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EFFECT OF HIGH PRESSURE PROCESSING ON THE SAFETY, SHELF LIFE AND QUALITY OF RAW MILK

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Keywords

Raw milk, high pressure, safety, shelf life, colour, stability

Abstract

High pressure processing (HPP) was investigated as an alternative to standard raw milk processing. Different pressure levels (400-600 MPa) and exposure times (1-5 min) were tested against artificially inoculated pathogenic *E. coli*, *Salmonella* and *L. monocytogenes*. HPP effectively inactivated bacterial concentration by 5 log CFU/ml. CFU/ml. The most effective/efficient/suitable HPP conditions were used to determine the effect of pressure on microbiological shelf life, particle size and colour of milk during refrigerated storage. Results were compared to pasteurised and raw milk. HPP (600 MPa for 3 min) also significantly reduced TVC, Enterobacteriaceae, lactic acid bacteria and *Pseudomonas* spp. in milk thus prolonging the microbiological shelf life of milk by 1 week compared to pasteurised milk. Particle size distribution curves of raw, pasteurised and HPP milk, showed that raw and HPP milk had more similar casein and fat particle sizes compared to pasteurised milk. The results of this study show the possibility of using HPP to eliminate pathogens present in milk while maintaining key quality characteristics similar to those of raw milk.

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Commented [3]: There is a lot of work done on HPP of milk. Some references below.

•Trujillo, A. J.; Capellas, M.; Saldo, J.; Gervilla, R. & Guamis, B. Applications of high-hydrostatic pressure on milk and dairy products: a review. *Innovative Food Science and Emerging Technologies* 2002, 3(4), 295-307.

•Datta, N. & Deeth, H. H. High pressure processing of milk and dairy products. *Australian Journal of Dairy Technology* 1999, 54(2), 41-48.

What is the novelty of this work?

1. INTRODUCTION

Recently, a strong preference for food products and ingredients that are natural has emerged amongst consumers (Murphy, Martin, Barbano, & Wiedmann, 2016; Melini, Melini, Luziatelli, & Ruzzi, 2017). Therefore, the demand for fresh-like food, with high nutrient content and high organoleptic quality has steadily increased (Hong & Wang, 2015). In this regard, the consumption of raw milk, and dairy products made from raw milk is increasingly considered desirable by some consumers. Raw milk has been identified as the cause of foodborne illness outbreaks in many cases. According to the European Food Safety Authority, 27 illness outbreaks took place within the EU between 2007 and 2012 which were linked with the consumption of raw milk (EFSA 2015). The presence and level of pathogens in milk is determined by different factors, such as season, farm size, farm hygiene and management practices and milking (Griffiths, 2010). Transmission to raw milk can take place either from zoonotic pathogens present within animals or from the environment. Specifically, raw milk can become contaminated with pathogenic bacteria by direct passage from the animal's blood into milk and externally via faecal contamination or contamination from humans. Thus, dairy farms are an important reservoir of various foodborne pathogens (Oliver, Jayarao, & Almeida, 2005). Pathogenic *Escherichia coli*, *Salmonella spp.* and *Listeria monocytogenes* are amongst the most common pathogenic bacteria found in milk and some of the most commonly reported gastrointestinal bacterial pathogens in humans in the European Union causing milk-borne infections, intoxications and toxicoinfections (Dhanashekar, Akkinepalli, & Nellutla, 2012; EFSA 2016; Melini et al., 2017). Therefore, pathogens in milk represent a safety risk that needs to be managed. The majority of the countries require raw milk to undergo some level of thermal processing in order to be rendered safe for the consumer (Griffiths 2010; Melini et al., 2017). However, conventional thermal treatment can have a detrimental effect on the nutrient content of milk as well as on

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its organoleptic and physicochemical properties (Buckow, Chandry, Ng, McAuley, & Swanson, 2014). The recent interest in the consumption of raw milk has led to the consideration of alternative processing technologies for production of milk that is safe but also minimally processed in order to be perceived as fresh by the consumer (Román, Sánchez-Siles, & Siegrist, 2017). High-pressure processing (HPP) is a food preservation technology and promising alternative to conventional thermal pasteurization as it can inactivate foodborne pathogens while minimising the loss of nutrients, such as vitamins, and maintaining the fresh-like characteristics of food products (Lee & Kaletunç 2010; Yang et al. 2012; Yao et al. 2014; Sheen, Cassidy, Scullen, & Sommers, 2015). HPP, although very efficient in eliminating vegetative microorganisms can also influence the physicochemical and technological characteristics of milk by modifying the structure of milk components (Patterson, 2005; Cadesky, Walkling-Ribeiro, Kriner, Karwe, & Moraru, 2017). Pressurization can result in conformational changes of milk proteins as it can disrupt milk casein micelles as well as the structure of whey proteins (Chawla, Patil, & Singh, 2011). It does not seem to affect lactose in milk which suggests that no Maillard or lactose isomerization reaction takes place in milk as a result of pressure treatment (Lopez-Fandino, Carrascosa, & Olano, 1996).

In the current study different levels of HPP were evaluated and compared with thermal pasteurisation and a raw milk control to determine the effect on microbiological safety, microbiological shelf life and quality. Specifically, the objectives were: (i) to identify HPP conditions that can achieve a 5-log reduction in the levels of pathogenic *E. coli*, *Salmonella* and *L. monocytogenes* inoculated in raw milk (ii) to determine the effect of HPP on milk microbiological shelf life and (iii) determine the effect of HPP on milk colour and physicochemical stability.

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I suggest ‘ The aim of the present study was to demonstrate the inactivation of *E. coli*, *Salmonella* and *L. monocytogenes* in milk using HPP while evaluating any potential impacts on product quality.

2. MATERIALS AND METHODS

2.1. Preparation of *E. coli*, *Salmonella* and *L. monocytogenes* inoculum

5 strain cocktail of the three pathogenic microorganisms was inoculated into raw milk samples separately in three different inoculation studies. The cocktail of *E. coli* consisted of NCTC 11601, NCTC 11602, NCTC 11603, NCTC 9706 and NCTC 9707. The *Salmonella* cocktail consisted of *Salmonella* Senftenberg, *Salmonella* Typhimurium, *Salmonella* Anatum, *Salmonella* Agona and *Salmonella* Saint Paul. The *L. monocytogenes* cocktail consisted of FMT 1750, NCTC 11994, NCTC 5214, NCTC 10888 and NCTC 19118 strains. These cocktails contained some relatively pressure-resistant strains, a *L. monocytogenes* strain associated with an outbreak in soft cheese and a *L. monocytogenes* strain isolated from a dairy processing environment.

