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Treated Cow Milk Quality Analysis in High-Temperature Short Time (HTST) Thermal Treatment using F-Value and Methylene Blue Reduction Test (MBRT)

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ABSTRACT

This study was conducted to evaluate raw and pasteurized cow milk regarding physical properties, microbiological quality, and lethality value of*Mycobacterium Paratuberculosis*(MAP)at different temperature and time combinations of the pasteurization process.Cow milk samples were pasteurized at high-temperature (70°C, 75°C, and 81°C) and short-time (15s and 25s) high temperature and short time (HTST) combinations. Raw and pasteurized (HTST) cow milk was analyzed, while commercial cow milk that undergo proses (HTST) was used as control. High-temperature short time (HTST) pasteurization showed a significant effect on the colour of raw and pasteurized cow milk ($p<0.05$) at every temperature. In addition, cow milk also indicated an increase in lightness and yellowness after HTST pasteurization.The microbiological quality of raw, pasteurized, and commercial cow milk is evaluated using the Methylene Blue Reduction (MBRT) test, a common, rapid, simple, and inexpensive method for microbiological quality evaluation.The MBRT on raw milk samples revealed that it was of poor quality. On the other hand, all pasteurized samples were good quality, and the commercial sample was excellent.Based on the evaluated F-values, the most suitable temperature and time combinationsin this study was70°C and 25s.

Keywords: HTST Pasteurization, Cow milk,colour measurement, Methylene blue reduction test, *Mycobacterium avium subsp. Paratuberculosis*.

INTRODUCTION

Milk is a popular beverage because of its nutritional value, a pleasing aroma, mouthfeel, and slightly sweet flavour. Due to its high nutritional value for humans, milk is an essential part of the diet of a large portion of the world's population. However, according to Huppertz.(2017)the abundant nutrient content in milk makes it an excellent medium for microbe growth. Therefore, it is easily polluted and spoiled if it is made unhygienic and treated carelessly. The discovery of a few milk pathogens has become more severe as milk can sustain massive increases in bacterial numbers (Chatterjee et al., 2006). *Mycobacterium paratuberculosis* (MAP) is present in raw cows' milk. Johne's disease (JD) is a disease that develops in ruminants due to *Mycobacterium paratuberculosis* (MAP), which causes chronic diarrhea, malnutrition, and muscle wasting(Rathnaiah et al., 2017). In order to inhibit this from happening, milk is introduced with thermal treatment to prevent microbedevelopment, prolong the shelf life of products, and improve their consistency.Two types of thermal treatment processes are applied in food processing: long temperature long-time (LTLT) 63^{o}C 30mins and high-temperature short time (HTST)72°C 15s pasteurization. LTLT pasteurization is also known as batch pasteurization, where the milk is heated up to a temperature of 63°C for 30 min. Meanwhile, HTST pasteurization is more familiar with continuous and flash pasteurization and high-temperature short-time combinations (Deak, 2014).

Milk pasteurization did not become popular until the late 1800s. Back then, milk used to be a famous carrier of tuberculosis. Pasteurization that Louis Pasteur discovers is then applied to milk, and it is still the most crucial step in the milk processing operation. Pasteurization of milk is usually carried out at a temperature below boiling point. This is because casein micelles will become irreversibly accumulated (or "curdle") at very high temperatures (Deak, 2014). The low-temperature long time (LTLT) process, known as batch pasteurization, was the first pasteurization process that can destroy tuberculosis pathogen. Vat pasteurizer is used in the batch pasteurization process. The product needs to be heated up to 62- 64 °C for 30 minutes and quickly cooled after that. Thermal shock occurs to microorganisms when the product is suddenly cooled. In a refrigerated condition, vat pasteurized products have a shelf life of about 2-3 weeks (Sebastian, 2019).

HTST has been applied in the industry for large-scale milk handlers. It is also known as flash pasteurization. Pasteurization kills harmful microorganisms, including bacteria, viruses, protozoa, moulds, and yeasts in milk products to lower microbial content and to ensure a safe product for consumers (Zwirner, 2016).HTST pasteurization consists of stainless-steel heat exchanger plates with product flowing on one side and heating media flowing on the opposite side to heat milk at least temperatures (72°C) for at least 15 seconds and then follow by rapid cooling.

Despite the fact that almost all psychrotrophic bacteria are destroyed during the pasteurization at temperature 72°C and time 15 s, some microbes survive before the expected expiry date, which could encourage the spoilage of pasteurized milk (Zwirner, 2016).The milk's colour can be detected and used to indicate the changes in the milk's physicochemical properties. This study aims to evaluate the influence of temperature and holding time on the color of cow milk and to determine the microbiological quality of raw and pasteurized milk. Additionally, we want to find suitable process conditions from the suggested time-temperature combinations based on the evaluated results.

