* and b*). The changes were statistically significant only for yellowness (b* value). Lightness (L* value) increased from 88.15 for the untreated sample to 90.61 for the sample treated at 250 MPa, i.e. ΔL* was +2.46 (Table 4). In case of the sample treated at 300 MPa, L* value increased to 89.22, i.e. ΔL* was only +1.07 which was less than the effect of the 250 MPa treatment. The L* value of the sample treated at 350 MPa decreased slightly in comparison to that of the untreated sample, i.e. ΔL* was -0.22. It can therefore be concluded that HP treatments at values up to 300 MPa will slightly increase milk lightness, while further increase in pressure will reverse the effect and will lead to a slight decrease in its lightness. A drop in L* value is mainly caused by the fragmentation of casein micelles by pressure leading to an increase in the translucency of the milk (Gervilla *et al.,* 2001). On the other hand, a small increase in the greenness (a* value) of camel's milk occurred after HP treatment. The a* value of the samples treated at 250, 300 and 350 MPa decreased from -1.17 for the untreated sample to -1.26, -1.21 and -1.30, respectively. Δa* of the sample treated at 250 MPa was -0.09, treatment at 300 MPa reduced this value to -0.04 while treatment at 350 MPa increased it to -0.13 (Table 2). HP treatment caused a significant increase in the yellowness (b* value) of camel milk (Table 3). The b* value of the untreated sample was 1.40, treatments at 250, 300 and 350 MPa, increased this value to 2.73, 2.31 and 2.18, respectively. Treatments at 250, 300 and 350 MPa reduced Δb* value of the milk sample to +1.33, +0.91 and +0.78,

respectively (Table 3). This effect was similar to the effect on ΔL* value which decreased with increasing pressure. The total color difference (ΔE) decreased from 2.80 at the 250 MPa treatment to 1.41 at 300 MPa and to 0.82 at 350 MPa (Table 3). Eszter (2009) reported that the color of bovine milk was not affected by high hydrostatic pressure processing at a large extent. Ewe milk color was found to be slightly affected by HP treatment (Gervilla *et al.* 2001). The b* value of untreated ewe milk reported by Gervilla *et al* (2001) was much higher than the one recorded in our study for camel milk, scoring 9.88 for ewe compared to 1.4 for camel milk. The HP treatment caused an increase in the b* value of ewe milk up to 11.26 at 500 MPa. In contrast to our findings for camel milk, the total color difference (ΔE) of ewe milk increased from 0.13 at 100 MPa to 3.40 at 500 MPa, whereas it decreased in camel milk from 2.8 at 250 MPa to 0.82 at 350 MPa.

Table 4: Effect of HP on the color of camel milk (40°C, 5 min). Mean ± SD

Pressure (MPa)	L*	ΔL*	a*	Δa*	b*	Δb*	ΔE
U	88.15^{a}±1.45		- 1.17^{a}±0.03		1.40^{b}±0.18		
250	90.61^{a}±2.89	2.46	- 1.26^{a}±0.04	-0.09	2.73^{a}±0.27	1.33	2.8
300	89.22^{a}±2.21	1.07	- 1.21^{a}±0.06	-0.04	2.31^{a}±0.49	0.91	1.41
350	87.93^{a}±2.87	-0.22	- 1.30^{a}±0.07	-0.13	2.18^{a}±0.32	0.78	0.82

U = untreated. L*, a* and b* = Hunter color scale. Means in a column followed by different letters are significantly different ($p<0.05$)

Effect of HP Treatment on Sensory Properties of Camel's Milk

Regarding acceptability, the sensory properties namely color and taste differ significantly from the untreated sample. Five panelists found all samples have typical camel milk color. Two panelists found the untreated sample and the samples treated at 250 and 300 to have typical camel milk color. One panelist found only the sample treated at 300 MPa to have typical camel milk color. Two panelists found all samples to have typical camel milk taste. Four panelists found the untreated sample and the samples treated at 250 and 300 MPa to have typical camel milk taste. One panelist found untreated sample and the sample treated at 250 MPa to have typical camel milk taste. One panelist found only the sample treated at 250 MPa to have typical camel milk taste.

Conclusion

It can be concluded that HP treatment could be used as an alternative method for camel milk preservation to avoid damage of some nutrient components of milk caused by heat preservation. A treatment at 300 MPa for 5 minutes at 40°C reduced the total bacterial count of milk to less than 10^3 cfu/ml and this count remained almost constant for 15 days in samples stored at 3°C. The microbial load of untreated samples increased quickly to reach spoilage level in about one week. The chemical composition and organoleptic characteristics of the treated milk were not significantly affected. The treatment also reduced contamination with Enterobacteriaceae to non-detectable levels. Treatments at 350 MPa caused milk clotting.