For each *E. coli*, *Salmonella* and *L. monocytogenes* strain used, a loopful of a fresh tryptone soya agar (Oxoid code CM0131) + 0.6% yeast extract (Oxoid code LP0021) (TSAYE) slope culture was inoculated into 10 ml of brain heart infusion broth (BHI) (Oxoid code CM1135) and incubated at 37 °C for 24 h. Subsequently 100 µl of a 10⁻⁴ dilution of this broth was inoculated into another 10 ml BHI broth and incubated at 37 °C for either 24 h or 48 h, until the stationary phase of growth was reached. The final 10 ml cultures were centrifuged at 3600 × g, for 30 min, washed twice in phosphate-buffered saline (PBS) and the pellet re-suspended in a final volume of 1 ml PBS to give approximately 10⁹-10¹⁰ CFU/ml. The suspensions of all 5 strains for each pathogenic microorganism were combined and mixed well. The combined suspensions were inoculated (100 µl) into different raw milk samples

(10 ml), to give a level of approximately 7-8 log CFU/ml. The 10 ml samples were transferred to polyethylene/polyamide pouches (Somerville Packaging, Lisburn, Northern Ireland) and the pouches heat sealed, excluding as much air as possible. For pressure treatment, the pouches were vacuum packed in a larger pouch and the vacuum pouches were packed in an outer bag containing 5% Anistel disinfectant. Inoculated samples were held for 24 h before pressure treatment to allow time for the bacteria to acclimatise to the substrate.

48 h after HPP, three samples in total for each of the 3 different treatments and each pathogenic microorganism were opened aseptically and the contents were aseptically transferred to a sterile plastic test-tube. If required, decimal dilutions were prepared in maximum recovery diluent (MRD) (Oxoid code CM733).

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2.2. Raw milk sample preparation and processing

Milk was supplied by The Village Dairy, Clonmore, Killeslin, Co. Carlow, Ireland. For all analyses conducted raw milk samples were placed either in plastic bottles or in polyethylene/polyamide pouches and heat sealed, excluding as much air as possible. Inoculated packaged raw milk samples were heat pasteurised (controls) in a water bath at $72^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 5 min. Pressure treatment of inoculated packaged raw milk samples was performed in a commercial-scale high pressure press (Quintus 35L, Avure Technologies, U.S.A.), with a pressure vessel of 35 L volume. The pressure transmission fluid used was potable water. The pressure come-up time was approximately 25 s per 100 MPa and the pressure release time was approximately 10 s. The initial temperature of the water was approximately 18 °C and the temperature increase due to adiabatic heating was approximately 2-3°C per 100 MPa. The samples were pressure treated at 400, 500 and 600 MPa with a hold time at pressure of 1, 3 and 5 min.

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The heat-treated and HPP milk was stored for 48 h at 4°C before enumeration as this gives a better estimate of survivors, as injured cells may either recover or die during subsequent cold storage. Unprocessed inoculated samples were enumerated at the time of pressure processing (i.e. 24 h after inoculation).

2.3. Enumeration of *E. coli*, *Salmonella* and *L. monocytogenes*

For enumeration of pathogenic *E. coli* an aliquot of 100 µl of each of the appropriate 10-fold dilutions was spread plated on TBX agar plates (Oxoid, CM0945) and the plates incubated at 37 °C for 24 h. For enumeration of pathogenic *Salmonella* an aliquot of 100 µl of each of the appropriate 10-fold dilutions was spread plated on brilliant green agar plates (Oxoid, CM0329) and incubated at 37 °C for 24 h. For enumeration of *L. monocytogenes* an aliquot of 100 µl of each of the appropriate 10-fold dilutions was spread plated on Palcam agar (Oxoid, code CM0877) supplemented with Palcam selective supplement (Oxoid SR0150) and incubated at 37 °C for 48 h. Each sample was plated in duplicate.

2.4. Microbial Shelf-life assessment

After processing, raw, pasteurised and HPP milk was stored in one litre bottles at 4± 0.5 °C for the duration of the 28 days shelf life study. Shelf life assessment of samples treated at 600 MPa for 3 min was determined as it was found to be the most promising in terms of pathogen reduction. Ten-fold dilutions of milk samples were prepared in MRD (Oxoid, Basingstoke, Hampshire, U.K.) and serially diluted further. Total mesophilic aerobic bacteria (TVC), were enumerated by spread plating 100 µl from each dilution on standard plate count agar (PCA, Oxoid Ltd., Basingstoke, Hampshire, U.K.). Plates were incubated at 30 °C for 48±2 h. Numbers of *Pseudomonas spp.* were determined by spread plating on Pseudomonas agar base with CFC supplement (Oxoid Ltd., Basingstoke, Hampshire, U.K.) incubated for 72±2 h at

25 °C. *Enterobacteriaceae* were enumerated by pour plating using violet red bile glucose agar (VRBG, Oxoid Ltd., Basingstoke, Hampshire, U.K.) incubated for 24±2 h at 37°C. Lactic acid bacteria were enumerated on de Man, Rogosa, Sharpe Agar (MRS, Oxoid Ltd., Basingstoke, Hampshire, U.K.), incubated for 48±2 h at 30 °C. Results were reported as Log₁₀ CFU ml⁻¹. Samples were taken on days 0, 5, 7, 14, 21 and 28 for microbiological, particle size and color analysis. Day 0 was set as the first day after high pressure treatment.

2.5. Particle size analysis

Particle size analysis was carried out on day 0 and after 7 days of storage for raw, pasteurised and HPP treated milk (600 MPa for 3 min) using a Malvern Mastersizer 3000 laser diffraction particle size analyser (Malvern Instruments, GB). The sample was added in drops (approximately 4-5 drops) into the dispersant (distilled water). Refractive Index (*nr*) of the sample was 1.33 for the dispersant, 1.38 and 1.45 for casein and fat particle sizes respectively. The particle diameters were expressed as: D [(3,2)], the area mean weighted average surface diameter, which measured spherical particles of the same surface area (Sauter mean diameter, according to eq. 1); D[(4,3)], the volume moment mean weighted average volume diameter, which measure the spherical particles having the same volume (De Brouckere mean diameter, according to eq. 2); d(0.9), indicates that 90 % of the volume distribution is below observed diameter and d (0,5) or median diameter, which indicates that 50 % of the volume distribution is above, and 50 % is below the observed diameter.

$$D(3, 2) = \frac{\sum_i n(i) \times d(i)^3}{\sum_i n(i) \times d(i)^2} \quad [1]$$

$$D(4, 2) = \frac{\sum_i n(i) \times d(i)^4}{\sum_i n(i) \times d(i)^3} \quad [2]$$

where (*n*) is the number of fat and casein globules having a diameter [m] identical to *d(i)*. Particles size measurements were performed in triplicates at Day 0 and Day 7 for raw, thermally and HPP milk.

2.6. Color Measurement

Instrumental colour analysis was performed at day 0, 5, 7, 14, 21 and 28 of storage at 4°C for all the samples. Before each measurement samples were mixed by shaking and 200 ml of milk poured into a 50 mm glass bottle so that it was filled to the top. Colour readings were taken in triplicate by emptying and refilling the bottle at each measurement. Measurements were performed using a dual beam spectrometer Hunter Lab system (UltraScan XE, Hunter Lab., VA, USA). Measurements were reported as distribution of CIE L* (lightness), a* (redness) and b* (yellowness) and the value used to calculate the total color difference between the samples ($\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$). Depending on the value of ΔE the color difference between treated and untreated samples could be estimated such as not noticeable (0–0.5), slightly noticeable (0.5–1.5), noticeable (1.5–3.0), well visible (3.0–6.0) and great (6.0–12.0) according to Cserhalmi, Sass-Kiss, Tóth-Markus, and Lechner (2006).

