

# A Study of Different Heat Effects on the Processing of Raw milk to Drinking Milk for Commercialization

Siti Nur Hamraa' Binti Mohd Isa, and DR. Siti Noor Suzila Binti Maqsood Ul- Haque,

*Faculty of Chemical Engineering, Universiti Teknologi Mara*

**Abstract**— Heat treatment is applied on the raw milk in order to decrease the microbiological risk, reduce the effect of protein denaturation, lower the acidity in milk and longer the shelf life. The study is conducted in order to treat the raw milk with different heat treatment method, such as pasteurization and in-container sterilisation, to investigate the effect of the heated raw milk with microbiology examination, protein denaturation, sensory evaluation and pH and to determine the best heat treatment method with or without preservative of raw milk to drinking milk to fulfil the customer demands. Low Temperature- Short Time (LTST) with incorporation with honey and cinnamon which act as preservatives in milk is the best heat treatment method due to prolong shelf life, less of microbial colony, does not affect the protein consumption and has optimum pH. The equipment used to heat the raw milk in laboratory is not suitable for processing milk for commercialization thus it will affect the hygienity, loss of nutrients and decrease the shelf life. It was concluded that application of the hygiene rules, combined with better equipment of heat treatment method will improve quality of the milk.

**keywords**—LTST, heat treatment, preservative, raw milk, shelf life.

## I. INTRODUCTION

Throughout the world, milk is used as a human food due to its high nutritive value and it is considered as one of the most important diet items of many people. Milk also defined as “the most nearly perfect food”. Thus, the demand of consumers for safe and high quality milk has placed a significant responsibility on dairy producers, retailers and manufacturers to produce and market safe milk. (Teshome Gemechu, 2015)

Milk is a good medium for the growth of various microorganisms, especially bacterial pathogens such as *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella spp.*, *Streptococcus*, *Streptophylococcus*, *Campylobacter spp.* etc. Those bacteria are case milk borne diseases for example

tuberculosis, brucellosis and disease about gastroenteritis. Raw milk from healthy cow may contain a low microbial load, but the microbial may increase multiply if it stored in some temperature. (Khanitta Ruangwittayanusorn, 2016) These microorganisms are harmful for consumers.

In many countries, raw milk is required to undergo thermal processing so that the milk is safe to consume. Recent interest in the consumption of raw milk and raw milk products has led to the consideration of alternative dairy processing technologies that will not compromise milk quality and safety.

Heat treatment of milk is considered as one of the essential steps of milk production. Heat treatment is used in order to improve the quality of milk itself, prolong its shelf life and reducing the microorganism by pasteurization or in container sterilization. However, heat treatment will affect functional properties in milk and induces the changes in their sensorial properties and pH. (Mohammad Kamal, 2017)

Heat treatment involves three distinct periods which are heating period, a holding period and cooling period. Based on theory those three periods may contribute to the reactions takes places. The heating and cooling periods are the most significant. Thus, two kinetic parameters are rate of reaction or inactivation at a constant temperature and the effect of temperature change on the reaction rate.

The production of heated raw milk for human consumption covers from pasteurization to in-container sterilization with respect to the pH, protein denaturation, microbial examination and shelf life.

Pasteurisation is a mild heat treatment in which food is mostly heated to below 100°C. Pasteurisation can be done as batch or continuous

process It is used to minimise health hazards from pathogenic microorganisms in low-acid foods and to extend the shelf-life of acidic foods by several days or weeks by destruction of spoilage microorganisms and/or enzyme inactivation. (Fellows, 2017)

There are many types of pasteurization such as Vat Pasteurisation, High Temperature Short Time (HTST), Ultra High Temperature (UHT), Low Temperature Short Time (LTST) and Low Temperature Longer Time (LTLT). These types of methods have their own conditions. The most common method of pasteurization is High Temperature Short Time (HTST), which uses metal plates and hot water to raise milk temperature until 71.5°C for 15 seconds, followed by rapid cooling. (Making A Difference for Dairy , 2017)

Furthermore, Bruce Applegate, associated professor were collaborated with researcher in the Department of Food Science from Purdue University has conducted the Low Temperature Short Time (LTST) method in sprayed tiny droplets of pasteurized milk which was inoculated with Lactobacillus and Pseudomonas bacteria, through heated, pressurized chamber, rapidly raising and lowering their temperature below 70°C. Thus, the treatment has lowered the bacterial level and extended shelf life. LTST also does not gives any difference in color, aroma and taste with the raw milk. (Phillip R. Myer, 2016)

