

Effect of cold chain interruptions on the shelf-life of fluid pasteurised skim milk at the consumer stage

Efeito de interrupções na cadeia de frio na vida útil de leite desnatado fluido e pasteurizado na etapa de consumidor

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Abstract

This study aimed to verify the effect of time and temperature abuse on bacterial numbers in fluid pasteurized skim milk by simulating the real-life scenario, which usually occurs when cold chain is interrupted by consumers prior to consumption that affect the shelf-life of milk. Total three trials were carried out in this study. Thermal abuse was simulated with temperature fluctuations from 5 °C. In the first trial, the information about holding the milk samples for 8 hours at three different temperatures of 15 °C, 20 °C and 25 °C was obtained using a data logger to predict the effect of temperature abuse on the milk microbial quality. Further, in the second and third trial, the effect of temperature abuse on bacterial numbers was examined by holding milk at 5 °C and then shifts temperature to 25 °C for 8 h and 6 h. The pH was monitored during storage. The total bacterial count was examined by the Standard Plate Count (SPC). The mean air temperature had the greatest impact on milk temperature. It took 3.0 h, 3.9 h and 4.2 h to warm up when exposed to the temperatures of 15 °C, 20 °C and 25 °C, respectively. The holding time of 8 h at 25 °C showed that bacterial numbers (1×10^5 CFU mL⁻¹) were higher after 14 days of storage, but control samples at 5 °C ($< 1 \times 10^4$ CFU mL⁻¹) were still within the acceptable level (5×10^4 CFU mL⁻¹). A holding time of 6 h at 25 °C showed much higher bacterial numbers (1×10^9 CFU mL⁻¹) compared to control samples (1×10^7 CFU mL⁻¹) which were held at 5 °C for 11 days. The pH of the milk decreased with increasing bacterial growth during the extended storage time. The results show that temperature abuse has a significant effect on milk microbial stability and shelf life. It is important to maintain the milk temperature at 5 °C or less as the bacterial growth directly depend on increasing temperature and holding time, which pose the potential risk of microbial hazards leading to foodborne illness. Thus, consumers must understand the factors associated with the safe handling of milk to keep it safe to use before the expiry date.

Keywords: Bacteria; Temperature abuse; Holding time; Spoilage; Shelf-life.

Resumo

O objetivo deste estudo foi verificar o efeito do abuso de temperatura e tempo na contagem bacteriana em leite fluido desnatado e pasteurizado, que ocorre geralmente quando a cadeia de frio é interrompida pelo consumidor antes do consumo, e que afeta a vida útil do leite. Três experimentos foram realizados para esse fim. O abuso térmico foi simulado com flutuações de temperatura a partir de 5 °C. No primeiro experimento, o leite foi mantido por 8 horas em três temperaturas (15 °C, 20 °C e 25 °C), usando-se um registrador de dados para prever o efeito do abuso térmico na qualidade microbiológica do leite. Nos outros dois experimentos, o efeito do abuso de temperatura sobre as contagens microbianas foi avaliado mantendo-se o leite a 5°C e depois aumentado a temperatura para 25 °C por 6 horas e 8 horas. O pH foi avaliado durante estocagem. A contagem total de bactérias foi determinada pela Contagem Padrão em Placa. A temperatura média do ambiente de estocagem apresentou o maior impacto na temperatura do leite. Quando o leite foi exposto a ambientes a 15 °C, 20 °C e 25 °C verificou-se o aquecimento após 3,0 h, 3,9 h e 4,2 h, respectivamente. Um tempo de incubação de 8 horas a 25°C resultou em contagens bacterianas mais altas (1×10^5 UFC mL⁻¹) após 14 dias de estocagem, comparativamente ao produto controle mantido a 5°C ($< 1 \times 10^4$ UFC mL⁻¹), que se manteve dentro de um nível aceitável



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(5×10^4 UFC mL⁻¹). Um tempo de 6 horas a 25°C resultou em maiores contagens (1×10^9 CFU mL⁻¹) comparativamente às amostras controle (1×10^7 CFU mL⁻¹) mantidas a 5 °C durante 11 dias. O pH do leite diminuiu à medida que o crescimento bacteriano aumentou, durante a estocagem. A flutuação de temperatura exerceu um efeito pronunciado na estabilidade microbiológica do leite e na sua vida útil. É importante manter o leite a temperatura de 5 °C ou menor, visto que o crescimento microbiano está associado à história térmica do produto e pode representar riscos à saúde pública, com o crescimento de microrganismos patogênicos. Portanto, os consumidores devem ser alertados sobre os fatores associados com o manuseio seguro do leite fluido pasteurizado, para garantir sua segurança antes do fim da validade.