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2.7. Statistical analysis

The entire experiment was replicated on three different occasions. Data were subjected to an analysis of variance (ANOVA) with treatment and storage time as the main effects and their interaction. Differences between groups were assessed by the Tukey's test. A significance level of 0.05 was used.

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3. RESULTS AND DISCUSSION

3.1. Initial considerations on experimental design

This study focused on the pathogens *E. coli*, *Salmonella* and *L. monocytogenes* because they have been linked to many outbreaks in raw milk and thus of concern for the food industry (Rodriguez, Arques, Nunez, Gaya, & Medina 2005; Oliver et al. 2005; Tambekar, & Bhutda,

2010). Literature has shown that bacterial cells in the stationary phase of growth exhibit greater pressure tolerance than exponentially-growing cells (Hayman, Anantheswaran, & Knabel, 2007; McClements, Patterson, & Linton, 2001). Therefore, bacteria were inoculated at the stationary phase in order to assess the efficiency of pressure to simulate the worst case scenario. In some cases, HPP can result in sub-lethally injured cells which cannot be detected on selective media. These cells can potentially repair themselves and cause disease. Repair of foodborne pathogens during storage is important for HPP low-acid foods such as milk because it can cause overestimation of safety (Jordan, Pascual, Bracey, & Mackey, 2001; Russell, 2002). It has also been shown that in some cases sub-lethally injured pathogens such as *E. coli* can recover even in a nutrient-free environment (Koseki & Yamamoto, 2006). To tackle that in the present study the pressure-treated milk was held for 48 h at 4°C to allow time for sub-lethally injured cells to either recover or die off. These samples were then enumerated. Here, raw milk was inoculated with individual cocktails of the three pathogenic bacteria at a high level in order to determine which pressure conditions are able to give a 5-log reduction in CFU. Specifically, *E. coli*, *Salmonella* and *L. monocytogenes* were inoculated at 8.11, 8.33 and 7.19 log CFU/ml of milk, respectively. Pasteurisation resulted in a reduction of *E. coli*, *Salmonella* and *L. monocytogenes* below the detection limit, which corresponds to a >7.11, >7.33 and >6.19 log CFU/ml reduction, respectively.

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3.2. Influence of HPP on the inactivation of *E. coli*, *Salmonella* and *L. monocytogenes*.

The effect of increasing pressure (400-600 MPa) and exposure time (1-3 min) from 400 to 600 MPa on the survival of the three artificially inoculated pathogens in raw milk is presented in Fig. 1. In general, for all three microorganisms a more pronounced inactivation was obtained with increasing pressure levels and increasing exposure time ($P < 0.05$). In all cases, HPP application even at the lower pressure level (400 MPa) and exposure time (1 min) resulted in a significant reduction ($P < 0.05$) in the levels of *E. coli*, *Salmonella spp.* and *L.*

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monocytogenes (0.85, 1.09 and 1.42 log reduction, respectively) compared to the control (raw milk). With regards to pathogenic *E. coli*, although HPP at 400 MPa and 500 MPa for 1 min did not result in statistically significant differences in reduction levels, at longer exposure times (3 and 5 min) there was a significantly higher reduction between the 400 and 500 MPa treatments. Application of pressure at 600 MPa for 3 and 5 min resulted in a reduction of 5.6 and 6.8 log CFU/ml, respectively. Linton, McClements and Patterson (2001) observed that pressure inactivation of pathogenic *E. coli* in skimmed milk varied between 3.4 and 6.7 log using a pressure treatment of 600 MPa for 15 min. Ramaswamy, Jin, & Zhu, (2009) demonstrated that HPP at 200 MPa for 15 min or 300 MPa for 5 min resulted in similar reduction of *E. coli* K12 counts (approx. 1.2 logs) in milk. In general, *Salmonella* exhibited the same trend as pathogenic *E. coli* (Fig. 1B). Reduction for 400 MPa for 1-5 min ranged from 1.09 to 2.36 log CFU/ml and for 500 MPa for 1-5 min ranged from 1.17 to 3.28 log CFU/ml. Significantly higher reductions were achieved at 600 MPa compared to the lower pressure levels ($P < 0.05$). Specifically, HPP at 600 MPa for 1, 3 and 5 min resulted in 2.48, 5.06 and 6.27 log CFU reduction in *Salmonella* counts, respectively. Similar results were obtained by Guan, Chen, & Hoover (2005) when pressure treated UHT whole milk. They found that *S. typhimurium* was reduced by 0.6, 1.8, and 5.0 log₁₀ CFU/ml, at pressures of 350, 400, and 450 MPa for 30 min, respectively. Whereas pressures of 500, 550, and 600 MPa for 10 min reduced counts of *S. typhimurium* by approx. 4.5 - 5.1 logs. *L. monocytogenes* survival after HPP is presented in Fig. 1C. In this case as well increasing pressure and exposure time resulted in more pronounced pathogen reduction. The milder conditions that could achieve a > 5 log reduction in the pathogen levels were 500 MPa for 5 min (5.48 logs) and 600 MPa for 3 min (5.65 logs). Pressure applied at 600 MPa for 5 min resulted in 5.91 log CFU/ml which did not differ significantly to the 600 MPa for 3 min treatment ($P > 0.05$). The most pronounced reduction was observed when 600 MPa were applied to the raw milk.

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However, there were no statistically differences between the *L. monocytogenes* counts at 600 MPa for 3 min and 600 MPa for 5 min ($P > 0.05$). This suggests that *L. monocytogenes* was more sensitive to increasing pressure than increasing exposure time (Erkmen & Dogan 2004), at least in the higher pressure levels. Koseki, Mizuno, & Yamamoto, (2008) found that *L. monocytogenes* cells artificially inoculated in milk ($7 \log_{10}$ CFU/ml) can be reduced after HPP at 500 MPa for 5 min by 5 log CFU/ml. Whereas, HPP above 550 and 600 MPa reduced the number of *L. monocytogenes* cells to below the limit of detection (<1 CFU/ml) immediately after treatment. According to Erkmen & Dogan, (2004), HPP at 400 and 600 MPa for 10 min resulted in 2.76 and 6.47 log CFU/ml reduction in *L. monocytogenes* counts in raw milk. Misiou, van Nassau, Lenz, & Vogel (2017) inoculated *L. monocytogenes* in milk at similar inoculum level ($7.4 \log$ CFU/ml) as in the present study and found that 300 MPa for 10 min did not have any effect on the pathogen counts. When pressures of 400 and 500 MPa were applied reductions of approx. 4.7 and 6.2 logs were observed, respectively. Based on these results, the lowest HPP condition set that were capable of reducing the levels of all three pathogenic bacteria by >5 log was the 600 MPa for 3 min set. These conditions were therefore assessed in subsequent experiments.