In-container sterilization process is by heating milk in a container in a commercial cooker/ retort. The purpose of this process is to destroy microbial and enzymatic activity. The length of time and magnitude of temperature employed during processing depend on the type of the product, number and heat resistance of microorganisms and enzymes present in milk. (Azaquar, 2011)

At present time, ultrasonic devices for homogenization are used at milk processing. Ultrasound treatment with high amplitude (power) hands an impotent effect on milk homogenization compared to conventional homogenization. As exposure time and power levels increase homogenization efficiency also rise. The highest homogenization efficiency and the smallest fat globule diameter are 3.22 and 0.725  $\mu\text{m}$  at power level 100 (450 W) for 10 min. respectively. Homogenization must always be sufficiently efficient to prevent creaming. The effect of homogenization on the physical structure of milk has many advantages: Smaller fat globules leading to no cream-line formation, whiter and more appetizing color,

reduced sensitivity to fat oxidation, more full-bodied flavor, better mouth feel and better stability of cultured milk product. (Asaad Rehman Saeed Al-Hilphy, 2012)

Preservation is used in order to maintain the quality, physic-chemical properties by providing safe product with having low spoilage potential during their shelf life. Hydrogen peroxidase was found to prevent souring of raw milk. Honey consists of major anti-bacterial factor which is hydrogen peroxide. Hydrogen peroxide is formed due to the oxidation of glucose and other monosaccharides by glucose oxidase present in hydropharyngeal glands of the honey bees.

The challenges being face today in commercializing raw milk to drinking milk are raw milk is easily to contaminate with spoilage bacteria and some microorganism even although it contain low of microbial count, different types of heat treatment method consist of limitation which contributes to the raw milk cannot stand longer. Therefore, there is need to conduct heat treatment method plus with the addition of preservatives, so that the milk is in good quality and extend the shelf life.

## II. METHODOLOGY

### A. Materials

2000 mL of fresh raw milk, nutrient agar, honey and cinnamon

### B. Apparatus

Water bath, autoclave, refrigerator, pH meter, incubator, weighing balance, conical flask, measuring cylinder, test tube, beaker, petri dish, colony counter, thermometer, heating plate, microwave oven, ultrasonic homogenizer, tissue and disposal plastic bottles.

### C. Methods

#### i) Preparation of Sampling Raw milk

About 2000 ml of fresh cow's raw milk from VKJ Enterprise is stored in refrigerator for about 4oC and were transported to the laboratory without delay until required.



**Figure i:** Fresh raw milk is obtained from VKJ Enterprise.

ii) Pasteurization

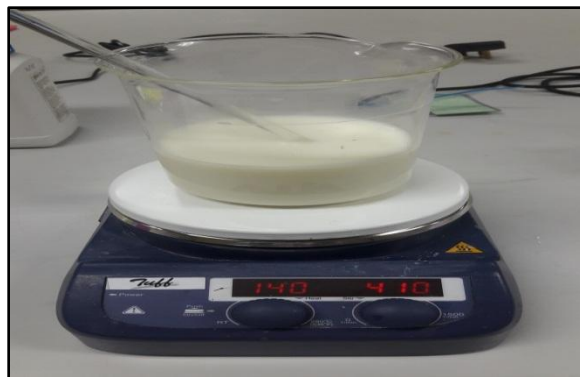
Pasteurization undergoes two condition processes which are high temperature-short time and low temperature short time.

Both High Temperature Short Time (HTST) and Low Temperature Short Time (LTST) of milk was heated in the water bath for 3 hours at 37°C. Then, the milk is heated until 71.7°C for 15s using heating plate.

Otherwise, a viable process, Low Temperature Short Time (LTST) which is low heat and short time duration were heated with heating plate at 61.7°C for 0.05s into 500 ml of milk.



**Figure ii. a)** Milk is heated in the water bath for 3 hours at 37°C.



**Figure ii. b)** Milk is heated by heating plate

iii) In- container sterilization

1000 ml of milk was heated with microwave oven at 71.7°C for 15s and then it is homogenized under ultrasonic homogenizer at about 40W and 80W with the same frequency 20Hz for 15 minutes.