Acknowledgement

The authors are grateful to King Abdul Aziz City for Science and Technology (KACST), Saudi Arabia for its financial support through the grant for the project number ARP-29-225.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The authors do not have any conflict of interest.

References

1. Abu-Tarboush, H. M. (1994). Growth behavior of Lactobacillus acidophilus and biochemical characteristics and acceptability of acidophilus milk made from camel milk. Milchwissenschaft. 49:379-382.
2. Aganovic, K., Hertel, C., Vogel, R. F., Johne, R., Schlüter, O., Schwarzenbolz, U., Jäger, H., Holzhauser, T., Bergmair, J., Roth, A., Sevenich, R., Bandick, N., Kulling, S. E., Knorr, D., Engel, Karl-Heinz, Heinz, Volker. (2021). Aspects of high hydrostatic pressure food processing: Perspectives on technology and food safety. *Compr. Rev Food Sci. Food Safety.* 20:3225–3266
3. Ahvenainen R. (2000): Ready-to-use fruit and vegetables. Flair-Flow Europe, Technical Manual 376A/00. http://www.teagasc.
4. Altuner EM, Alpas H, Erdem YK, Bozoglu F. (2006): Effect of high hydrostatic pressure on physicochemical and biochemical properties of milk. *Eur Food Res Technol.,* Vol. 222:392–396.
5. Anema, S. G. (2008). Effect of milk solids concentration on whey protein denaturation, particle size changes and solubilization of casein in high- pressure treated skim milk. *International Dairy Journal.* Vol. 18:228-235.
6. Beresford T. and Lane, C. (1999): High pressure processing of dairy foods. End of project report: DPRC No. 22. (http://www.teagasc.ie/research/reports).
7. Buffa, M., Guamis, B., Royo, C. and Trujillo, A.J. (2001) Microbiological changes throughout ripening of goat cheese made from raw, pasteurized and high-pressure-treated milk. *Food Microbiology,* Vol. 18:45-51.
8. Campus, Marco (2010): High pressure processing of meat, meat products and seafood. *Food Engineering Review,* Vol. 2:256–273.
9. Carlez, A., Rosec, J.P., Richard, N. and Cheftel, J.C., (1994). Bacterial Growth during chilled storage of pressure treated minced meat. *Lebensmittal Wiss u Technologie,* Vol. 27:48–54.
10. Chawla R., G. R. Patil and A. K. Singh (2011): High hydrostatic pressure technology in dairy processing: a review. *Journal of Food Science and Technology,* Vol. 48:260–268.
11. Cheftel, J.C. and Culioli, J., (1997). Effects of high pressure on meat: A review. *Meat Science,* Vol. 46:211–234.
12. Deliza R., A. Rosental, F.B.D. Abadio, C.H.O. Silva and C. Castillo (2005): Application of high pressure technology in the fruit juice processing: benefits perceived by consumers. *Journal of Food Engineering,* Vol. 67:241-246.
13. Drake, M.A., Harrison, S.L., Asplund, M., Barbosa-Canovas, G. and Swanson, B.G. (1997) High pressure treatment of milk and effects on microbiological and sensory quality of cheddar cheese. *Journal of Food Science,* Vol. 62:843-845.
14. Eszter, Kálmánné Tuboly (2009): Effects of the use of high hydrostatic pressure technology on the microbiology and other quality parameters of some selected foodstuffs. PhD thesis, Corvinus University of Budapest, Faculty of Food Science, Department of Refrigeration and Livestock Products Technology.
15. Garcia-Risco, M. R., E. Cortes, A. V. Carrascosa and R. Lopez-Fandino, (1998): Microbiological and chemical changes in high-pressure-treated milk during refrigerated storage. *Journal of Food Protection,* Vol. 61:735–737.
16. Gaucheron F., M. H. Famelart, F. Mariette, K. Raulot, F Michel and Y. Le Graet, (1997): Combined effects of temperature and high-pressure treatments on physicochemical characteristics of skim milk. *Food Chemistry,* Vol. 59:439–447.
17. Gervilla, R., V. Ferragut, and B. Guamis (2001): High hydrostatic pressure effects on color and milk-fat globule of ewe's milk. Journal of Food Science, Vol. 66: 880-885.
18. Gould G.W. (2000) Strategies for food preservation. In The Microbiological Safety and Quality of Food Vol. 1 (eds. Lund, B.M., Baird-Parker, T.C., and Gould, G.W.). Aspen Publishers, *Inc., Gaithersburg, Maryland.* Pp. 19-35.