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3.3. Effect of HPP on microbiological shelf life

As soon as the raw milk is obtained from the animal it can be contaminated by a complex spoilage bacterial microbiota which can be present on the animal itself and/or the environment. These microorganisms can affect the nutritional and organoleptic characteristics of milk (Melini et al. 2017). The TVC, Enterobacteriaceae, lactic acid bacteria (LAB) and *Pseudomonas* spp. counts of raw milk were determined immediately after treatment and during refrigerated storage (Fig. 2). The TVC counts for the raw milk were approx. 6 log CFU/ml at the beginning of storage. Pasteurisation led to a significant reduction of 1.19 log

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CFU/ml whereas HPP (600 MPa at 3 min) led to a more pronounced decrease of 3.95 log CFU/ml, immediately after treatment. After 5 days storage, the TVC of the pasteurised milk, did not differ significantly compared to the raw milk ($P > 0.05$) for the remaining storage period. The TVC for HPP milk was always lower compared to the other two treatments with the TVC in HPP milk reaching 7.05 log CFU/ml after 28 days compared with 14 days to reach >7 log for raw and pasteurised milk. Pasteurisation also resulted in a significant reduction in *Enterobacteriaceae* counts by approx. 1.7 log CFU/ml compared to the raw milk and reached 7.87 log CFU/ml after 21 days. Whereas HPP was able to reduce the levels to below the detection limit, and the counts remained at this level throughout storage. LAB levels in raw milk were 4.26 log CFU/ml at the beginning of storage and reached 7.93 log CFU/ml after 14 days. Pasteurisation reduced the LAB counts by 2.2 log CFU/ml and increased during storage reaching 7.92 log CFU/ml after 21 days. On the other hand, HPP reduced the LAB levels below the detection limit and were detected again at 14 days storage, reaching 7.17 log CFU/ml after 28 days, which was significantly lower ($P < 0.05$) compared to LAB levels of the pasteurised milk at day 21. *Pseudomonas spp.* in the untreated raw milk increased during storage and reached 8.16 log CFU/ml after 14 days. Pasteurisation reduced *Pseudomonas spp.* by 1.28 log CFU/ml immediately after treatment. Its levels increased during storage and after 21 days it reached 7.45 log CFU/ml. On the other hand, HPP reduced the *Pseudomonas spp.* to below the detection limit, where it remained for at least 7 days. After 21 days, *Pseudomonas spp.* levels were 5.63 log CFU/ml, which was significantly lower compared to the pasteurised milk. At 28 days, *Pseudomonas spp.* counts reached 6.91 log CFU/ml for the HPP treatment. Results clearly showed that HPP (600 MPa for 3 min) was able to significantly reduce TVC, *Enterobacteriaceae*, LAB, and *Pseudomonas spp.* and prolong the microbiological shelf life of milk by 7 days compared to pasteurised milk. Erkmen & Dogan (2004) found that HPP at 400 and 600 MPa for 10 min could reduce the

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aerobic bacteria counts in raw milk by 2.09 and 5.09 log CFU/ml, respectively. High pressure homogenisation has also been applied to raw milk to increase its shelf life and has been found to reduce psychrotrophs, lactococci, and total bacteria count by approx. 4 log CFU/ml in raw milk. When the high pressure homogenised milk was stored at 4°C, the microbiological shelf life was 14-18 days, similar to that of pasteurised milk (90°C for 15 s) (Pereda, Ferragut, Quevedo, Guamis, & Trujillo, 2007).

3.3. Effect of HPP on casein particles

It is well known that HPP can affect milk constituents such as proteins and fat whereas compounds such as vitamins, amino acids, simple sugars and flavour compounds tend to remain unaffected (Chawla et al., 2011). The effects of HPP on the particle sizes of milk are particularly important since they influence its microstructure and define many properties such as colloidal stability, texture, colour etc. Differences in milk particle size can significantly affect milk quality and its further processing.

Average volume diameter $D[(4,3)]$ and average surface diameter $D[(3,2)]$ for all the three treatments tested, along with the percentile values of distribution $d(0.5)$ and $d(0.9)$ are presented in Table 1. For casein particle sizes, HPP treatment significantly ($P < 0.05$) increased all size parameters at day 0 and day 7, compared to thermally treated milk, showing similarities in $D[(4,3)]$ and $D[(3,2)]$ to those observed for raw milk. From the particle size distribution curve of raw, thermal and HPP treated milk, it can be seen that raw and HPP milk had similar peaks at 2.2 μm and $\sim 2 \mu\text{m}$, while pasteurised milk has a major peak at $\sim 0.5 \mu\text{m}$ corresponding to the smaller casein micelles (Fig. 3). A similar pattern was observed after 7 days of storage for raw and HPP milk showing the same peaks at 1.88 μm , while the peak for pasteurised milk appeared was at 0.46 μm , suggesting that the effect of HPP on casein sizes are irreversible during storage time. It has been previously reported that

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when HPP is applied the size and number of casein micelles tend to increase due to the dissociation of casein micelle into sub-micelles (Huppertz, Fox, de Kruif, & Kelly, 2006). However, diverse effects on milk proteins have been reported based on different pressures and holding times; for example, the average size of casein micelles of milk treated at 100–200 MPa at ambient temperature was comparable to untreated milk, while a pressure of 250 MPa, yielded considerably larger casein micelles than untreated milk (Huppertz, Fox, & Kelly, 2004; Regnault, Thiebaud, Dumay, & Cheftel, 2004). Decreases in micelle diameter were observed after treatment of raw or pasteurized skim milk at 400 and 600 MPa, with treated samples having ~50% smaller casein micelles than those in untreated milk (Needs, et al., 2000; Needs, Stenning, Gill, Ferragut, & Rich, 2000; Regnault et al., 2004). However, increases in average casein micelle size were observed after treatment at 200 MPa for 60 min at 30 or 40 °C or after treatment at 300 MPa for 5 min at 40 °C (Anema, Lowe, & Stockmann, 2005). Cadesky et al. (2017) reported similar changes in particle sizes as a result of pressure treatment at pressures greater than 250 MPa; increasing the pressure in low milk proteins concentration (2.5%) resulted in progressively smaller particle sizes, while for higher protein concentration (10%) a significant increase in particle size was observed. Increase in the average micelle size induced by HPP is most likely due to the presence of large casein aggregates in the milk; the results of the present study seem to support this view and are consistent with other studies where the presence of large casein aggregates in HPP treated milk was determined by electron microscopy (Considine, Patel, Anema, Singh, & Creamer, 2007; Garcia-Risco, Olano, Ramos, & Lopez-Fandino, 2000; Gaucheron et al., 1997; Needs et al. 2000).

