

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.703.268>

Microbiological Quality and Safety of Unfinished UHT Milk at Storage Time-Temperature Abuse

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ABSTRACT

The objective of this study is to determine the effect of storage time-temperature abuse on the microbiological quality and safety of unfinished UHT milk. Therefore, the present study attempts to imitate the condition of unfinished UHT milk during consumption. The UHT milk was opened and drunk and then the UHT milk was kept at three different storage temperature of $15 \pm 1^\circ\text{C}$, $25 \pm 1^\circ\text{C}$, $35 \pm 1^\circ\text{C}$ for 2, 4, and 6 hours. The microbiological analysis had been conducted which includes the account of the number of bacteria regarding Total Plate Count (TPC), Yeast and moulds count, Mesophilic sporeformers count, *Bacillus Cereus*, *Staphylococcus aureus*, Total and Fecal Coliform, *Listeria monocytogenes*. At the 35°C storage temperature for 6 hours storage time for unfinished UHT milk, results showed mean of TPC $7.91 \log_{10}$ CFU/mL, Yeast and Moulds counts $6.84 \log_{10}$ CFU/mL, Mesophilic sporeformers counts $7.55 \log_{10}$ CFU/mL, *Bacillus cereus* counts $7.73 \log_{10}$ CFU/mL, *Staphylococcus aureus* counts $8.30 \log_{10}$ CFU/mL and *Listeria monocytogenes* counts 100 CFU/mL. This indicates that unfinished UHT milk is not safe to consume at this condition since value of all bacteria counts exceeded the maximum limit (100 CFU/mL for *L. monocytogenes* and $5.00 \log_{10}$ CFU/mL for others) permitted by Food Act 1983 (Act 281) and Food Regulations 1985 and Netherlands National Food and Commodities Law. Interestingly, there is no detection of total and fecal coliform in the sample.

Keywords

UHT milk,
Unfinished, Storage
temperature,
Storage time

Article Info

Accepted:
20 February 2018
Available Online:
10 March 2018

Introduction

The milk demand increase globally due to the awareness to choose nutritional food in daily meals. Milk is a nutritious food and suitable for all range consumer. It is a source of protein and calcium which important to our body needs. Milk and dairy products provided more than 70% of calcium in the US diet (Ding *et al.*, 2016; Huth *et al.*, 2006). In

Malaysia, 'Program Susu 1Malaysia (PS1M)' under Ministry of Health Malaysia tend to increase the awareness and help students in primary school to get sufficient nutrition by consuming UHT (Ultra-high temperature) milk supplied in individual boxes for each student.

However, milk is a perishable food which susceptible in rapid spoilage by the action of

the naturally enzyme and contaminating microorganisms. Thus, it becomes unsafe to consume. Foodborne disease will depend on the extent of food safety control in place through food production, processing and distribution keeping food clean, separation of raw and cooked, and cooking thoroughly, keeping food at safe temperature and using safe water and raw materials are some of the important points especially for safety of food of humans (Addis and Sisay, 2015). Painter *et al.*, (2013) stated that foodborne outbreaks cases associated with the consumption of milk and dairy products occur each year and an estimated 6,561,951 annual foodborne illnesses are attributed to dairy products caused by a variety of pathogens in the United States, resulting in an estimated 7464 hospitalisations and 121 deaths. Many food poisoning cases in Malaysia were reported was involving foodborne disease after consuming milk, but the causes are still unknown. However, one possible reason could be due to student practices, that prone to open and drink some of the milk, but not finish it. The unfinished milk is just left at room temperature for few hours until they drink it again.

Ultra High Temperature (UHT) processing heats the milk at a temperature of 138°C for a few seconds destroys all microbes present in milk as well as inactivates all the enzymes, thus gives the milk a better shelf-life and a more acceptable sensory perception (Bylund, 1995). UHT milk in aseptic packaging is a shelf stable product. Safety of UHT milk depends primarily upon ensuring that the heat-processing is adequate and that container integrity is maintained (ICMSF, 1978). The prolong shelf life will secure the industries and consumer risk toward spoiled products and foodborne disease. Heat treatment as one of the processing steps in the manufacturing of milk that will give an impact to its microbiological quality before packaged as a final product.

Milk also contains microflora as the milk characteristics itself is a suitable medium for microbial growth. This microflora can induce the spoilage of milk together with suitable temperature and time condition. In addition, presumptive bacteria that are alive and able to grow in milk are *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, *Clostridium* and *Bacillus cereus*. Several outbreaks of Listeriosis have been associated with contaminated food such as, vegetables, dairy products as soft cheeses, pasteurised milk and meat products, on which *L. monocytogenes* can multiply even at low temperatures (Chaturongakul and Boor, 2006; Consuelo *et al.*, 2009). Besides, *C. peffrigens* and *B. cereus* both can survive the heat treatment.

Storage temperature and time together with pH will greatly influence the survival and growth of microorganisms. Microbial growth in the milk that is shelf stable for many months also can be influenced by factors such as moisture content, pH, processing parameters, and temperature of storage (Ledenbach and Marshall, 2010). There are researches on milk spoilage, and the factors contribute to the spoilage for raw milk (AbdElrahman *et al.*, 2013; Schmidt *et al.*, 2012). Nonetheless, there is a research gap for the effect of microbiological and physicochemical quality of unfinished UHT milk after being susceptible to the favourable condition. Therefore, this study was done in order to determine the effect of storage time-temperature abuse on microbiological quality and safety of unfinished UHT milk.

Materials and Methods

Samples

The commercial UHT milk was purchased. Each sample contains 200 mL of UHT milk. Imitation of unfinished milk followed by storage at certain temperature and time on the

milk sample was conducted at Food Microbiology Laboratory, Faculty of Food Science and Technology, Universiti Putra Malaysia.

The unfinished sample was defined by the unfinished milk, which was opened and drank by the one person.