**Figure iii. a)** Milk is heated via microwave oven for 15s for 71.7°C



**Figure iii. b)** Milk is homogenized by ultrasonic homogenizer at 40W and 80W with the same frequency for 15 minutes.

iv) Addition of Preservatives

After the heat treatment process, four (4) different samples such as HTST, LTST and microwave oven with different power (40W and 80W) of samples is added with preservatives which are honey and cinnamon.

v) pH

After the heating done, the pH is measured by pH meter at day 1, 3, 9, 16, 18, 21 and 27.

vi) Protein Denaturation

The turbidity test is used into the samples of raw milk. 1 ml of raw milk with 99 ml of distilled water is added into the measuring cylinder and shake it simultaneously. Then, put into the small glass bottles before read by turbidity meter.



Figure v. Value of turbidity is obtained by inserted the sample into turbidity meter

vii) Microbial examination

0.01ml of milk samples is drop onto the nutrient agar plate and streaks it rotationally and then incubates it for 48 hours in incubator. After 48 hours, read the colony using counting meter.



Figure vii. Conducting microbial test in laminar flow

viii) Sensory evaluation

The sensory test is done by observe the appearance, smell the aroma and taste the sweetness of milk and then ranked it as table below:

Rank	Sweetness	Appearances	Aroma
1	Too strong	Wonderful aroma	Most wonderful aroma
2	Little strong	Moderately attractive	Wonderful aroma
3	Strong	Attractive	Less wonderful aroma
4	Moderate	Less attractive	Appealing aroma
5	Weak	Unappetizing	Acceptable aroma
6	Little too weak	Unattractive	Less acceptable aroma
7	Much too weak	Moderately unattractive	Aroma is not appealing
8	Not at all	Extremely unattractive	Unappetizing aroma

### III. RESULTS AND DISCUSSIONS

#### A. The effects of pH

PH meter is used in order to measure the pH after the samples of milk is cooled. The test completed after the milk was stale.

Property of milk acidity is measured by pH. pH is a direct measurement of  $H^+$  activity and may

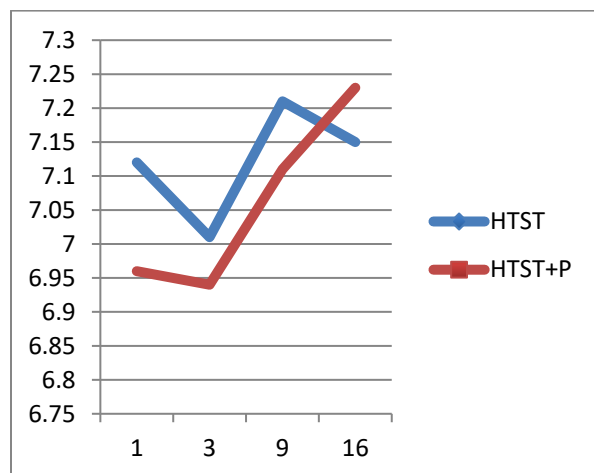
influence many other aspects related to milk quality, the colloidal stability of milk and other heat induced action such as Maillard browning and lactulose formation.

The pH of raw milk is usually between 6.6 to 6.9 and the exact value depends on protein, mineral and acid contents. The pH of milk could fall to below 6.0 when heating condition and it will increase when cooling.

**Table 1:** The different heat treatment methods with day based on pH at 18°C

Metho d/ Day	1	3	9	16	18	21	27
HTST	7.1 2	7.0 1	7.2 1	7.1 5			-
HTST+ P	6.9 6	6.9 4	7.1 1	7.2 3			-
LTST	7.0 8	6.9 8	6.5 1	7.1 3			-
LTST+ P	7.1 0	7.0 1	6.3 3	6.4 1	5.5 4	5.9 4	5.6 2
40W	6.9 9	6.9 1	6.9 5				-
40W+P	7.0 0	7.0 0	6.2 4				-
80W	7.1 5	6.8 7	6.8 2				-
80W+P	7.2 2	7.0 1	6.5 5				-

LTST: Low Temperature Short Time, HTST: High Temperature Short Time, P: Preservatives