Palavras-chave: *Bactéria; Temperatura abusiva; Tempo de incubação; Deterioração; Vida útil.*

1 Introduction

Milk is unacceptable to the consumer when organoleptic changes resulting from microbiological spoilage occur, because it is an ideal medium for the growth of different microorganisms resulting in its early deterioration (NICOLAOU; GOODACRE, 2008; SILVA et al., 2010). Milk is highly perishable and the high water activity, near neutral pH (6.4 - 6.7) and nutritional composition make milk an ideal medium for microbial growth (ADAMS; MOSS, 2008; WALKLING-RIBEIRO et al., 2009; SILVA et al., 2010). Low-temperature storage is necessary for every stage in the cold chain to maintain quality and safety. Pasteurization is an effective method for eliminating food borne pathogens and other bacteria from milk. Shelf-life is the time during which the pasteurised milk can be kept at a given temperature without undesirable changes (WALSTRA et al., 2006). JANZEN et al. (1982a,b) found that every 2.77 °C rise in temperature cuts the shelf-life in half.

The shelf-life of milk and milk products is not completely related to deterioration, but to a complex situation depending on the interaction with the consumer food choice (HOUGH et al., 2003). Survival analysis is one of the most used statistical tools by the dairy industries to determine the right formulation according to consumer acceptability. This method is also applied to estimate the shelf-life of foods when the event of interest was substituted by the storage time (ESMERINO et al., 2015).

The cold chain refers to all operations in the production, transportation, handling, storage and retailing of refrigerated milk, which have an effect on the shelf-life of the milk (LIKAR; JEVSNIK, 2006). Inadequate refrigeration, handling practices, and temperature abuses occurring during any stage of the cold chain can cause unexpected loss of quality and a significant decrease in the expected shelf-life, due to the rapid growth of bacteria (KOUTSOUMANIS et al., 2006).

Temperature significantly influences the growth of microorganisms and deterioration of the milk, unless stored at controlled low temperatures (CHANDLER; McMEEKIN, 1985). The microbiological quality of milk depends on both the types of bacteria present in the milk and the numbers of microorganisms (FSANZ, 2006). Spoilage microorganisms

can be active at temperatures between 2 °C and 30 °C (NICOLAOU; GOODACRE, 2008) and refrigeration favours the presence of psychrotrophic bacteria in milk, which can grow slowly at low temperatures (< 7 °C). However, such bacteria show optimum growth at 15 °C to 30 °C and are responsible for milk spoilage (CHAMPAGNE et al., 1994; GLEESON et al., 2013).

Changes caused by the growth of bacteria in milk do not usually become noticeable until the count reaches between 5×10^6 and 20×10^6 CFU mL⁻¹ (WALSTRA et al., 2006). Considering the shelf-life and safe consumption, most countries have legal limits for the maximum number of bacteria in milk, for example, Canada: 10,000 CFU mL⁻¹; USA: 20,000 CFU mL⁻¹; European Economic Community: 50,000 CFU mL⁻¹ (INSTITUTE OF MEDICINE NATIONAL RESEARCH COUNCIL OF THE NATIONAL ACADEMIES, 2003). Food Standards Australia New Zealand (FSANZ) sets the microbiological limits for pasteurized milk at 5×10^4 CFU mL⁻¹ and should not exceed the level of 10^5 CFU mL⁻¹ (FSANZ, 2001). Milk becomes less acceptable to consumers when the microbial count exceeds 10^7 CFU mL⁻¹ (GRIFFITHS; PHILLIPS, 1988). The shelf-life of milk indicates the level of quality marked on the package with the 'best before date' up to which the milk should remain safe and suitable for consumption. Pasteurized fluid milk has a shelf-life of 14 days if it is stored at an appropriate refrigeration temperature (BARBANO et al., 2006; FSANZ, 2006). From the consumer's point of view, the end of the shelf-life of fluid milk occurs when they notice the milk is no longer palatable based on its sensory attributes (CAPLAN; BARBANO, 2013), mainly odours.