3.4. Effect of HPP on fat particles

The particle size of the fat droplets present in dairy products is important in defining properties such as flavor release, mouth feel and the emulsion stability. Along with changes in milk proteins, HPP has been also linked with modifications of fat globules. In particular, the use of HPP has been observed to contribute to homogenization of dairy products due to a reduction of fat globule size; smaller globules cannot form large enough clusters for creaming to occur, resulting in an increased shelf-life for the milk. According to the literature, typical parameters for the size distributions of particles for homogenized milk at pressure of 100 MPa for D [(4, 3)] and a D [(3, 2)] are of about 0.5 μm and 0.2 μm . For non-homogenized milk, respective values of 4.5 μm and 1 μm are usually observed (Tobin, Heffernan, Mulvihill, Huppertz, & Kelly, 2015). Table 2 shows the fat particle size distribution of raw, pasteurised and HPP milk samples after 0 and 7 days of storage at 4°C. In the present study, HPP of milk at 600 MPa for 3 min did not result in a significant reduction of the fat particle size. Pasteurised milk displayed significant smaller ($P < 0.05$) average size distribution for fat globules compared to raw and HPP milk, (Fig. 3). Studies have shown that minimum fat particle sizes are observed after pressure application at 200-250 MPa (Picart et al., 2006; Serra, Trujillo, Quevedo, Guamis, & Ferragut, 2007), while above 250 MPa the size of the fat globules may actually increase. This has been attributed to the formation of a too large surface area which would cause the formation of cluster between the fat globules (Pereda et al., 2007; Serra et al., 2007).

3.5. Colour evaluation

The white colour of milk is due to scattering of light particles by fat globules and casein micelles and generally, the Hunter Luminance value (L^* value) is used as a measure of the whiteness of a liquid (Harte, Luedecke, Swanson, & Barbosa-Cánovas, 2003). As discussed previously, different treatments can cause changes in the size of fat particles and micelle

disintegration, resulting in different light scatter and therefore differences in colour. Results of the colour parameters distribution during the storage time of milk samples are shown in Table 3. Pasteurised milk presented the highest L^* values; significant changes ($P < 0.05$) could be detected after HPP with L^* value closer to raw milk L^* values. This is in agreement with Chawla et al. (2011) and Tao, Sun, Hogan, and Kelly (2014). A similar trend was found by Naik, Sharma, & G. (2013) in skimmed milk after treatment at 250–450 MPa, where a significant decrease in the L^* values was observed, and in ewe's milk, by Gervilla, Ferragut, & Guamis (2001). Also, Harte et al. (2003) reported that milk subjected to HPP or thermal treatment followed by high pressure, loses its white colour and turns yellowish. Significant differences ($P < 0.05$) were observed in the colour parameter $-a^*$ (greenness) of raw milk (-0.34 ± 0.05) compared to HPP (-0.61 ± 0.08) and thermal treated (-0.72 ± 0.06) milk. For the $+b^*$ value (yellowness), HPP caused a significant (14.03 ± 0.30) increase ($P < 0.05$) compared to raw milk (12.49 ± 0.26) and to pasteurised milk samples (9.79 ± 0.19). The total colour difference (ΔE) parameter is used to indicate the degree of colour difference between treated/untreated samples or before/after storage (Barba, Esteve, & Frígola, 2012) and values can be classified as not noticeable (0–0.5), slightly noticeable (0.5–1.5), noticeable (1.5–3.0), well visible (3.0–6.0) and great (6.0–12.0) (Cserhalmi et al., 2006). According to this, noticeable colour differences could be observed at the beginning of the shelf life between HPP and raw milk (ΔE 2.82) and between raw and thermally-treated milk (ΔE 2.95), while well visible differences could be seen between HPP and thermally-treated milk (ΔE 5.69). Moving towards the end of shelf life (based on LAB bacterial count), the perceived colour difference between HPP and raw milk decreased to slightly noticeable (ΔE 1.41) while remained in the range of well visible for HPP compared to thermally treated milk (ΔE 4.98) and raw to thermal milk samples (ΔE 3.65). These observations are in line with previous studies where optical parameters were reported not to be affected after treatment of milk at

100-200 MPa, but were reduced progressively with treatment pressures of 200–400 MPa, with further reduction when pressures >400 MPa was applied. Moreover, changes in optical parameters became irreversible during subsequent storage at 5 °C (Huppertz et al. 2004; Huppertz et al., 2006).

3. CONCLUSION

This study demonstrated that HPP was effective in achieving 5 log reductions for pathogenic *E. coli*, *Salmonella* and *L. monocytogenes* respectively. It is envisaged that HPP prolonged the shelf life of raw milk by reducing TVC, Enterobacteriaceae, LAB and *Pseudomonas* spp. levels compared to pasteurised and raw milk.. The particle size and color analysis of HPP milk compared to raw and pasteurized milk, revealed that HPP milk seem to preserve the quality attributes which characterize raw unprocessed milk, such as color and mouth feel sensation due to particle size. Since the demand for unpasteurized raw milk appears to be growing, HPP could be a viable alternative for the dairy industry in order to produce microbiologically safe milk with fresh-like characteristics.

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REFERENCES

- Anema, S. G., Lowe, E. K., & Stockmann, R. (2005). Particle size changes and casein solubilisation in high-pressure-treated skim milk. *Food Hydrocolloids*, *19*, 257-267.
- Barba, F. J., Esteve, M. J., & Frígola, A. (2012). High Pressure Treatment Effect on Physicochemical and Nutritional Properties of Fluid Foods During Storage: A Review. *Comprehensive Reviews in Food Science and Food Safety*, *11*, 307-322.
- Buckow, R., Chandry, P. S., Ng, S. Y., McAuley, C. M., & Swanson, B. G. (2014). Opportunities and challenges in pulsed electric field processing of dairy products. *International Dairy Journal*, *34*, 199–212.
- Cadesky, L., Walkling-Ribeiro, M., Kriner, K. T., Karwe, M. V., & Moraru, C. I., (2017). Structural changes induced by high-pressure processing in micellar casein and milk protein concentrates. *Journal of Dairy Science*, *100*, 7055-7070.
- Chawla, R., Patil, G. R., & Singh, A. K., (2011). High hydrostatic pressure technology in dairy processing: a review. *Journal of Food Science and Technology*, *48*, 260-268.
- Considine, T., Patel, H. A., Anema, S. G., Singh, H., & Creamer, L. K. (2007). Interactions of milk proteins during heat and high hydrostatic pressure treatments — A Review. *Innovative Food Science & Emerging Technologies*, *8*, 1-23.
- Cserhalmi, Z., Sass-Kiss, Á., Tóth-Markus, M., & Lechner, N. (2006). Study of pulsed electric field treated citrus juices. *Innovative Food Science & Emerging Technologies*, *7*, 49-54.
- Dhanashekar, R., Akkinapalli, S., & Nellutla, A., (2012). Milk-borne infections. An analysis of their potential effect on the milk industry. *Germs*, *2*, 101.
- EFSA (European Food Safety Authority), (2016). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. *EFSA Journal*, *14*, 4634.

EFSA Panel on Biological Hazards (BIOHAZ). Scientific Opinion on the Public Health Risks Related to the Consumption of Raw Drinking Milk: Public Health Risks Related to Raw Drinking Milk. *EFSA Journal*, 13, 3940.