Experimental Design

The unfinished milk was stored at three temperatures (15, 25 and 35°C) for three storage time (2, 4 and 6 h). The samples were conducted and analyzed within 12 hours. The interactions between the microbial growth of bacteria and pH with three different storage temperatures and three different storage times were analysed. All analysis was conducted by independent triplicated. Each replicated represents nine boxes of milk samples.

Microbiological analysis

UHT milk samples were analyzed using standard procedures (APHA, 2001). A 25 mL of unfinished UHT milk was aseptically transferred to a sterile stomacher bag and mix thoroughly, with 225 mL of sterile 0.1% peptone water. Appropriate decimal serial dilutions of the sample were prepared using the same diluents to 10^{-7} and spread on different growth media. Total plate counts (TPC) were determined using the Plate Count Agar (PCA) (OXOID), incubated at 37°C for 48 hours. Yeast and mould counts were determined using the Potato Dextrose Agar (PDA) (OXOID), incubated at 32°C for five to seven days. Mesophilic sporeformer counts were determined using the Dextrose Tryptone Agar (DTA) (OXOID), incubated at 37°C for 48 hours, after heating the inoculated agar at 80°C for ten minutes to destroy vegetative cells. *Bacillus cereus* inoculated using *Bacillus Cereus* Selective Agar Base (OXOID) with Egg Yolk Emulsion, incubated at 37°C for 48 hours. *Staphylococcus aureus*

was enumerated using the Baird-Parker Agar (BPA)(OXOID) with Egg Yolk Tellurite Emulsion which was incubated at 37°C (IDF 145A:1997) for 48 hours; while total coliform and fecal coliform conducted by using MacConkey Agar (OXOID), incubated at 37°C for 48 hours. *Listeria monocytogenes* was enumerated using PALCAM Agar Base (OXOID), incubated at 30°C for 48 hours (IDF143A:1995) by using Buffered Listeria Enrichment Broth (OXOID), incubated at 30°C for 48 hours. All results were expressed as \log_{10} colony forming unit/gram (\log_{10} CFU/mL).

Determination of pH

Methods used for the determination of pH were adopted from the Microbiological Laboratory Guidebook of USDA/FSIS (Dey and Lattuada, 1998).

The pH meter (Mettler Toledo Seven Multi pH) was warmed up before measuring the sample. The calibration of this pH meter is conducted by using buffered solutions pH 4.00 and pH 7.00. Then a sample is prepared in sterile 25mL stork bottle. The electrode of the pH meter was rinsed and blotted. After that, the electrode was immersed in the sample. The pH reading for the sample measured was recorded after the pH meter was stabilized for one minute. The means of the two measurements were recorded. Measurement of pH for the sample is repeated in triplicate.

Statistical analysis

All data collected were analyzed using the Minitab 16 statistical software (MANITAB Inc., State College, PA), using two-way analysis of variance (ANOVA) to identify the significant differences between factors in the present study. Thus, all the data reported were the means of triplicates.

Results and Discussion

Microbiological quality and safety of unfinished UHT milk at different storage temperature and time

Total plate count, yeast and moulds count and mesophilic sporeformers count of the UHT milk (control) were 4.48 ± 0.25 ; 4.43 ± 0.21 and $4.32 \pm 0.10 \log_{10}$ CFU/mL (Fig. 1), respectively. *Bacillus cereus*, *Staphylococcus aureus*, Total and Fecal Coliform and *Listeria monocytogenes* were not detected. The microbiological quality of the unfinished UHT milk with different storage temperature and time was tabulated in Table 1.

The microbial load of yeast and moulds in UHT milk was in contrast with the finding from the study by Al-Tahiri (2005), who reported absent of yeast and moulds in their UHT milk samples (Gamal *et al.*, 2015). Microbial load of the tested sample may differ where the UHT milk may come from different bulk tank and pipelines. Furthermore, borderline for microbial growth in TPC of UHT product must be absent (Centre for Food Safety, 2014). UHT milk should not contain any viable microorganisms (Carl and Mary, 2014). Contamination during the UHT milk processing could be the reason for the present of microorganisms in the end product.

Table 1 shows the microbiological quality and safety of unfinished UHT milk at three different temperatures and three storage time. The findings reveal an increase of bacteria counts at different storage temperature and time. As expected, there are a higher number of microbial loads at the 35°C storage temperature for 6 hours storage time of unfinished UHT milk tested. This explains that the unfinished UHT milk is not safe to consume when it stored (or left) at 35°C for 6 hours. The unfinished UHT milk turns to be slimy, viscous and fermented off-flavour at

this critical condition as gelation of milk started (by observation).

Total Plate Count (TPC) of unfinished UHT milk at 15°C for 2, 4, and 6 hours were 4.56 ± 0.42 ; 4.85 ± 0.59 and $6.24 \pm 0.34 \log_{10}$ CFU/mL, at 25°C for 2, 4, and 6 hours were 6.05 ± 1.04 ; 5.97 ± 0.50 and $7.54 \pm 0.86 \log_{10}$ CFU/mL, at 35°C for 2, 4, and 6 hours were 5.27 ± 0.59 ; 6.00 ± 0.86 and $7.91 \pm 1.11 \log_{10}$ CFU/mL (Fig. 2), respectively. From the graph of Figure 2, it shows the microbial growth increase as storage temperature and time increase in unfinished UHT milk.

The TPC at 5 and 10°C as stated by Abd Elrahman *et al.*, (2013) are 2.45 and 2.53 \log_{10} CFU/mL, lower than the values from the present study. In this study, an increase of microbial growth of TPC was observed started at 15°C for 2 hours. Based on the Food Act 1983 (Act 281) and Food Regulations 1985 (2016), the maximum growth value of microbiological standard for TPC is 5.0 \log_{10} CFU/mL of heat-treated milk. In this study, the values of the TPC for the unfinished UHT milk had exceeded the maximum values starting from 25°C for 2 hours (6.05 \log_{10} CFU/mL). Koushki *et al.*, (2016) stated that total microbial count of pasteurised milk on an expired date is 4.88 \log_{10} CFU/mL.