Once the milk is removed from the retail display cabinet the susceptibility to microbial spoilage is increased. Temperature abuse occurs when milk is left too long at temperatures that are ideal for microbial growth, which can shorten the printed 'best before date' of the milk. Thus, the authors of the present study were interested in looking at the temperatures that milk is exposed to during the consumer stage over a longer period of time. Few studies are available on the impact of temperature abuse on fluid pasteurized skim milk during the consumer stage. Since bacterial spoilage causes significant losses of milk quality and reduces the shelf-life, there is a constant need to develop awareness amongst consumers regarding milk

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spoilage due to temperature abuse. Therefore, it is important to have a better understanding of the consequences of consumer food-handling practices with respect to bacterial growth during transport, handling and storage and under domestic conditions, in order to preserve the original bacteriological milk quality. This study focused on bacterial numbers that cause spoilage either at low temperatures or after temperature abuse. The objective of this study was to examine the effect of cold chain interruptions on the bacterial growth and shelf-life of pasteurized fluid skim milk, and also determine the impact of the duration of temperature abuse on the milk at the consumer stage for different holding times.

2 Materials and methods

2.1 Milk samples

A total of sixty-two one-litre bottles of pasteurized skim milk were randomly collected from a local supermarket in Dunedin on the basis of a 'best before date' in August 2012. On arriving at the laboratory, the milk bottles were immediately stored at below 5 °C until analysed.

2.2 Experimental design

Altogether, three trials were carried out under different temperature abuse conditions. In the first trial three temperature regimes were evaluated as follows, with a basic storage temperature of 5 °C and i) temperature shifts to 15 °C before returning to 5 °C, ii) temperature shifts to 20 °C before returning to 5 °C, and iii) temperature shifts to 25 °C before returning to 5 °C. The holding time was considered to be 8 hours in the first trial. The second and third trials were carried out to determine the effects of temperature abuse on the bacterial numbers and pH values of the pasteurized skim milk. In these latter two trials, the milk samples were stored as a control scenario with a constant storage temperature of 5 °C and were exposed to room temperatures of 25 °C before returning to 5 °C. The temperatures of the milk samples were changed for different durations in each trial, the total time at an abusive temperature being 8 hours in the second trial and 6 hours in the third trial. The end point of the shelf life was considered to be when the microbial numbers reached between 10^6 to 10^7 CFU mL⁻¹, which is generally considered to be the number of microorganisms needed for spoilage to occur in milk (GRIFFITHS; PHILLIPS, 1988).

2.3 Preparation of the microbial media

The medium for the standard plate count (SPC) was prepared using the Standard Method Agar (Difco, Becton, Dickinson & Company, France). Diluents were prepared using Bacto peptone and distilled water. In this study, visible total bacterial populations were counted using SPC, but specific flora were not identified. The Petri dishes were incubated at 25 °C for 72 h for the

detection of the viable bacterial counts in refrigerated milk (VAN DER ZANT; MOORE, 1955; MOYER; MORITA, 2007). The colonies were then counted and recorded for later analysis and interpretation.

2.4 Incubation and temperature regime tests

The temperature abuse of the milk samples was monitored during incubation using thin wire thermocouples connected to a Grant 800 data logger (Squirrel data logger, Grant Instrument, Cambridge, UK). The data logger was connected to temperature sensors which recorded the air and milk temperatures. It is mainly used to reduce the milk cooling costs by monitoring and ensuring optimum efficiency of the milk pre-cooling equipment and vat refrigeration performance. This device also provides ready information for dairy farmers and milk processors about Hazard Analysis and Critical Control Points (HACCP) and Good Manufacturing Practices (GMP) throughout the milk chain in the dairy industry to allow for efficient control, which is essential to ensure milk safety.

In the first trial, in order to quantify the temperature changes, three milk bottles were attached to temperature probes, and placed in an incubator at 25 °C for 8 hours along with another twenty-five milk bottles, simulating the cold chain from the supermarket to domestic storage by consumers. After 8 hours, the milk bottles were removed from the incubator and stored at 5 °C. The second trial was carried out using the fluctuating temperature scenario, which occurs in the homes of consumers. On the day of testing, a total of six bottles (three bottles of milk with the probe and another three without probes) were placed in the 25 °C incubator and left for 6 hours. Three other milk bottles which were used as controls (without incubation) were stored at 5 °C. After 6 hours, 100 mL samples of milk were collected from each of the nine bottles, and all nine bottles were then stored at 5 °C for 18 hours.