MATERIALS AND METHODS

Materials

Raw cow milk purchased from the local market in Besut, Terengganu, was quickly stored in a sterilized glass bottle at a storage temperature (≤ -18 °C) before further analysis. One litre of raw cow milk was treated with High-Temperature Short Time (HTST) at 70°C, 75°C, or 81°C for 15s or 25s. This experimental design limitation was overcome by imitating a vat pasteurizer,as shown in Fig 1 under the HTST process condition. First, 100ml raw cow milk is added into a beaker and was heated using a hot plate. Then, a thermometer is used to monitor the changes in the temperature, and a magnetic stirrer has added the beaker to mimic the agitation in vat pasteurization

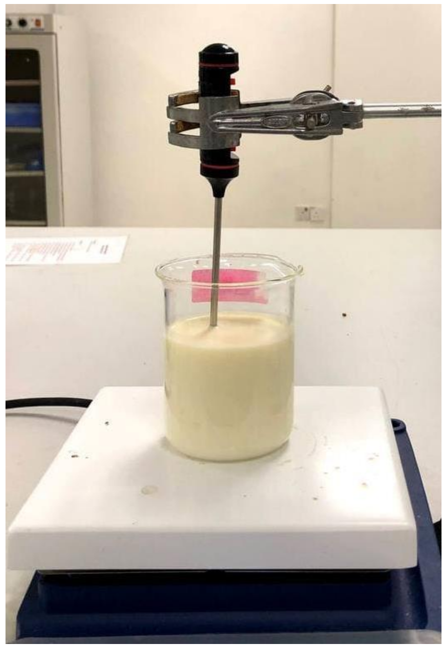

Fig 1: Pasteurization of Cow milk

Colour Analysis

The colour of the milk sample was determined using a colourimeter (Chroma Meter CR-400 (Konica Minolta, Japan). The colour measurement of the sample was carried out to study the L* (lightness), a* (red-green), and b* (yellow-blue) values. The colour of the samples was examined using the following equations (Gul et al., 2018):

chroma (C*ab):

$$C^*ab = \sqrt{a^{*2} + b^{*2}} \qquad \textbf{Eqn. 1}$$

Whiteness index (WI):

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \qquad \textbf{Eqn.2}$$

Methylene Blue Reduction Test

Methylene blue reduction test (MBRT) test in which the blue colour of a dye solution added to milk starts to decolourize as the milk's oxygen is reduced by microbial activity. The faster the milk is discoloured, the lower the bacteriological content is considered to be. All glassware and cap should be sterilized in an autoclave or boiling water, and all glassware should be free from chemicals. First, 10 ml of milk sample is added to a clean, dry test tube. Then, 1 ml of MBRT dye solution is added to the test tube. The test tube is covered using a cap and put in a water bath that needs to maintain the temperature at 37±1^{o}C. The time required to record at this stage as the incubation phase has started. When only a faint blue ring (about 5mm) remains at the top of the test tube, the decolourization process is considered complete (Anwer et al., 2018).It is considered complete when the blue colour of the dye solution added which is shown in Fig 2,to the milk decolorizes.

Lethality value (F-value)

Lethality value or pasteurization value (F-value) is when the number of minutes needed under the specified condition destroys a population of microorganisms in a given food. Usually, 12 D-value is set for F-value. It allows a theoretical reduction in the 12-log cycle of the most heat-sensitive mesophilic spore species in a can of food (Pharmastate Blog, 2017). Providing that the F-value at Tref and the z value are known, then the F value at the required temperature, T, can be calculated using Eqn3 below:

$$F = dt \sum 10^{\frac{T-T_{ref}}{z}} \qquad \textbf{Eqn.3}$$

Statistical Analysis

All of the laboratory tests were carried out in triplicate. The effects of temperature, holding time, and interaction on raw and pasteurized cow milk samples were evaluated using a two-factor factorial randomized design (3 temperatures 2 holding durations) with repeated measures. The data were analyzed using one-way ANOVA and Duncan's multiple ranges to test whether there was a significant difference at the $P<0.05$ level and using the SPSS 13.0 software (Statistical Package for the Social Sciences) (Zakaria et al., 2020).

RESULTS AND DISCUSSION

Colour Analysis

The colour results of raw, pasteurized, and commercial cow milk are presented in Table 1. There was a significant effect ($p<0.05$) of the HTST pasteurization process in every temperature on the colour of cow milk in terms of L*(lightness), a*(redness), and b*(yellowness). Pasteurized cow milk at 81 °C (25s) showed a higher L* and b* value but a lower a* value compared to raw and commercial cow milk. Our result was in agreement with the study of Chugh et al. (2014) that the milk increased in L* value and b* value after high-temperature short time (HTST) pasteurization process. HTST treatments slightly increased the L* (lightness) value of all samples because of the increasing number of dispersed components generated by the denaturation of milk proteins at high temperatures. Devi et al. (2015)stated that during thermal processing, the colour lightness of milk increases. This is due to the denaturation of b-lactoglobulin and its conjugation to j-casein.