Interestingly, TPC value of UHT milk at 15°C in 4 hours (4.85 \log_{10} CFU/mL) shows in Figure 2 is close to the value of microbial growth for expired date milk. The growth value of microbiological standard for TPC considered acceptable below 5.0 \log_{10} CFU/mL since the sample was opened and drank.

Although the bacterial count was provided in this study, the TPC is only used as an indicator of bacterial populations in unfinished UHT milk. El-kholy *et al.*, (2016), stated that most foods especially dairy

products should be regarded as unsatisfactory when a large number of microorganisms are present even though these organisms are not known to be pathogenic. They also stated that high aerobic plate counts indicate contaminated raw materials, unsatisfactory processing from a sanitary point of view or cross-contamination in milk. Specific microbiological testing was needed on pathogenic and spoilage bacteria of the sample. On the other hand, microbial growth regularly increased as storage temperature and time increased for TPC.

Yeast and moulds count of unfinished UHT milk at 15°C for 2, 4, and 6 hours were 4.99 ± 0.69 ; 5.06 ± 0.39 and 7.15 ± 0.77 log₁₀ CFU/mL, at 25°C for 2, 4, and 6 hours were 4.60 ± 0.53 ; 5.54 ± 0.52 and 7.31 ± 0.39 log₁₀ CFU/mL, at 35°C for 2, 4, and 6 hours were 4.50 ± 0.28 ; 5.56 ± 1.02 and 6.84 ± 0.44 log₁₀ CFU/mL (Fig. 3), respectively. Yeast and moulds count start to increase at 15°C for 4 hours (5.06 log₁₀ CFU/mL) as presented in Figure 3. It explained that yeast and moulds are able to survive at 15°C and required 4 hours after opened and drunk to grow under the same temperature. Thus, it is not safe for consumption as related to the Food Act 1983 (Act 281) and Food Regulations 1985 (2016).

Presumptive yeast and moulds identified (based on morphology) in the present study are *Saccharomyces cerevisiae*, *Hericium corolloides*, *Penicillium spp.*, *Aspergillus niger*, *Geotrichum candidum*, *Fusarium spp.*, *Rhizopus stolonifer* and *Rhizopus spp.*, and *Aspergillus flavus* as referred in the study by Pitt and Hocking, (2009).

Fusarium oxysporum is found in flavoured UHT milk in Australia owing to the production of thickly walled *Chlamydoconidia* and the ability to tolerate low oxygen tensions (Sørhaug, 2011). *Aspergillus spp.* and *Penicillium spp.* can grow in milk results from poor sanitation in the processing plant

and entry of mould spores from cross-contamination (Hubert, 2014). Yeasty and fermented off-flavours and gassy appearance are often detected when yeast grow to 5.0 to 6.0 log₁₀ CFU/mL (Ledenbach and Marshall, 2010). In Figure 3, yeast and moulds count slightly increased as storage temperature and time increased in unfinished UHT milk.

Mesophilic sporeformers count of unfinished UHT milk at 15°C for 2, 4, and 6 hours were 4.54 ± 0.56 ; 5.20 ± 0.28 and 7.04 ± 0.50 log₁₀ CFU/mL, at 25°C for 2, 4, and 6 hours were 4.49 ± 0.014 ; 5.56 ± 0.69 and 7.19 ± 0.59 log₁₀ CFU/mL, at 35°C for 2, 4, and 6 hours were 4.37 ± 0.52 ; 5.73 ± 0.98 and 7.55 ± 0.22 log₁₀ CFU/mL (Fig. 4), respectively.

The value of mesophilic sporeformers count (5.20 ± 1.36 log₁₀ CFU/mL) at 15°C for 4 hours exceeding the maximum limit stated by European Union (EU) standards. EU standards for the total count of mesophilic sporeformer in milk are ≤ 5.0 log₁₀ CFU/mL (Samaržija *et al.*, 2012). In this study, the microbial growth of mesophilic sporeformers exceeding the limit starting at 15°C for 2 hours. This explains the existed mesophilic sporeformers in UHT milk survived during UHT processing and increased in microbial growth when exposed to a favourable condition. Moreover, cross-contamination had occurred and increased microbial load in samples.

Spore-forming bacteria that are present in milk are important because the formation of the spore by the bacterium allows it to be resistant to heat, freezing, chemicals, and other adverse environments that milk had undergoes during processing and preparation (Cousin, 1989). In Figure 4, mesophilic sporeformers count increased as storage temperature and time increased in unfinished UHT milk. As stated in a study by Set low (2003), spores will remain dormant until the conditions become favourable for the change.

Table.1 The microbiological quality and safety (Total Plate Count, Yeast and Moulds, Mesophilic spore formers, *Bacillus cereus*, *Staphylococcus aureus*, Total and Fecal Coliform and *Listeria monocytogenes*) (log₁₀ CFU/mL) of unfinished UHT Milk at different storage time-temperature abuse