2.5 Microbiological analysis and pH measurement

Following the temperature abuse, the effects of the different temperatures and holding times on bacterial growth in the milk was determined in the shelf-life study. In the first trial, the milk bottles were divided into two groups, with twenty-five one-litre bottles being stored under controlled conditions with a constant storage temperature of 5 °C, and another twenty-five bottles being held at 25 °C for 8 hours of time-temperature abuse, and then returned to 5 °C. In the second trial three 100 mL aliquots of milk were subjected to time-temperature abuse by placing the milk in a 25 °C incubator, and allowing it to reach the target temperature. At each sampling time, 100 mL aliquots were removed from the incubator, and another three 100 mL aliquots from the 5 °C chamber, which were used after 6 hours to determine the bacterial numbers.

The effect of fluctuating temperature on bacterial growth was measured by the standard plate count (SPC).

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In each trial, the total number of bacteria that could grow and form countable colony forming units (CFU) was determined in milk samples on a Standard Methods Agar plate (Difco, Becton, Dickinson and Company, France). Ten-fold serial dilutions were carried out for each experiment by preparing 0.1% peptone water in 9.0 volumes. An aliquot (0.1 mL) of milk was transferred aseptically into serial dilution bottles from which further dilutions were made. All plates were aerobically incubated in triplicate at 25 °C for 72 hours before counting (MOYER; MORITA, 2007). The temperature of 25 °C was used in this study because it is more favourable to the growth of bacteria that grow at refrigeration temperatures, while also permitting many other microorganisms to grow. The pH of the milk samples was measured at 5 °C and at 25 °C using a pH meter (Hanna, Fisher Scientific Company, Pittsburgh, PA, USA).

2.6 Statistical analysis

All experiments were repeated in triplicate. The results of the microbiological and pH analyses were compared by ANOVA using the Excel 2010 software program (Microsoft, CA, USA). The statistical significance was determined at $p < 0.05$.

3 Results and discussion

Fresh milk samples were tested after collection for total bacterial numbers, and low SPC ($<1 \times 10^3$ CFU mL⁻¹) were observed. The bacterial counts remained with acceptable numbers (1×10^4 CFU mL⁻¹) for up to 14 days at these storage temperatures. The results of the statistical analyses showed that the differences in bacterial numbers were not significant ($p > 0.05$) up to 14 days of storage for milk samples stored at 2 °C and 5 °C. Milk samples stored at 2 °C continued to show low bacterial numbers and acceptable levels up to day 17 as compared to 5 °C. However, after 17 days, the bacterial numbers in the milk stored at 5 °C exceeded the acceptable bacterial level as compared to the milk samples stored at 2 °C. At 5 °C the bacterial numbers continued to grow rapidly after 22 days ($>1 \times 10^6$ CFU mL⁻¹), and reached $>1 \times 10^9$ CFU mL⁻¹ after 31 days of storage. In contrast, the bacterial numbers in the milk stored at 2 °C reached 1×10^6 CFU mL⁻¹ on day 28, and after 31 days were still lower ($>1 \times 10^7$ CFU mL⁻¹) as compared to the samples stored at 5 °C.

3.1 Simulated temperature regime test at 15 °C, 20 °C and 25 °C

The temperature normally starts to change as soon as the milk leaves the refrigerator cabinet and increases while the consumer completes the shopping and during the drive home, before being returned to a refrigerator bin the consumer's home. Information about holding milk samples for 8 hours at three different temperatures (15 °C, 20 °C and 25 °C) was obtained using a data logger shown in Figure 1.