Table 1: The colour value of cow milk samples

Sample	L*	a*	b*	C	WI
Raw milk	72.76±0.58^{d}	-2.48±0.01^{a}	8.91±0.03^{d}	6.40±0.91^{d}	72.01±0.73^{e}
Commercial	76.90±0.77^{c}	-3.42±0.31^{bc}	7.00±0.69^{bcd}	7.79±0.74^{bc}	75.61±0.53^{c}
70°C (15s)	73.57±0.06^{bc}	-3.00±0.07^{ab}	6.39±0.36^{cd}	7.06±0.32^{ab}	72.64±0.14^{de}
70°C (25s)	74.16±0.64^{b}	-3.19±0.88^{ab}	6.77±0.29^{cd}	7.51±0.61^{ab}	73.09±0.69^{d}
75°C (15s)	77.26±0.12^{c}	-3.57±0.87^{bc}	7.00±0.74^{bcd}	7.89±0.78^{bc}	75.92±0.21^{c}
75°C (25s)	77.84±0.67^{c}	-3.85±0.08^{bc}	6.77±0.29^{bc}	8.18±0.81^{bc}	76.37±0.69^{cd}
81°C (15s)	79.07±0.89^{b}	-3.87±0.15^{bc}	8.01±0.01^{ab}	8.90±0.08^{ab}	77.25±0.84^{b}
81°C (25s)	94.68±0.190^{a}	-4.28±0.16^{c}	8.91±0.03^{a}	9.88±0.04^{a}	88.77±0.08^{a}

C, chroma; WI, whiteness index. Values are means ± standard deviation. Mean within the same column with different letters are different at $P < 0.05$.

Aside from other necessary nutrients, milk is also a good source of carotenoids. According to recent research byUllah Rahat et al. (2017), carotene is present in cow milk but not in buffalo, goat, or ewe milk (Ullah Rahat et al., 2017). Carotenoids can also cause the fat globules in milk to turn yellow. The b* value also shown increasing, which indicates the yellowness in pasteurized milk after heat treatment. This could be due to heatinduced browning interactions between lactose and amino acids in milk. In milk, the two primary browning reactants are lactose and casein.Chroma (C*) indicates the colour sensation. When colour is fully saturated, it is regarded to be in its purest form(Gul et al., 2018). The lower the chroma value the less pure the colour, which is much closer to the white colour. That makes the result in Table 1 in line with the concept of chroma, as chroma value increaseds, from temperature 70 °C to 81 °C.

Methylene Blue Reduction test

This method is not very accurate in detecting the contamination of dairy products. Thus, this method needs to be used in combination with any other methods to evaluatebacteria quality of the milk. Table 2 shows grading of milk samples based on methylene-blue reduction test in different milk samples. The quality of raw pasteurized and commercial milk sample is shown in Table 3, depiciting the decolorizing time and grading of milk samples.

Table 2: Grading of milk samples based on methylene-blue reduction test in different milk samples

Quality of milk	Decolourization time
Excellent	More than 8 h
Good	Between 6 hours and 8 h
Fair	Between 2 to 6 h
Poor	Less than 2 h

Source:Anwer et al. (2018)

Table 3: Decolorizing time and grading of cow milk samples

Sample	Decolourization time (h)	Grade
Raw milk	1:37	Poor
Commercial	12:43	Excellent
Treatment 1 70^{o}C (15s)	6:10	Good
Treatment 270^{o}C (25s)	6:27	Good
Treatment 375^{o}C (15s)	6:46	Good
Treatment 475^{o}C (25s)	6:53	Good
Treatment 581^{o}C (15s)	7:28	Good
Treatment 681^{o}C (15s)	7:44	Good

MBRT performed on the raw milk revealed that it had poor quality. It decolourized within two hours during the methylene blue reduction test and was classified as class 4 milk. Chatterjee et al. (2006) stated that raw milk may have a larger number of microorganisms, most likely due to animal contamination. During milking, collection, and transport procedures, the milk may get contaminated with various organisms due to direct or indirect contact with any source of external contaminants. Direct physical contact of milk can be with surfaces that are not properly cleaned, such as milking utensils, teats and also it could be from the milk handler's hand. Furthermore, environmental factors such as design, cleanliness of the building, and water supply probably contribute to milk contamination. Besides that, bacterias in fertilizer, soil, and water that are in contact with dairy utensils may contaminate the milk. In terms of pasteurized milk, mostly 100% of the samples were considered good quality. This is due to the destruction of microorganisms during pasteurization.