19. Griffin, Michael (2000): Value added dairy products: an international perspective. Paper presented at: North Atlantic Treaty Organization advanced research workshop on "economics of the dairy industry in Central and Eastern Europe", Rydzyna, Poland.
20. Hayes, M. G. and A. L Kelly (2003). High pressure homogenization of milk (b) effects on indigenous enzymatic activity. *Journal of Dairy Research,* Vol. 70:307-313.
21. Heinz V. and Roman Buckow (2010): Food preservation by high pressure. J. Verbr. Lebensm, Vol. 5:73-81.
22. Huang, H.W., Hsu, C.P., and Wang C.Y. (2020): Healthy expectations of high hydrostatic pressure treatment in food processing industry. *Journal of Food and Drug Analysis,* Vol. 28:1-13.
23. Huppertz, T., M. A Smiddy, V. K. Upadhyay and A. L. Kelly (2006): High-pressure-induced changes in bovine milk: a review. *International Journal of Dairy Technology,* Vol. 59:58-66.
24. Huppertz, T., Zobrist, M.R., Uniacke, T., Upadhyay, V., Fox, P.F. & Kelly, A.L. (2005). Effects of high pressure on some constituents and properties of buffalo milk. *Journal of Dairy Research,* Vol. 72: 226–233.
25. Huppertz, T., P. F. Fox and A. L. Kelly (2004a): High-pressure treatment of bovine milk: effects on casein micelles and whey proteins. *Journal of Dairy Research,* Vol. 71:97–106.
26. Huppertz, T., P. F. Fox and A. L. Kelly (2004b). High-pressure induced denaturation of α-lactalbumin and β-lactoglobulin in bovine milk and whey: a possible mechanism. *Journal of Dairy Research,* Vol. 71:489–495.
27. Jay, J.M., M.J. Loessner and D.A. Golden (2005): Modern Food Microbiology, 7th edition. Springer Science+Business Media Inc. New York. P. 82. http://books.google.com.
28. Juan, B., V. ferragut, M. Buffa, B. Guamis and A. J. Trujillo (2007). Effects of high pressure on proteolytic enzymes in cheese: relationship with the proteolytic of ewe milk cheese. *Journal of Dairy Science,* Vol. 90:2113-2125.
29. Kadam PS, Jadhav BA, Salve RV, Machewad GM (2012): Review on the High Pressure Technology (HPT) for Food Preservation. *Journal of Food Processing Technology,* Vol. 3:135-139. DOI: 10.4172/2157-7110.1000135.
30. Katsaros, G. I., G. Tavantzis, and P. S. Taoukis (2010): Production of novel dairy products using actnidin and high pressure as enzyme activity regulator. *Innovative Food Science and Emerging Technologies,* Vol. 28:47-51.
31. Lado, Beatrice H. and Ahmed E. Yousef (2002). Alternative food-preservation technologies: *efficacy and mechanisms. Microbes and Infection,* Vol. 4:433-440.
32. Larsson-Raznikiewicz, M. and Mohamed, M. A. (1986). Analysis of casein content in camel (Camelus dromedarius) milk. *Swedish Journal of Agricultural Research,* Vol. 16:13 18.
33. Lim, J. (2011). Hedonic scaling: A review of methods and theory. Food Quality and Preference, Vol. 22: 733-747.
34. Linton, M., J. M. J. McClements, and M. F. Patterson (2004). Changes in the microbiological quality of vacuum- packaged, minced chicken treated with high hydrostatic pressure. *Innovative Food Science and Emerging Technologies,* Vol. 5:151-159.
35. Lopez-Fandino, R., A. V. Carrascosa and A. Olano (1996). The effects of high pressure on whey protein denaturation and cheese-making properties of raw milk. *Journal of Dairy Science.* 79:929–936.
36. Mandava, R., Dilber, E., and Fernandez, J. (1995). Meat and meat products: use of high pressure treatment. *Meat Focus International.* Pp 147–151.
37. Mehaia, M. A. and M. A. Al-Kanhal (1992). Taurine and other free amino-acids in milk of camel, goat, cow and man. *Milchwissenschaft,* Vol. 47:351-353.
38. Mehaia, M. A., M. A. Hablas, K. M. Abdel-Rahman and S. A. El-Mougy (1995). Milk composition of Majaheim, Wadah and Hamra camel in Saudi Arabia. *Food Chemistry,* Vol. 52:115–122.
39. Naik L., Sharma R., Rajput Y. S. and Manju G (2013): Application of High Pressure Processing Technology for Dairy Food Preservation - Future Perspective: A Review. *J. Anim. Prod. Adv.,* Vol. 3:232-241.
40. Orlien, V., J. C. Knudsen, M. Colon and L. H. Skibsted (2006). Dynamics of casein micelles