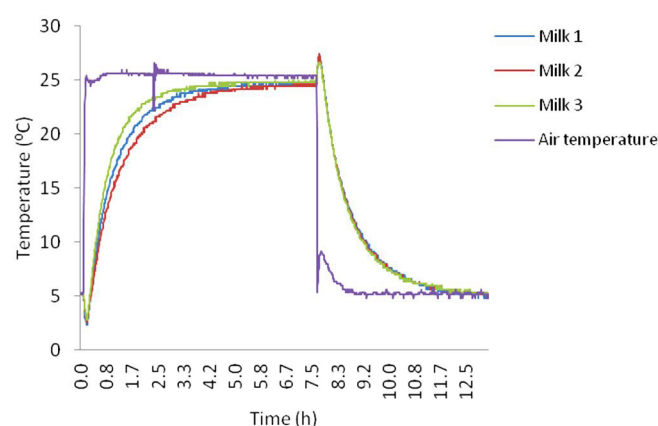


Figure 1. Holding time and air temperature fluctuation cycles for 3 milk samples subjected to temperature abuse at 25 °C for 8 hours and subsequently held at 5 °C for 18 hours.

The results showed that any increase in the temperature of the environment had an adverse effect on the temperature of the milk. In the simulated temperature regime test, the milk temperatures were observed to rise quickly up to 15 °C, 20 °C or 25 °C when the samples were exposed to warm temperatures. The milk samples took 4.2 h, 3.9 h and 3.0 h to warm up when exposed to the temperatures of 15 °C, 20 °C and 25 °C, respectively. Tamime (2009) found that ambient outdoor temperatures of 15-20 °C increased the milk temperature by 0.4 to 4 °C per hour, and that air temperatures of 25-30 °C could cause the milk temperature to increase 6 to 7 °C per hour. In the present study, when the milk was replaced into a refrigerator at a temperature of 5 °C, the temperature of the milk started to cool down slowly. The results revealed that the temperature changes in milk samples at 15 °C, 20 °C and 25 °C took 4.0 h, 4.9 h and 6.7 h respectively. The recovery time for the milk samples may be longer due to multiple opening and closing of the doors that could result in heat entering the refrigerator. Thus, the time taken for the temperature of the milk to cool down in this test may vary. Tamime (2009) reported that if the temperature of the milk rises to 15-20 °C, it is difficult to obtain quick re-cooling, even with cold air circulation. This finding highlights the importance of not allowing milk to warm up after purchase.

3.2 Bacterial numbers in milk subjected to temperature abuse at 25 °C for 8 hours prior to being stored at 5 °C

In this trial, since the aim was to obtain information on bacterial growth in the simulation experiment, a room temperature of 25 °C was selected for the bacterial growth study.

Figure 2 shows that initially the bacterial counts increased slowly at both 25 °C and 5 °C. On day 6, the milk samples stored at 25 °C (test sample) and 5 °C

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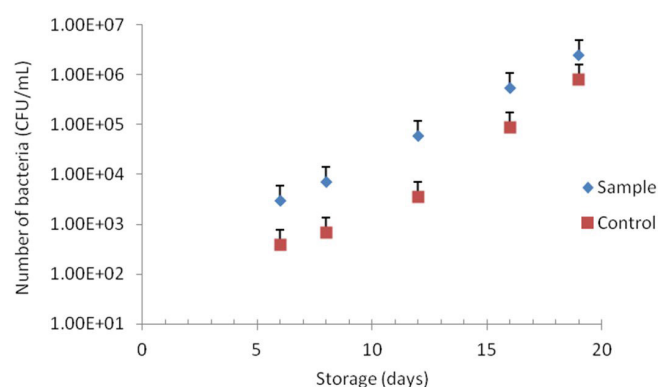


Figure 2. Effect of the holding time and storage temperature on bacterial numbers.

(control) showed bacterial counts of $< 1 \times 10^4$ CFU mL⁻¹ and $< 1 \times 10^3$ CFU mL⁻¹, respectively. A rapid increase in the SPC at 7 °C was observed by Zahar et al. (1996), who found that on day 5 the total aerobic bacterial count reached from 1×10^5 to 1×10^6 CFU mL⁻¹. If the storage temperature of pasteurised milk is increased by 2 °C, the storage capacity of milk is reduced by 50% (RYSSTAD; KOLSTAD, 2006). A comparison of the total bacterial numbers was made on day 14 of the shelf life and showed that the total count for the temperature abused milk had reached 1×10^5 CFU mL⁻¹, whilst the control milk samples had counts of 1×10^4 CFU mL⁻¹, still within an acceptable level. On day 19, the bacterial numbers in both the temperature abused and control milk samples had extrapolated the shelf life limit, which typically ends when the SPC reaches above 1×10^6 CFU mL⁻¹. The differences between the control and temperature abused milk samples were found to be significant ($P < 0.05$) at the end of the shelf life. The results obtained in this trial confirmed that exposure to an abusive storage temperature (25 °C) reduced the shelf life of pasteurised skim milk since the bacteria could grow if the cold chain was not maintained and the milk allowed to warm up during transport to home (CHANDLER; McMEEKIN, 1985). These authors further reported that the shelf-life of milk would be longer and safer if the milk was stored at low temperatures.