Erkmen, O., & Dogan, C., (2004). Effects of ultra high hydrostatic pressure on *Listeria monocytogenes* and natural flora in broth, milk and fruit juices. *International Journal of Food Science & Technology*, 39, 91-97.

Garcia-Risco, M. R., Olano, A., Ramos, M., & Lopez-Fandino, R. (2000). Micellar changes induced by high pressure. influence in the proteolytic activity and organoleptic properties of milk. *Journal of Dairy Science*, 83, 2184-2189.

Gaucheron, F., Famelart, M. H., Mariette, F., Raulot, K., Michela, F., & Le Graeta, Y. (1997). Combined effects of temperature and high-pressure treatments on physicochemical characteristics of skim milk. *Food Chemistry*, 59, 439-447.

Gervilla, R., Ferragut, V., & Guamis, B. (2001). High Hydrostatic Pressure Effects on Color and Milk-Fat Globule of Ewe's Milk. *Journal of Food Science*, 66, 880-885.

Griffiths, M. W. (2010). The microbiological safety of raw milk. In: Griffiths MW, (Ed.), *Improving the Safety and Quality of Milk, Volume 1: Milk Production and Processing*. (27–63). Boca Raton, FL: CRC Press.

Guan, D., Chen, H., & Hoover, D. G. (2005). Inactivation of *Salmonella typhimurium* DT 104 in UHT whole milk by high hydrostatic pressure. *International Journal of Food Microbiology*, 104, 145-153.

Harte, F., Luedecke, L., Swanson, B., & Barbosa-Cánovas, G. V. (2003). Low-fat set yogurt made from milk subjected to combinations of high hydrostatic pressure and thermal processing. *Journal of Dairy Science*, 86, 1074-1082.

Hayman, M. M., Anantheswaran, R. C., & Knabel, S. J., (2007). The effects of growth temperature and growth phase on the inactivation of *Listeria monocytogenes* in whole

milk subject to high pressure processing. *International Journal of Food Microbiology*, 115, 220-226.

Hong, X., & Wang, J., (2015). Use of electronic nose and tongue to track freshness of cherry tomatoes squeezed for juice consumption: comparison of different sensor fusion approaches. *Food and Bioprocess Technology*, 8, 158-170.

Huppertz, T., Fox, P. F., & Kelly, A. L. (2004). Properties of casein micelles in high pressure-treated bovine milk. *Food Chemistry*, 87, 103-110.

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

Jordan, S. L., Pascual, C., Bracey, E., & Mackey, B. M. (2001). Inactivation and injury of pressure-resistant strains of *Escherichia coli* O157 and *Listeria monocytogenes* in fruit juices. *Journal of Applied Microbiology*, 91, 463-469.

Koseki, S., & Yamamoto, K., (2006). Recovery of *Escherichia coli* ATCC 25922 in phosphate buffered saline after treatment with high hydrostatic pressure. *International Journal of Food Microbiology*, 110, 108-111.

Koseki, S., Mizuno, Y. & Yamamoto, K. (2008). Use of mild-heat treatment following high-pressure processing to prevent recovery of pressure-injured *Listeria monocytogenes* in milk. *Food Microbiology*, 25, 288-293.

Kumar, R., & Prasad, A., (2010). Detection of *E. coli* and *Staphylococcus* in milk and milk products in and around Pantnagar. *Pakistan Journal of Nutrition*, 1, 151-52.

Lee, J., & Kaletunç, G. (2010). Inactivation of *Salmonella Enteritidis* strains by combination of high hydrostatic pressure and nisin. *International Journal of Food Microbiology*, 140, 49-56.

- Lopez-Fandino, R., Carrascosa, A. V., & Olano, A., (1996). The effects of high pressure on whey protein denaturation and cheese-making properties of raw milk. *Journal of Dairy Science*, 79, 929-936.
- Lucey, J. A. (2015). Raw Milk Consumption: Risks and Benefits. *Nutrition Today*, 50, 189-193.
- McClements, J. M. J., Patterson, M. F., & Linton, M., (2001). The effect of growth stage and growth temperature on high hydrostatic pressure inactivation of some psychrotrophic bacteria in milk. *Journal of Food Protection*, 64, 514-522.
- Melini, F., Melini, V., Luziatelli, F., & Ruzzi, M., (2017). Raw and Heat-Treated Milk: From Public Health Risks to Nutritional Quality. *Beverages*, 3, 54.
- Misiou, O., van Nassau, T. J., Lenz, C. A., & Vogel, R. F., (2017). The preservation of Listeria-critical foods by a combination of endolysin and high hydrostatic pressure. *International Journal of Food Microbiology*, 266, 355-362.
- Murphy, S. C., Martin, N. H., Barbano, D. M., & Wiedmann, M., (2016). Influence of raw milk quality on processed dairy products: How do raw milk quality test results relate to product quality and yield? *Journal of Dairy Science*, 99, 10128-10149.
- Linton, M., McClements, J. M. J. & Patterson, M. F., (2001). Inactivation of pathogenic *Escherichia coli* in skimmed milk using high hydrostatic pressure. *Innovative Food Science & Emerging Technologies*, 2, 99-104.
- Naik L., Sharma R., S., R. Y., & G., M. (2013). Application of High Pressure Processing Technology for Dairy Food Preservation - Future Perspective: A Review. *Journal of Animal and Veterinary Advances*, 3, 232-241.
- Needs, E. C., Capellas, M., Bland, A. P., Manoj, P., Macdougall, D., & Paul, G. (2000). Comparison of heat and pressure treatments of skim milk, fortified with whey protein

- concentrate, for set yogurt preparation: effects on milk proteins and gel structure. *Journal of Dairy Research*, 67, 329-348.
- Needs, E. C., Stenning, R. A., Gill, A. L., Ferragut, V., & Rich, G. T. (2000). High-pressure treatment of milk: effects on casein micelle structure and on enzymic coagulation. *Journal of Dairy Research*, 67, 31-42.
- Oliver, S. P., Jayarao, B. M., & Almeida, R. A. (2005). Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications. *Foodborne Pathogen and Disease*, 2, 115-129.
- Olson, D. W., White, C. H., & Richter, R. L. (2004). Effect of Pressure and Fat Content on Particle Sizes in Microfluidized Milk. *Journal of Dairy Science*, 87, 3217-3223.
- Patterson, M. F., (2005). Microbiology of pressure-treated foods. *Journal of Applied Microbiology*, 98, 1400-1409.
- Pereda, J., Ferragut, V., Quevedo, J. M., Guamis, B., & Trujillo, A. J. (2007). Effects of Ultra-High Pressure Homogenization on Microbial and Physicochemical Shelf Life of Milk. *Journal of Dairy Science*, 90, 1081-1093.
- Pereda, J., Ferragut, V., Quevedo, J.M., Guamis, B., & Trujillo, A. J., 2007. Effects of ultra-high pressure homogenization on microbial and physicochemical shelf life of milk. *Journal of Dairy Science*, 90, 1081-1093.
- Picart, L., Thiebaut, M., René, M., Pierre Guiraud, J., Cheftel, J. C., & Dumay, E. (2006). Effects of high pressure homogenisation of raw bovine milk on alkaline phosphatase and microbial inactivation. A comparison with continuous short-time thermal treatments. *Journal of Dairy Research*, 73, 454-463.
- Ramaswamy, H. S., Jin, H., & Zhu, S. (2009). Effects of fat, casein and lactose on high-pressure destruction of *Escherichia coli* K12 (ATCC-29055) in milk. *Food and Bioproducts Processing*, 87, 1-6.