in skim milk during and after high pressure treatment. *Food Chemistry,* Vol. 98:513-521.

41. Pásztor-Huszár, Klára (2008): Protein changes of various types of milk as affected by high hydrostatic pressure processing. PhD Thesis, Corvinus University of Budapest Faculty of Food Science, Department of Refrigeration and Livestock Products Technology.
42. Patterson, M. F., Quinn M., Simpson R., and Gilmour A. (1995). Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate-buffered saline and foods. *Journal of Food Protection,* Vol. 58:524-529.
43. Polydera, A., C., Galanou E., Stoforos, N.G. and. Taoukis P.S. (2004): Inactivation kinetics of pectin methylesterase of Greek Navel orange juice as a function of high hydrostatic pressure and temperature process conditions. *Journal of Food Engineering,* Vol. 62:291-298.
44. Rahmeh, R., Alomirah, H., Akbar, A. and J.S. Sidhu. 2018. Online Book Chapter on "Composition and Properties of Camel Milk" for the online book, "Milk Production, Processing and Marketing" Published by "Infotech Limited", London, UK.
45. Ritz, M., J.L. Tholozan, M. Federighi, and M.F. Pilet (2002): Physiological damages of Listeria monocytogenes treated by high hydrostatic pressure. *International Journal of Food Microbiology,* Vol. 79:47-53.
47. Schrader, K., W. Buchheim, and C. V. Morr (1997): High-pressure effects on the colloidal calcium phosphate and the structural integrity of micellar casein in milk. Part 1. High-pressure dissolution of colloidal calcium phosphate in heated milk systems. *Nahrung,* Vol. 41:133–138.
48. Scollard, P. G., T. P. Beresford, P. M. Murphy and A. L. Kelly (2000). *Barostability of milk plasmin activity. Lait.,* Vol. 80:609-619.
49. Simonin, H., F. Duranton and M. de Lambaallerie (2012): New insights into the high-pressure processing of meat and meat products. *Comprehensive reviews in food science and food safety,* Vol. 11:285-306.
50. Steel, R.G.D. and J.H. Torrie, (1980): Principles and procedures of statistics. A biometrical approach, 2nd Edition, McGraw-Hill Book Company, New York
51. Trujillo, A.J., B. Royo, B. Guamis and V. Ferragut, (1999): Influence of pressurization on goat milk and cheese composition and yield. *Milchwissenschaft,* Vol. 54:197-199.
52. Trujillo, A.J., M. Capellas, J. Saldo, R. Gervilla and B. Guamis (2002): Application of high-hydrostatic pressure on milk and dairy products: a review. *Innovative Food Science and Emerging Technologies,* Vol. 3:295-307.
53. Voigt, D. D. , F. Chevalier, J. A. Donaghy, M. F. Patterson, M. C. Qian and A. L. Kelly (2012): Effect of high-pressure treatment of milk for cheese manufacture on proteolysis, lipolysis, texture and functionality of Cheddar cheese during ripening. *Innovative Food Science and Emerging Technologies.* 13:23–30.
54. Wrolstad, R.E., T.E. Acree, E.A. Decker, M.H. Penner, D.S. Reed, S.J. Schwarz, C.F. Shoemacker, D. Smith and P. Sporns (2005): *Handbook of Food Analytical Chemistry, John Wiley and Sons,* Inc., pp. 547-565.
55. Zare, Zahra (2004): High pressure processing of fresh tuna fish and its effects on shelf life. M.Sc. thesis, Department of Food Science and Agricultural Chemistry, Macdonald Campus of McGill University, Montreal, Quebec, Canada.
56. Zobrist, M. R., T. Huppertz, T. Uniacke, P. F. Fox and A. L Kelly (2005): High-pressure-induced changes in rennet-coagulation properties of bovine milk. *International Dairy Journal,* Vol. 15:655–662.