| Microbial Profile | Time (Hour) | Log ₁₀ CFU/mL | | |
|--------------------------------|-------------|--------------------------|--------------------------|--------------------------|
| | | Temperature (°C) | | |
| | | 15 | 25 | 35 |
| Total plate count | 2 | 4.56±0.42 ^{Ba} | 6.05±1.04 ^{Ba} | 5.27±0.59 ^{Ba} |
| | 4 | 4.85±0.59 ^{Ba} | 5.97±0.50 ^{Ba} | 6.00±0.86 ^{ABa} |
| | 6 | 6.24±0.34 ^{Aa} | 7.54±0.86 ^{Aa} | 7.91±1.11 ^{Aa} |
| Yeast and moulds count | 2 | 4.99±0.69 ^{Ba} | 4.60±0.53 ^{Ba} | 4.50±0.28 ^{Ba} |
| | 4 | 5.06±0.39 ^{Ba} | 5.54±0.52 ^{Ba} | 5.56±1.02 ^{Ba} |
| | 6 | 7.15± 0.77 ^{Aa} | 7.31±0.39 ^{Aa} | 6.84±0.44 ^{Aa} |
| Mesophilic spore formers count | 2 | 4.54±0.56 ^{Ba} | 4.49±0.014 ^{Ba} | 4.37±0.52 ^{Ba} |
| | 4 | 5.20±0.28 ^{Ba} | 5.56±0.69 ^{ABa} | 5.73±0.98 ^{ABa} |
| | 6 | 7.04±0.50 ^{Aa} | 7.19±0.059 ^{Aa} | 7.55±0.22 ^{Aa} |
| <i>Bacillus cereus</i> | 2 | 4.48±0.000 ^{Ba} | 4.88±0.81 ^{Ba} | 4.92±1.29 ^{Aa} |
| | 4 | 4.84±0.77 ^{ABa} | 5.48±1.05 ^{Ba} | 6.12±0.39 ^{Aa} |
| | 6 | 6.62±0.83 ^{Aa} | 7.68±0.00 ^{Aa} | 7.73±1.02 ^{Ba} |
| <i>Staphylococcus aureus</i> | 2 | 0.00±0.00 ^{Aa} | 4.93±0.11 ^{Ba} | 4.30±0.00 ^{Ba} |
| | 4 | 0.00±0.00 ^{AB} | 4.87±0.23 ^{Ba} | 5.56±0.62 ^{BaB} |
| | 6 | 8.15±0.21 ^{Ba} | 7.53±0.92 ^{Aa} | 8.30±0.00 ^{Aa} |
| Total and fecal coliform | 2 | N/D | N/D | N/D |
| | 4 | N/D | N/D | N/D |
| | 6 | N/D | N/D | N/D |
| <i>Listeria monocytogenes</i> | | | CFU/mL | |
| | 2 | N/D | N/D | 100 |
| | 4 | N/D | N/D | 100 |
| | 6 | N/D | N/D | 100 |

^{A-B} Means with different uppercase superscripts are significantly different (p<0.05) against row

^{a-b} Means with different lowercase superscripts are significantly different (p<0.05) against column

Table.2 The pH of unfinished UHT Milk at different storage time-temperature abuse.

| | | pH value | | |
|-------------|---|--------------------------|----------------------------|----------------------------|
| | | Temperature (°C) | | |
| | | 15 | 25 | 35 |
| Time (Hour) | 2 | 6.50±0.31 ^{Aa} | 6.43±0.0058 ^{ABa} | 6.46±0.10 ^{Aa} |
| | 4 | 6.55±0.044 ^{Aa} | 6.44±0.0058 ^{Ab} | 6.42±0.0057 ^{ABb} |
| | 6 | 6.52±0.021 ^{Aa} | 6.41±0.010 ^{Bb} | 6.29±0.00 ^{Bc} |

A-B Means with different uppercase superscripts are significantly different (p<0.05) against row
 a-b-c Means with different lowercase superscripts are significantly different (p<0.05) against column

FIGURES

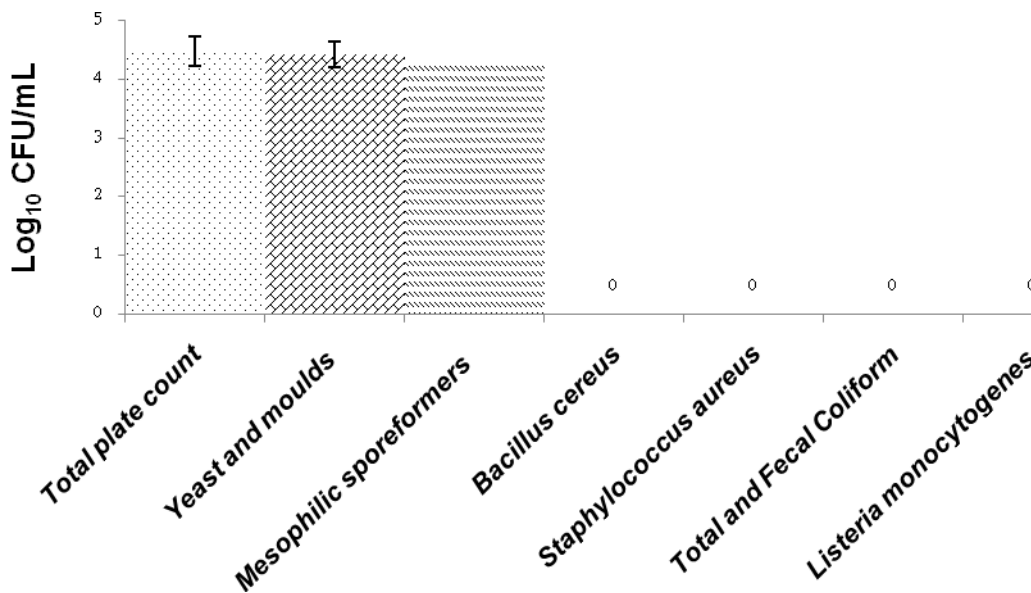


Fig.1 The microbiological quality and safety of UHT milk (Total Plate Count, Yeast and Moulds, Mesophilic sporeformers, *Bacillus cereus*, *Staphylococcus aureus*, Total and Fecal Coliform and *Listeria monocytogenes*) (log₁₀ CFU/mL).