- Regnault, S., Thiebaud, M., Dumay, E., & Cheftel, J. C. (2004). Pressurisation of raw skim milk and of a dispersion of phosphocaseinate at 9°C or 20°C: effects on casein micelle size distribution. *International Dairy Journal*, *14*, 55-68.
- Rodriguez, E., Arques, J. L., Nunez, M., Gaya, P., & Medina, M. (2005). Combined effect of high-pressure treatments and bacteriocin-producing lactic acid bacteria on inactivation of *Escherichia coli* O157:H7 in raw-milk cheese. *Applied and Environmental Microbiology*, *71*, 3399-3404.
- Román, S., Sánchez-Siles, L. M., & Siegrist, M. (2017). The importance of food naturalness for consumers: Results of a systematic review. *Trends in Food Science and Technology*, *67*, 44–57.
- Russell, N. J., (2002). Bacterial membranes: the effects of chill storage and food processing. An overview. *International Journal of Food Microbiology*, *79*, 27-34.
- Serra, M., Trujillo, A. J., Quevedo, J. M., Guamis, B., & Ferragut, V. (2007). Acid coagulation properties and suitability for yogurt production of cows' milk treated by high-pressure homogenisation. *International Dairy Journal*, *17*, 782-790.
- Sheen, S., Cassidy, J., Scullen, B., & Sommers, C. (2015). Inactivation of a diverse set of shiga toxin-producing *Escherichia coli* in ground beef by high pressure processing. *Food Microbiology*, *52*, 84–87.
- Tambekar, D. H., & Bhutda, S. A., (2010). Prevalence of Bacterial Pathogens in Pedha (A Milk Product) Sold in Amravati (India). *International Journal of Dairy Science*, *5*, 173-176.
- Tao, Y., Sun, D.-W., Hogan, E., & Kelly, A. L. (2014). Chapter 1 - High-Pressure Processing of Foods: An Overview. In D.-W. Sun (Ed.), *Emerging Technologies for Food Processing (Second Edition)* (pp. 3-24). San Diego: Academic Press.

- Tobin, J., Heffernan, S. P., Mulvihill, D. M., Huppertz, T., & Kelly, A. L. (2015). Applications of High-Pressure Homogenization and Microfluidization for Milk and Dairy Products *Emerging Dairy Processing Technologies* (pp. 93-114): John Wiley & Sons, Ltd.
- Yang, B., Shi, Y., Xia, X., Xi, M., Wang, X., Ji, B., & Meng, J., (2012). Inactivation of foodborne pathogens in raw milk using high hydrostatic pressure. *Food Control*, 28, 273-278.
- Yao, J., Zhou, B., Wang, R., Wang, T., Hu, X., Liao, X., & Zhang, Y., (2015). Inactivation of *Staphylococcus aureus* by high hydrostatic pressure in saline solution and meat slurry with different initial inoculum levels. *Food and Bioprocess Processing*, 94, 592-600.

Tables

Table 1. Casein particle size (μm) of raw, thermally treated and HPP milk samples after 0 and 7 days of storage at 4°C.

Day 0	d(0.5)	d(0.9)	D[(4,3)]	D[(3,2)]
Raw	0.96 \pm 0.01 ^b	3.44 \pm 0.02 ^b	1.49 \pm 0.01 ^b	0.53 \pm 0.01 ^a
Thermal	0.39 \pm 0.00 ^c	0.99 \pm 0.00 ^c	0.49 \pm 0.00 ^c	0.27 \pm 0.00 ^b
HPP	1.21 \pm 0.19 ^a	4.05 \pm 0.21 ^a	2.15 \pm 0.15 ^a	0.54 \pm 0.14 ^a
Day 7	d(0.5)	d(0.9)	D[(4,3)]	D[(3,2)]
Raw	1.01 \pm 0.01 ^b	4.12 \pm 0.09 ^a	2.19 \pm 0.13 ^a	0.54 \pm 0.01 ^b
Thermal	0.40 \pm 0.00 ^c	1.01 \pm 0.01 ^c	0.61 \pm 0.07 ^c	0.28 \pm 0.00 ^c
HPP	1.17 \pm 0.01 ^a	3.72 \pm 0.04 ^b	1.67 \pm 0.01 ^b	0.71 \pm 0.00 ^a

^{a-c} Mean value \pm standard deviation; values without common superscripts were significantly different ($P < 0.05$).

* D (0.5): diameter below which 50% of the volume of particles are found, D (0.9): diameter below which 90% of the volume of particles are found, D[(4,3)]: volume-weighted mean diameter, D[(3,2)]: surface-weighted mean diameter.

Table 2. Fat particle size (μm) of raw, thermally treated and HPP milk samples after 0 and 7 days of storage at 4°C.

Day 0	d(0.5)	d(0.9)	D[(4,3)]	D[(3,2)]
Raw	1.60 \pm 0.11 ^b	6.07 \pm 0.09 ^b	2.88 \pm 0.27 ^b	0.12 \pm 0.00 ^a
Thermal	0.32 \pm 0.01 ^a	0.96 \pm 0.00 ^a	0.43 \pm 0.00 ^a	0.13 \pm 0.00 ^a
HPP	3.26 \pm 0.42 ^c	7.50 \pm 0.36 ^c	4.79 \pm 0.91 ^c	0.27 \pm 0.14 ^a
Day 7	d(0.5)	d(0.9)	D[(4,3)]	D[(3,2)]
Raw	2.38 \pm 0.06 ^b	8.78 \pm 0.76 ^a	4.24 \pm 0.47 ^a	0.14 \pm 0.00 ^a
Thermal	0.42 \pm 0.03 ^c	1.42 \pm 0.20 ^b	3.03 \pm 1.31 ^a	0.22 \pm 0.04 ^a
HPP	3.19 \pm 0.29 ^a	8.57 \pm 2.19 ^a	5.62 \pm 1.51 ^a	0.23 \pm 0.06 ^a

^{a-c} Mean value \pm standard deviation; values without common superscripts were significantly different ($P < 0.05$).

* d(0.5): diameter below which 50% of the volume of particles are found, d(0.9): diameter below which 90% of the volume of particles are found, D[(4,3)]: volume-weighted mean diameter, D[(3,2)]: surface-weighted mean diameter.