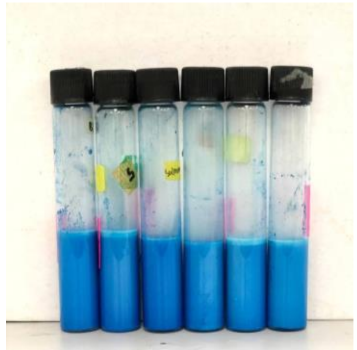

Fig 2: Cow milk samples after the addition of Methylene Blue and before decolorization

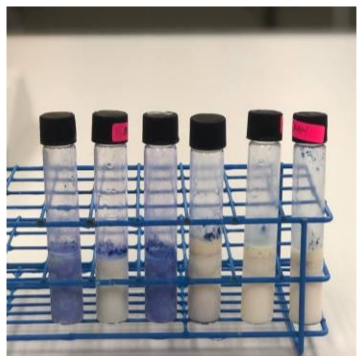

Fig 3: Cow milk samples after the decolorization

Lethality

In dairy manufacturing, milk is subjected to a temperature-time process that will lower the probability of survival of one of the heat-resistant bacteria, *Mycobacterium Paratuberculosis* (MAP), by 12 logs or 12 times D_{ref} for consumer safety consumption. $12D_{ref}$ is the minimum threshold of attaining a low population of MAP. The D value is proportional to a microorganism's thermal resistance, z. Therefore, the more resistant the microbe is to the heat treatment, the higher the D value. It is shown that the calculation of F value for every temperature achieves the minimum 12 D_{ref}, which is safe for human consumption (Pharmastate Blog, 2017).

Based on the result in Table 5 F value for 70 °C (15s and 25s), the most suitable temperature and holding time for cow milk with HTST pasteurization is 70 °C 25s. This is because the F values for this treatment was nearest to the approximate value with D value compared with other treatment conditions. This study is in line with Grant et al.(1999)where the inactivation entirely of MAP was more effective with a longer holding time of 25 seconds at 72°C. It was discovered that a longer holding time is more recommended rather than a higher pasteurization temperature to accomplish complete inactivation of (MAP) in cow milk. Other's temperature treatments are not suitable as the huge differences between F value and 12D value, which may lead to deformation of proteins, destruction of vitamins and minerals, and lousymilk taste.

Table 4:D value for 70 °C,75 °C, 81 °C (15s and 25s)used for lethality calculation (F-value)

Z-Value (°C)	6.90	7.11	7.70	8.60
D-Value(s) (70°C (15s))	29.23	28.67	27.28	25.62
D-Value(s) (70°C (25s))	48.73	47.78	45.47	42.71
D-Value(s) (75°C (15s))	15			
D-Value(s) (75°C (25s))	25			
D-Value(s) (81°C (15s))	15			
D-Value(s) (81°C (25s))	25			

T_{ref}=72°CT= 70 °C
Source of Z-Value:Sung & Collins, (1998)

Table 5: Lethality value for 70 °C (15s and 25s)

Z-Value (°C)	6.90	7.11	7.70	8.60
12 D value (70°C (15s))	350.85	344.01	327.35	307.49
F-Value (70°C (15s))	1130.48	1137.92	1158.63	1189.56
12 D value (70°C (25s))	584.76	573.34	545.58	512.48
F-Value (70°C (25s))	1064.69	1071.72	1091.13	1119.75

T_{ref}=70 °C dt= 180s

Table 6: Lethality value for, 75 °C (15s and 25s)

Z-Value (°C)	6.90	7.11	7.70	8.60
12 D Value (75°C (15s))	180			
F-Value (75°C (15s))	640.96	646.48	662.62	688.56
12 D Value (75°C (25s))	300			
F-Value (75°C (25s))	882.81	887.37	900.89	922.98

T_{ref}=75 °C dt= 180s

Table 7: Lethality value for, 81 °C (15s and 25s)

Z-Value (°C)	6.90	7.11	7.70	8.60
12 D Value (81°C (15s))			180	
F-Value (81°C (15s))	951.76	958.18	975.77	1001.56
12 D Value (75°C (25s))			300	
F-Value (81°C (25s))	1378.32	1381.89	1392.49	1409.84

T_{ref}=81 °C dt= 180s

CONCLUSION

This study revealed that increasing the temperature caused physical andmicrobiological changes in HTST pasteurized cow milk. The changes in colour and microbiology will lower the product quality and may lead to a decrease in acceptability towards pasteurized commercial cow milk. Furthermore, this study found that raw cow milkhad poor microbiological quality and was potentially harmful to humaningestion. However, it was seen that all pasteurize milk had good quality andwas safe to consume.

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