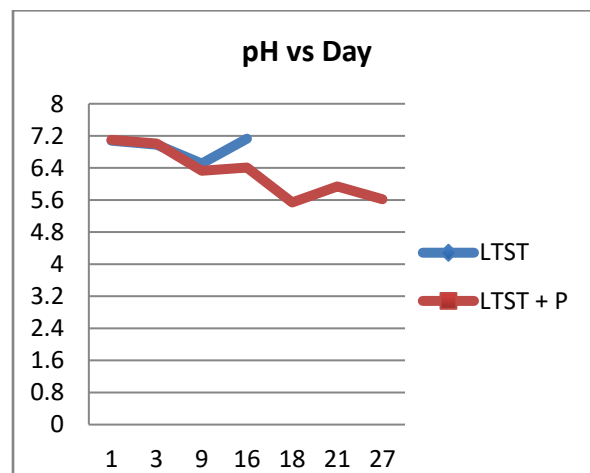


**Figure 1:** Comparison of heated raw milk by HTST at 71.7°C for 15s with and without added preservatives based on pH

Figure 1 above show the pH of raw milk heated with HTST without any preservative is higher compared with preservatives from 1<sup>st</sup> day until 9<sup>th</sup> day. But at last day when the milk has become stale, pH of HTST with preservatives is higher compared to HTST only.

From Figure 1, it show that pH of HTST is lead to alkaline due to alkaline phosphate enzyme. The alkaline phosphatase is occurring after the milk is being pasteurized and mostly is detect in raw milk to pasteurized milk. (Alkaline Phosphatase) pH of HTST is higher than HTST with preservatives from 1<sup>st</sup> day until it was stale.

According to the National Honey Board, the acidity for honey ranges from a pH 3.4 to 6.1, with an average 3.9 while cinnamon is alkalizing seasoning. From that, the pH of HTST with preservatives is less acidity due to the pH of preservatives from 1<sup>st</sup> day until 9<sup>th</sup> day and become alkaline at the last day due to stale of the milk.



**Figure 2:** Comparison heated raw milk by LTST at 61.7°C for 0.05s with and without added preservatives based on pH

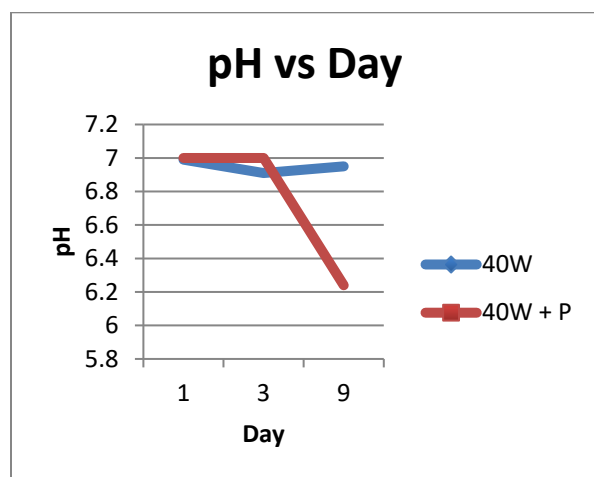
Figure 2 above show the raw milk heated with LTST at 61.7°C for 0.05s with and without preservatives share exactly same pH from 1<sup>st</sup> day until on the 9<sup>th</sup> day which is lead to alkaline phosphatase enzyme. This enzyme is appear when raw milk is being pasteurized. The addition of acidity honey will make the pH of LTST with preservative decreases. Then, pH for LTST is increased on 16<sup>th</sup> day and lead to stale. pH for LTST is decreases until pH 5.64 on the 27<sup>th</sup> day. According to the recent work by Guillane et.al. 2002, the effect of



acidification of milk to pH 5.8 after some of preservatives added into it.

Due to LTST was stale on 16<sup>th</sup> day, the LTST is compared to LTST with preservative during that day. From that, it was determined the pH of LTST is more acidic and near to optimal pH of milk compared to LTST with the preservatives.

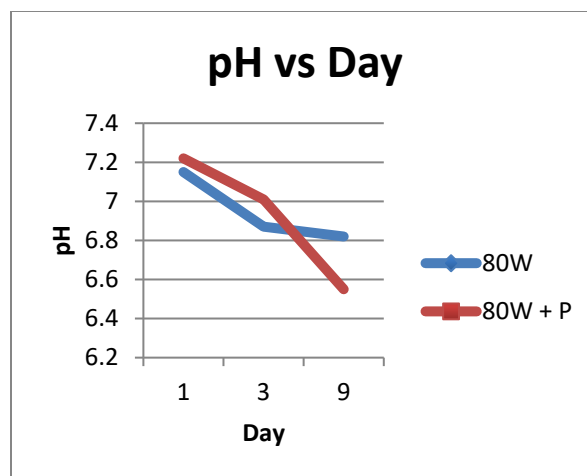
Low Temperature Short Time method will preserve the nutrient quality of the raw milk same like fresh raw milk thus kill the spoilage microorganism in raw milk.