*Means (SD from seven determinations)

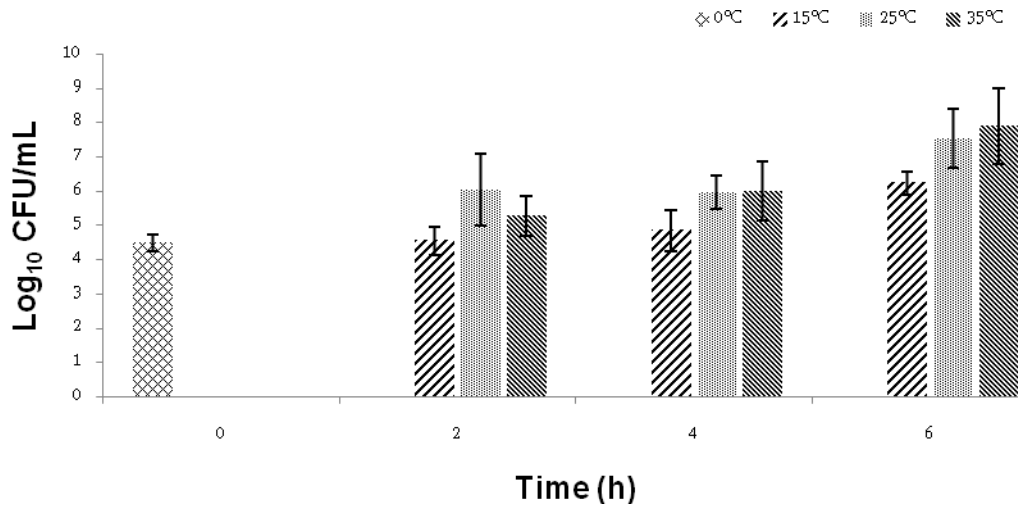


Fig.2 Total plate count (\log_{10} CFU/mL) of unfinished UHT milk at storage time-temperature of 15, 25 and 35°C for 2, 4 and 6 hours.
*Means (SD from three determinations)

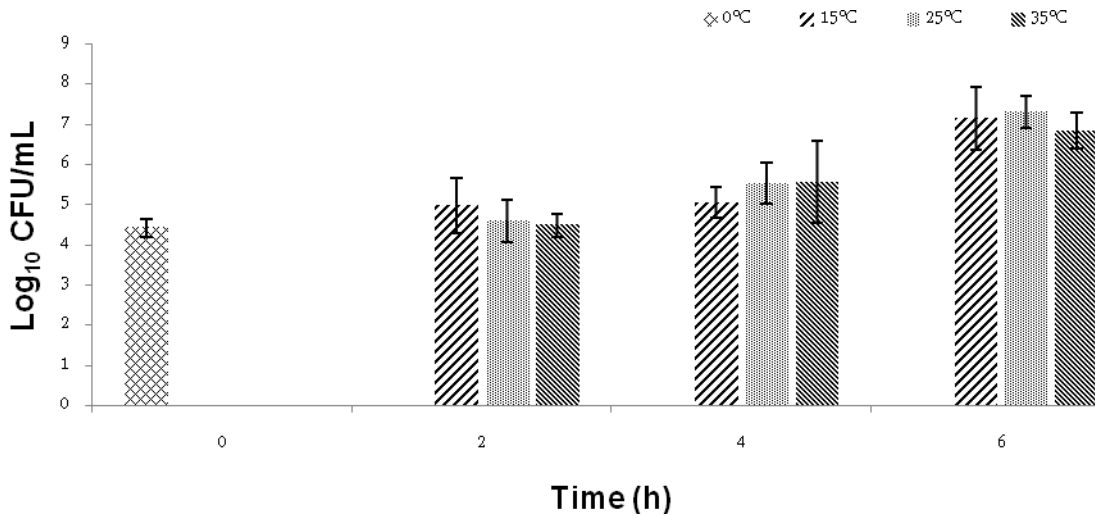


Fig.3 Yeast and moulds count (\log_{10} CFU/mL) of unfinished UHT milk at storage time-temperature of 15, 25 and 35°C for 2, 4 and 6 hours.
*Means (SD from three determinations)

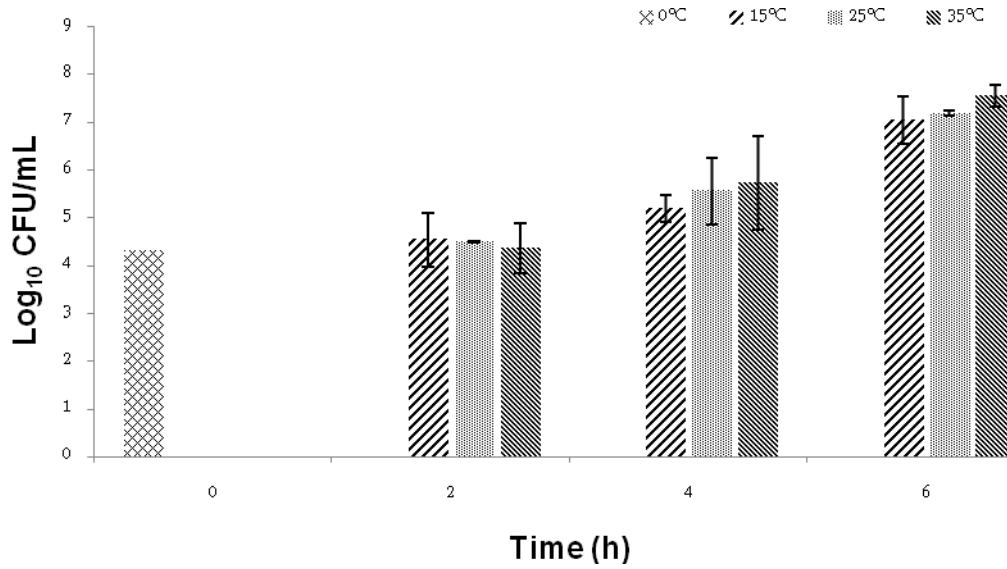


Fig.4 Mesophilic spore formers count (\log_{10} CFU/mL) of unfinished UHT milk at storage time-temperature of 15, 25 and 35°C for 2, 4 and 6 hours.
*Means (SD from three determinations)