The results shown in Figure 3 indicated that the pH of the milk decreased faster when the temperature was increased to 25 °C and held for 8 hours than the pH of the milk stored at low temperature (control). The average pH of the fresh milk samples was 6.75. Statistically, no significant difference ($P > 0.05$) was observed between the pH of the milk held at the two storage temperatures for up to 14 days. The pH value of the milk samples remained within the normal range of from 6.60 to 6.75 at the two temperatures up to 21 days.

On day 6, the pH of the milk samples held at 25 °C and 5 °C was 6.72 and 6.75, respectively. After day 8, the

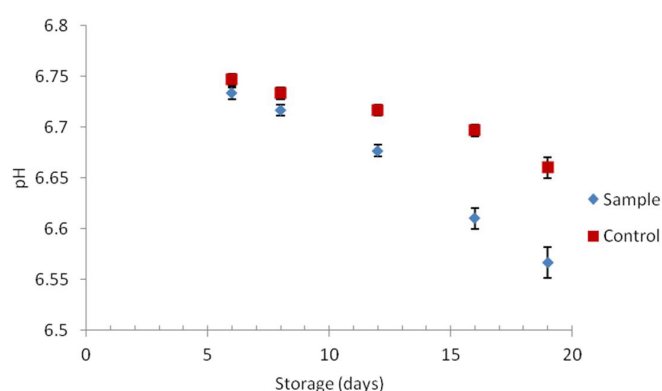


Figure 3. Effect of storage time and temperature abuse on the pH value.

pH values started to decrease gradually. On day 14, the pH of the temperature abused milk samples dropped to 6.66, whereas the pH of the control samples showed no significant change. When compared, it was observed that the pH of the temperature abused samples decreased constantly to 6.56 with higher bacterial counts, but the control samples were still within the normal pH range after storage for 19 days. The results confirmed that a prolonged storage period and temperature abuse can increase the acidity level and consequently affect milk quality.

3.3. Determination of the bacterial counts in skim milk subjected to temperature abuse at 25 °C for 6 hours and subsequently stored at 5 °C for 18 hours

In this trial, the temperature abuse regime was referred to as the fluctuating regime. The air temperature at both 5 °C and 25 °C showed the same pattern of temperature fluctuations when the milk samples were subjected to simulated temperature fluctuations for 7 days, which consisted of incubating the milk at 25 °C for 6 hours, followed by storage at 5 °C for 18 hours as shown in Figure 4. This pattern followed the same cycle every day of the experiment except for day 4. An attempt was made to simulate the real life scenario normally practiced by average consumers, where the milk is poured into a serving container after taking it out of the refrigerator, and then left at room temperature on the table for a period of time before returning to the original container in the refrigerator.

The results showed that the milk samples responded quickly and directly to the temperature fluctuations with respect to the length of the shelf life. The initial temperatures of the milk samples for each cycle were the same. However, on the first day, there was a slight temperature variation, which could have been due to the effect of the outside temperature that the milk experienced when the samples were transported from the supermarket to the laboratory. This study was designed to quantify how the milk temperature changes with room temperature when