Table 3. Distribution of the colour values of milk samples in CIE *Lab* system

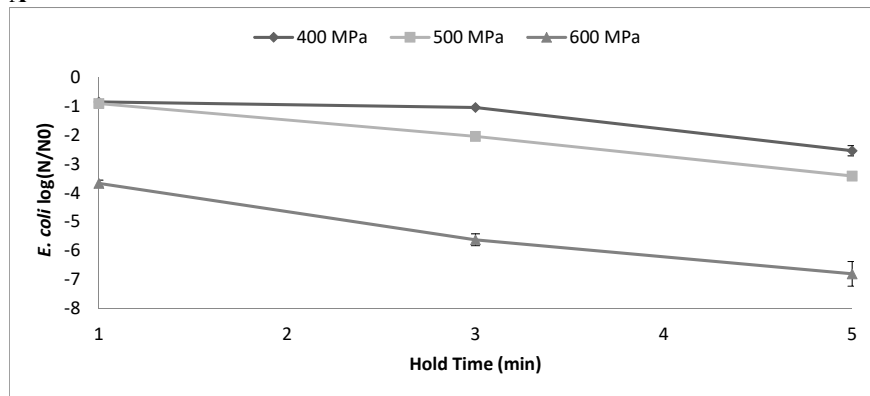
	L*	a*	b*
HPP	77.29±0.35 ^c	-0.61±0.08 ^a	14.03±0.30 ^c
Raw	78.94±0.31 ^b	-0.34±0.05 ^b	12.49±0.26 ^b
Thermal	80.80±0.32 ^a	-0.72±0.06 ^a	9.79±0.19 ^a

^{a-c} Mean value ± standard deviation; values without common superscripts were significantly different ($P < 0.05$).

Figures

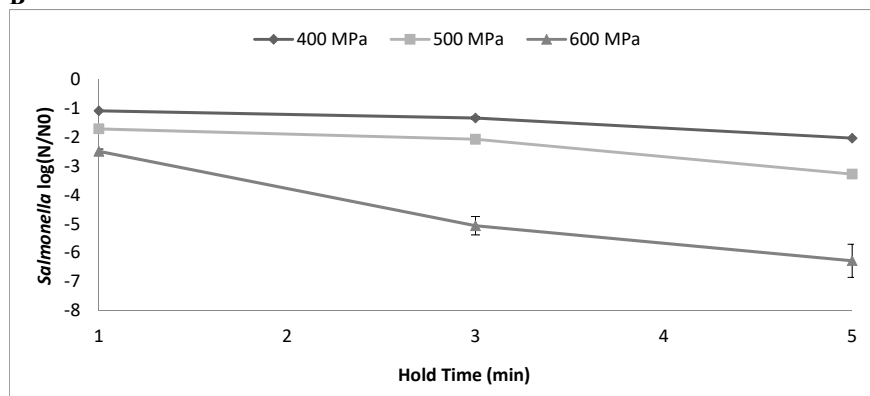
Figure 1

A



Commented [26]: Can these data sets be normalized?

B



C

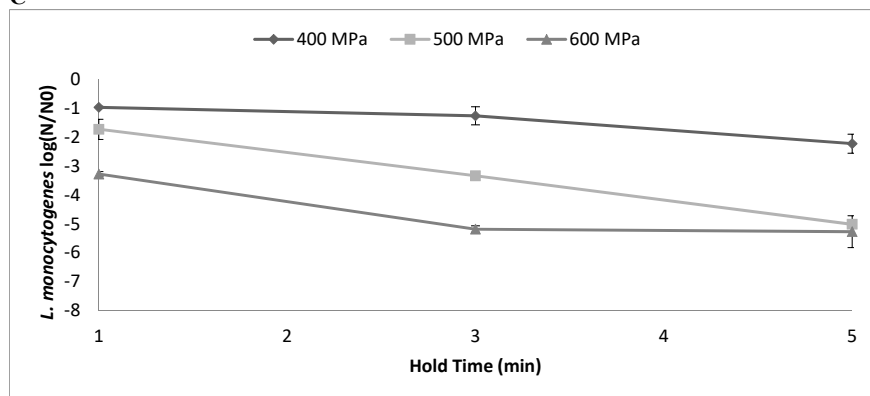


Figure 2

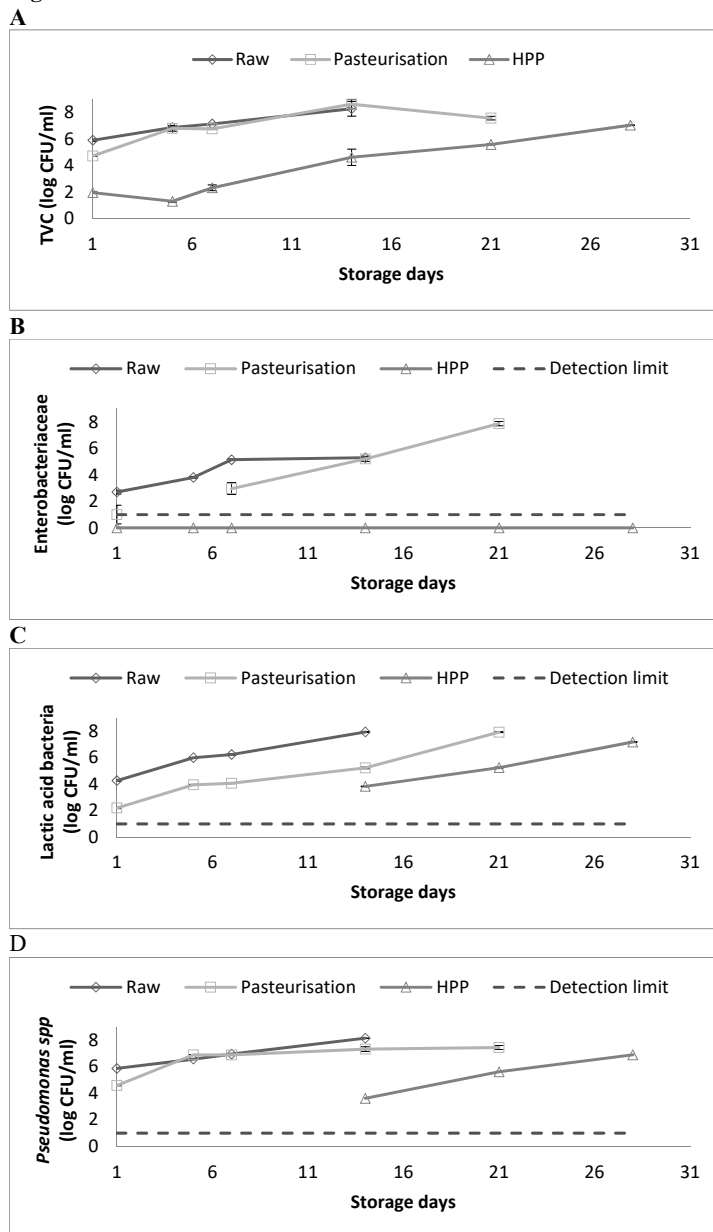


Figure 3