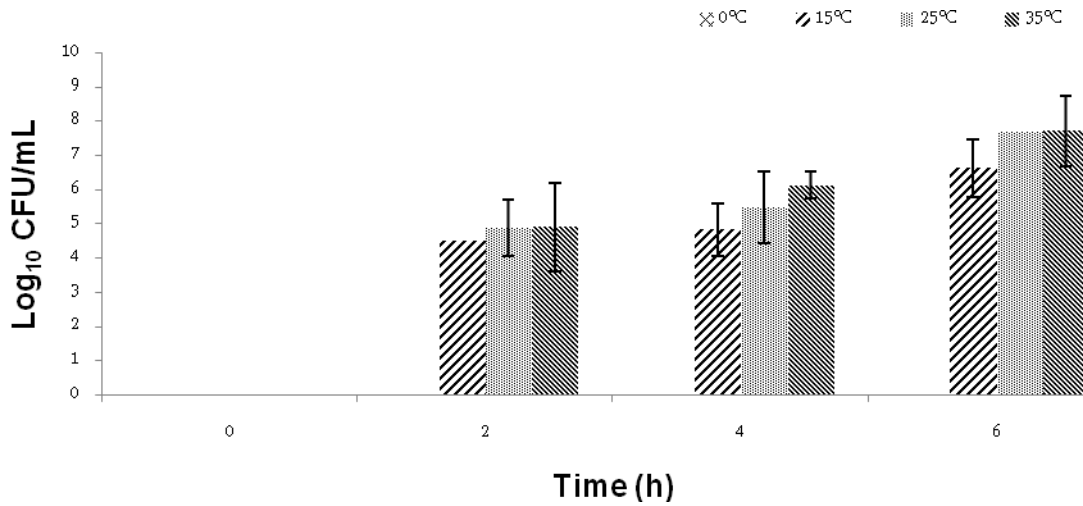


Fig.5 *Bacillus cereus* (\log_{10} CFU/mL) of unfinished UHT milk at storage time-temperature of 15, 25 and 35°C for 2, 4 and 6 hours.
*Means (SD from three determinations)

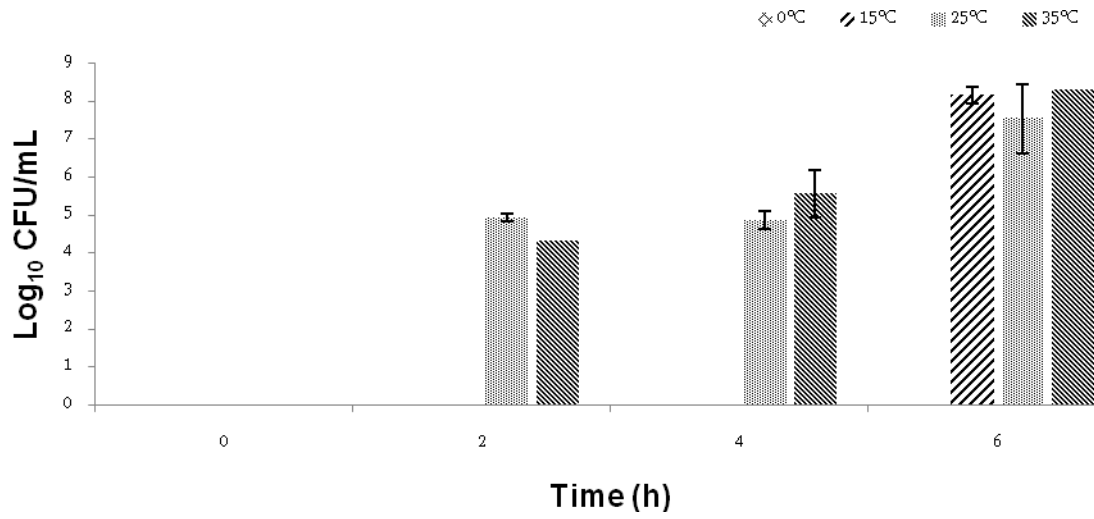


Fig.6 *Staphylococcus aureus* (\log_{10} CFU/mL) of unfinished UHT milk at storage time-temperature of 15, 25 and 35°C for 2, 4 and 6 hours.
*Means (SD from three determinations)

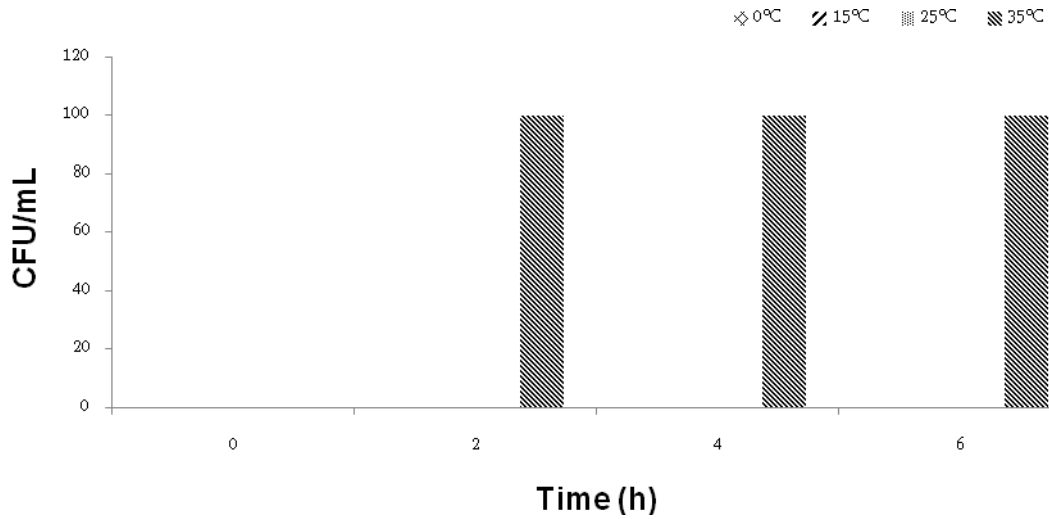


Fig.7 *Listeria monocytogenes* (CFU/mL) of leftover UHT milk of unfinished UHT milk at storage time-temperature of 15, 25 and 35°C for 2, 4 and 6 hours.
*Means (SD from three determinations)