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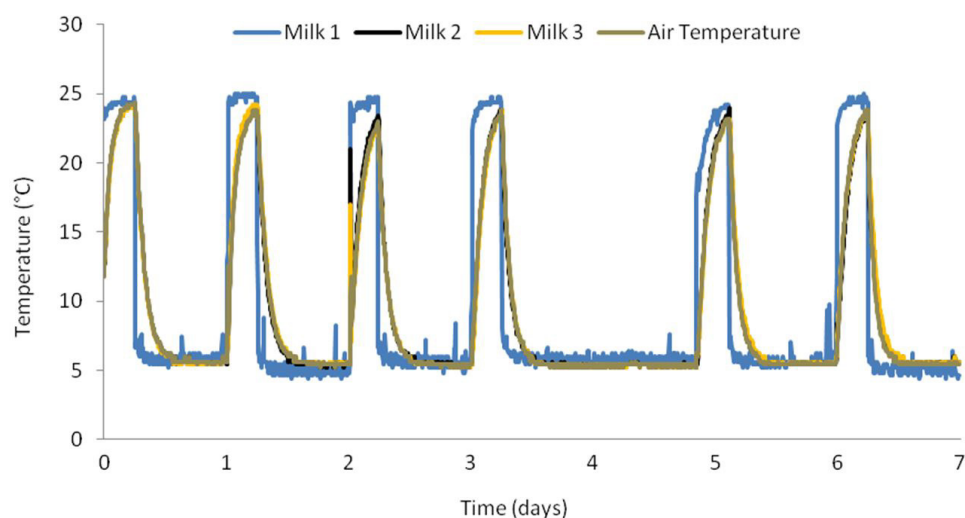


Figure 4. Holding time and air temperature fluctuation cycles for 3 pasteurised skim milk samples subjected to temperature abuse at 25 °C for 6 hours and subsequently held at 5 °C for 18 hours.

the milk is left outside the refrigerator. However, it cannot be suggested to what extent the milk temperatures are affected by the air temperature and cycles. It was found that the temperature of the milk samples was not lower than the air temperature. In this test, day 4 was exempted in an attempt to understand the fluctuation of the refrigerator temperature during the cycle. It was found that the milk temperature did not fluctuate at the same speed or to the same extent as the air temperature.

After completing the temperature fluctuation test, the milk samples were exposed to 25 °C to determine the effect of exposure of the milk to abusive conditions on the microorganisms, and compare the bacterial count in the milk with that in the milk stored at 5 °C. The fluctuating temperature regimes had a great effect on the potential bacterial growth in the milk as shown in Figure 5.

The highest bacterial counts were found in the temperature abused milk samples at 25 °C, whilst the lowest bacterial counts were found in the control samples kept at 5 °C. The results of this test indicated that the bacterial counts in the temperature abused milk at 25 °C were significantly different ($P < 0.05$) from those of the control milk samples that were held at 5 °C for 11 days. A gradual but steady increase in the total bacterial counts at 5 and 25 °C were found. The control and temperature abused samples were all within the acceptable microbial levels up to 5 days of storage.

On day 5, the bacterial numbers for the control and temperature abused milk samples were $< 1 \times 10^3$ CFU mL⁻¹ and $< 1 \times 10^4$ CFU mL⁻¹ respectively. After day 9, the control samples exceeded the acceptable level of 1×10^4 CFU mL⁻¹, and reached to 1×10^7 CFU mL⁻¹ on day 11. The milk samples subjected to room temperature (25 °C) showed that the microorganisms increased rapidly and soon reached

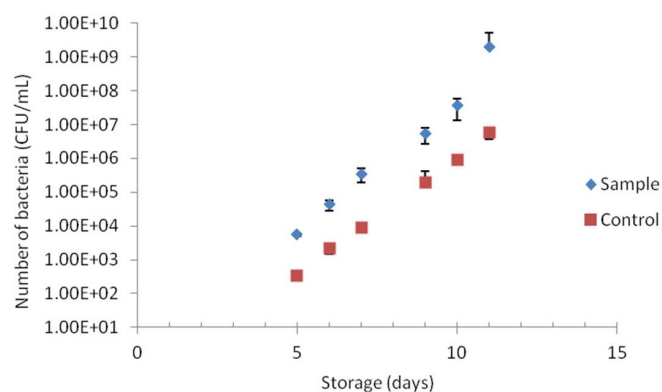


Figure 5. Effect of the holding time and storage temperature on the bacterial counts.

an unacceptable level ($> 1 \times 10^4$ CFU mL⁻¹). This rapid rise continued up to day 11 when the bacterial numbers reached $> 1 \times 10^9$ CFU mL⁻¹.

It is evident that higher bacterial counts are an indicator of time-temperature abuse in milk with higher bacterial counts resulting from holding the milk for too long at a high temperature, allowing the bacteria to grow faster. Thus the temperature abuse of milk can change the microbial growth in the milk (SCHAFFNER et al., 2003). Burdova et al. (2002) reported that a storage temperature of 10 °C reduced the shelf life of pasteurised milk by nearly two-thirds as compared to a storage temperature of 4 °C.