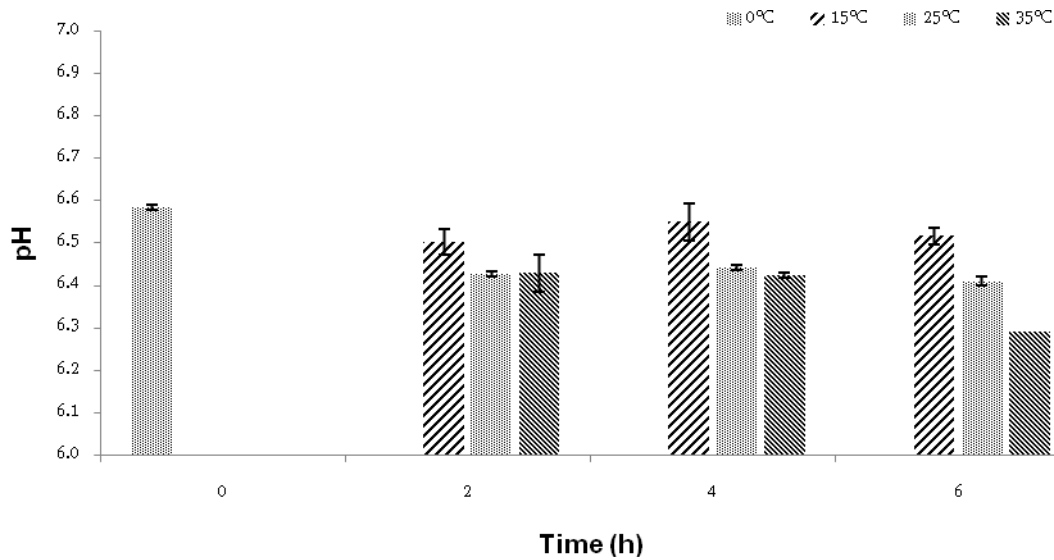


Fig.8 The pH of unfinished UHT milk at storage time-temperature of 15, 25 and 35°C for 2, 4 and 6 hours.*Means (SD from three determinations)

Meanwhile, several studies have reported that heat plays an important role in activation of spores and that its effect varies within species or even strains (Kim and Foegeding, 1990; Ghosh *et al.*, 2009; Anzueto, 2014).

Bacillus cereus of unfinished UHT milk at 15°C for 2, 4, and 6 hours were 4.48 ± 0.00 ; 4.84 ± 0.77 and 6.62 ± 0.83 log₁₀ CFU/mL, at 25°C for 2, 4, and 6 hours were 4.88 ± 0.81 ; 5.48 ± 1.04 and 7.68 ± 0.00 log₁₀ CFU/mL, at 35°C for 2, 4, and 6 hours were 4.92 ± 1.29 ; 6.12 ± 0.39 and 7.73 ± 1.03 log₁₀ CFU/mL (Fig. 5), respectively.

As reported by teGiffel *et al.*, (1996) and Kumari and Sarkar (2016), the incidence of higher levels of contamination by *B. cereus* in Netherlands for milk were between 1.0 to 4.0 log₁₀ CFU/mL. Thus, the value of *B. cereus* count (4.48 ± 1.04 log₁₀ CFU/mL) at 15°C for 2 hours exceeding the maximum limit stated. This explains unfinished UHT milk was not safe to consume starting at 15°C for 2 hours since the microbial growth increased from

this control point. Schmidt *et al.*, (2012) stated that the pasteurisation step eliminated all Gram-negative and lactic acid bacteria, leaving only high G + C Gram-positive and spore-formers. Presumptive *B. cereus* which is Gram-positive and spore-formers bacteria detected at 15°C from 2 to 6 hours incubation period range values from 4.48, 4.84 to 6.62 log₁₀ CFU/mL and increased to a maximum value at 35°C for 6 hours (7.73 log₁₀ CFU/mL) in this study. *B. cereus* group and *Bacillus subtilis* are the most important spoilage bacteria in dairy environments as stated by Lücking *et al.*, (2013). They are able to produce an extracellular enzyme that able to degrade the quality of milk by reducing the shelf life of processed milk and dairy products (Kumari and Sarkar, 2016). They also stated in their study that heat-resistant spores of *B. cereus* could survive heat treatment, be present in the milk, and able to germinate and grow during storage. This can lead to off-flavor, clotting, and gelation of milk (Chen *et al.*, 2003; Furtado, 2005; Kumari and Sarkar, 2016). In addition, the presence of *B. cereus*

indicates improper cleaning and sterilization of the UHT-Aseptic Packaging (UHT-AP) (Scott, 2008). In the study on food poisoning potential by *B. cereus* strains from Norwegian dairies, the strains were highly cytotoxic when grew at 25°C but less toxic when entering human body temperature, 37°C. Thus, these strains considered pose to minor risk about diarrhoeal food poisoning (Stenfors *et al.*, 2007) symptoms. This foodborne disease is caused by intoxication of diarrhoeal toxins after consumption of unfinished UHT milk. In Figure 5, presumptive *B. cereus* was able to grow up reaching to 8.0 log₁₀ CFU/mL at 35°C for 6 hours which is not safe for human consumption. Magalhães da Veiga Moreira *et al.*, (2016) reported that yeast, *B. cereus*, and *Bacillus spp.* are commonly isolated from fermentation of cocoa.

Staphylococcus aureus of unfinished UHT milk at 15°C for 2, 4, and 6 hours were 0.00 ± 0.00; 0.00 ± 0.00 and 8.15 ± 0.21 log₁₀ CFU/mL, at 25°C for 2, 4, and 6 hours were 4.93 ± 0.12; 4.87 ± 0.23 and 7.53 ± 0.92 log₁₀ CFU/mL, at 35°C for 2, 4, and 6 hours were 4.30 ± 0.00; 5.56 ± 0.62 and 8.30 ± 0.00 log₁₀ CFU/mL (Fig. 6), respectively. *S. aureus* shows a significant difference of its microbial growth in both storage temperature and time.