In this simulated test, the bottles were opened and the milk poured into containers, followed by storage of the milk bottles at a constant temperature of 5 °C to produce realistic results. When the bottles were opened multiple times the oxygen supply in the bottles increased facilitating growth of the microorganisms in the milk. This observable

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fact was supported by Sinclair and Stokes (1963), who found that the availability of oxygen resulted in higher bacterial counts in the milk, but they acknowledged that the growth rates were still dependent on the temperature at which the bacteria grow most rapidly.

The results obtained from this trial showed that the mean pH values of the milk samples temperature abused at 25 °C continued to decrease from 6.72 to 6.56 after 11 days of storage. The initial pH of the control milk samples was 6.75 and gradually decreased towards the end of the storage period, but still remained within the normal pH range of milk as shown in Figure 6. It was noticed that the decrease in pH was significant ($P < 0.05$) from day 5 to 11 during storage. The organoleptic changes in the milk were the indication of spoilage, which became very apparent with the high bacterial counts in this test. The spoilage of the milk samples was evidenced by curdling, a bitter smell, rancid and acidic pH. The reason for the fall in pH could be due to the growth of lactobacillus and the production of lactic acid in the milk. In addition to this, other chemical reactions possibly occurred due to psychrotrophic bacterial activity (CHAMPAGNE et al., 1994). A direct relationship between the temperature and shelf-life of the milk was observed, where the shelf-life was shortened with increasing temperature. Milk turns sour depending on the temperature, the milk sugar (lactose - $C_{12}H_{22}O_{11}$) being mainly responsible for the souring (i.e. growth of bacteria). The lactic acid ($C_3H_6O_3$) produced from the decomposition of the lactose by the lactase present in the lactobacillus reduces the pH of the milk and when the pH reaches 4.5 - 4.7, the milk protein casein coagulates. Thus the conversion of lactose into lactic acid turns the milk sour (WALSTRA et al., 2006). The milk samples showed a decrease in pH with increase in storage time at both temperatures, and consequently the bacterial counts also increased.

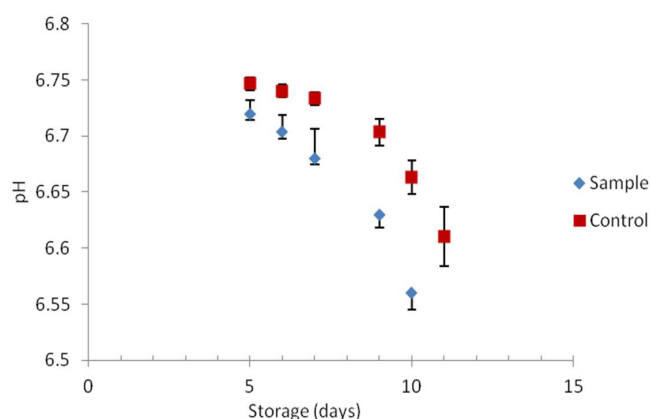


Figure 6. Effect of storage time and temperature abuse on the pH values.

Temperature fluctuations are not unusual during transportation, storage or transference of milk to and from the refrigerator or when leaving milk outside the fridge for an extended period of time. Many studies have reported that consumers set the refrigerator temperature above the recommended one of 4.4°C, due to a lack of awareness (JOHNSON et al., 1998; KOSA et al., 2007), which might limit the shelf-life of milk.

4 Conclusion

The simulated temperature abuse study provided evidence that holding milk for 8 or 6 hours at 25°C led to a reduction in the shelf-life as compared to storage at 5°C. This shows the importance of the low temperature storage of pasteurized milk in the home to maintain its quality, avoiding temperature fluctuations throughout the entire cold chain, thus preventing spoilage and extending the shelf life. Consumers require greater awareness of the potential effect of temperature abuse on milk. Moreover, there is a need for the dairy industry to adopt an assurance quality system, such as HACCP or GMP throughout the entire milk chain, since there is a real risk of milk spoilage during the shelf-life. In addition, since the sensory evaluation is the key factor to determine the shelf-life, the survival analysis is also needed to examine consumer acceptability and rejection of the milk under temperature abuse conditions. Storage temperatures below 5 °C are a key factor in extending the shelf-life of pasteurized milk.

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