Several studies have considered 5.0 log₁₀ CFU/mL as the threshold of concern for *S. aureus* and its concentration more than 5.0 log₁₀ CFU/mL are unacceptable as state in FDA (1992), Rho and Schaffner (2007) and Ding *et al.*, (2016) since *S. aureus* able to produce enterotoxin in food in that population. At 35°C for 4 hours (5.56 log₁₀ CFU/mL), the *S. aureus* count indicates the unfinished UHT milk is not safe for consumption. Fujikawa and Morozumi, (2006) and Ding *et al.*, (2016), reported that *S. aureus* enterotoxin was detectable if the organism concentration was greater than 6.5 log₁₀ CFU/mL and the temperature higher than 15°C.

In Figure 6, the present study shows the concentration of *S. aureus* higher than 6.50 log₁₀ CFU/mL at 35°C after 6 hours storage time (8.30 log₁₀ CFU/mL). Based on the study reported from Ding *et al.*, (2016), the mean of *S. aureus* slightly increased during storage period even after heat treatment had applied during processing as compared to the *S. aureus* concentration before processing.

Microbial count in this product is important since this is Ready-To-Drink (RTD) dairy food where there is no heat treatment followed afterward (Centre of Food Safety, 2014). This can be an issue in microbial quality and safety to the consumer. The milk sample was opened and drank (by one person) for all samples before exposed to the experimental condition. This contributed to cross-contamination of *S. aureus* and other bacteria from the person and air source. Mastitis and hygiene of equipment during the milking (Ninoslava, 2012) can cause the contamination of *S. aureus* in UHT milk since *S. aureus* is resistant to heat. Thus, the microbial growth of *S. aureus* will increase as temperature and time increased under this condition. *Listeria monocytogenes* of unfinished UHT milk at 15°C for 2, 4, and 6 hours were 0; 0; and 100 CFU/mL, at 25°C for 2, 4, and 6 hours were 0; 0; and 100 CFU/mL, at 35°C for 2, 4, and 6 hours were 0; 0; and 100 CFU/mL (Fig. 7), respectively. *L. monocytogenes*, a pathogen of concern, causing foodborne disease grows rapidly in chocolate milk at 13°C as reported by Pearson and Marth, (1990) and Kotzekidou *et al.*, (2008). UHT-processing of milk can stop the microbial activity of the bacteria but could not completely kill the pathogenic bacteria. The increasing of microbial growth can be observed in the TPC, yeast and moulds, mesophilic spore formers count, *B. cereus*, and *S. aureus*. But for *L. monocytogenes*, the growth is not increasing as temperature and time increased.

They grow at $1.0 \log_{10}$ CFU/mL and stagnantly grow in the samples without any multiplication of colonies even though the serial dilution decreased to 10^{-4} , as well as storage time and temperature increased. The present study shows presumptive *L. monocytogenes* positively detected at 35°C for 2, 4 and 6 hours. As state by Chaturongakul and Boor (2006), *L. monocytogenes* can multiply at low temperatures and able to enter the carrier animals that shed the organism in the milk and feces due to the microorganism resistance to adverse environmental condition (Chan *et al.*, 2007).

The presence of *L. monocytogenes* found in unfinished UHT milk in this study constitutes a potential hazard for the consumer due to the storage temperature at 35°C for 2 and 6 hours holding time. Elliot and Elmer (2007) state that under the Netherlands National Food and Commodities Law, *L. monocytogenes* should be <100 CFU/mL for all food types except raw food, based on European Union (EU) draft regulations.

In RTE foods that can support growth, absent in five (25 mL) samples are required, unless the manufacturer can show that numbers will not exceed 100 CFU/mL throughout the stated shelf life of the product (IDF, 2013). In the US, Australia and New Zealand, regulations require absence of *L. monocytogenes* in five (25 mL) samples in all cases (IDF, 2013). Limitation of pathogens bacteria allowable depends on the country population resistance towards cases fatality rates.

Determination of pH of unfinished UHT Milk at different storage temperature and time

The pH of milk at 15°C for 2, 4, and 6 hours were 6.50 ± 0.31 ; 6.55 ± 0.042 and 6.52 ± 0.021 , at 25°C for 2, 4, and 6 hours were 6.43

± 0.0058 ; 6.44 ± 0.0058 and 6.41 ± 0.01 , at 35°C for 2, 4, and 6 hours were 6.46 ± 0.10 ; 6.42 ± 0.12 and 6.29 ± 0.00 (Fig. 8), respectively. The pH readings of unfinished UHT milk are optimum for growth since the values are between pH 6.00 to pH 7.00 (Table 2). The pH of milk normally ranges from 6.4 to 6.8 as reported by Li (2011).

Milk samples should range from pH 6.5-6.7 and sample which out of the pH range considered acid milk and being rejected (Lai *et al.*, 2016). The lower the pH, the stronger the acidity of milk indicated the milk started to ferment. Storage time and temperature have a great effect on pH values of the stored samples as reported by Kocak and Zadow (1985). Therefore, acidity increases as milk spoil. Thus, acidity can be quantified to measure milk quality (Lu *et al.*, 2013).

There was a present of microorganism's growth in Total Plate Count (TPC), yeast and molds, mesophilic spore formers, *Bacillus cereus*, *Staphylococcus aureus*, and *Listeria monocytogenes* in unfinished UHT milk. The unfinished UHT milk reveals an increasing trend of microbial growth when stored within time-temperature abuse. This indicates the unfinished UHT milk is diminishing in quality and unsafe to drink.

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How to cite this article:

Siti Norashikin, A., M.A.R. Nor-Khaizura and Wan Zunairah, W.I. 2018. Microbiological Quality and Safety of Unfinished UHT Milk at Storage Time-Temperature Abuse. *Int.J.Curr.Microbiol.App.Sci*. 7(03): 2278-2296. doi: <https://doi.org/10.20546/ijcmas.2018.703.268>