**Figure 3:** Comparison heated raw milk at 71.7°C for 15s with the frequency 20Hz and the power 40W with and without added preservatives for 15 minutes.

Figure 3 show that both milk samples were stale on the 9<sup>th</sup> day. Milk is heated at 40W with preservatives decline sharply on the 9<sup>th</sup> day while 40W without preservatives increase to 6.95 which is normal. So that, it shows the milk with preservatives shows more acidity and near to optimal pH of milk due to acidity of preservatives compared to heated raw milk without preservatives. By that, the pH of heat treated raw milk of both samples is among average pH of untreated raw milk which is 6.1 to 6.9.

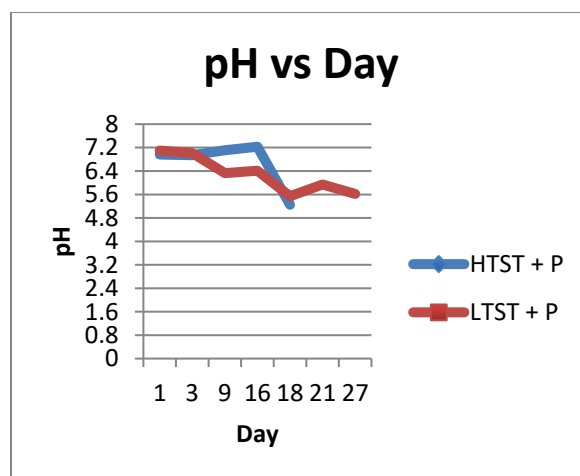
Furthermore, heat with microwave oven also preserved the nutrients and vitamins contents, as well as flavor, sensory characteristics, color of food of raw milk. Ultrasonic homogenizer wills smaller the fat globules in raw milk itself.



**Figure 3:** Comparison heated raw milk at 71.7°C for 15s using microwave and ultrasonic homogenized with the frequency 20Hz and the power 80W with and without added preservatives

Based on the graph above, both heated milk indicate pH neutral (pH = 7) but it decrease sharply on day until 9 and become stale on the 9<sup>th</sup> day. But, 80W with addition of preservatives seems better than 80W without preservatives. Hence, it can be concluded that the milk with the preservatives shows more acidity and near to optimal pH which is between 6.1 until 6.8 compared to without any preservatives.

Moreover, microwave oven combined with ultrasonic homogenizer with preservatives will optimize the quality of raw milk.

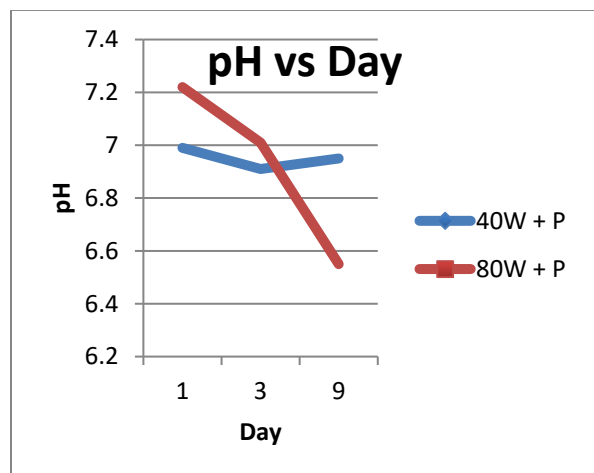


**Figure 4:** Comparison heated raw milk by HTST and LTST with added preservatives

Based on the graph above, the HTST and LTST with the addition of preservatives show almost same pH during 1<sup>st</sup> day. On the 9<sup>th</sup> day until 16<sup>th</sup> day, the HTST with preservatives is show higher pH compared to LTST with preservatives but on day 18, the pH of HTST and LTST with preservatives are more likely same. On 18<sup>th</sup> day, HTST with added preservatives is stale while LTST with preservatives is stale on 27<sup>th</sup> day and indicate pH stable for the milk.

Because of the milk by HTST with preservatives was stale during 18<sup>th</sup> day, the comparison was made between LTST with preservatives during 1<sup>st</sup> day until 18<sup>th</sup> day. The graph show that LTST with preservatives was more acidic compared to HTST with preservatives although it was stale.

The acidity of LTST with preservatives is occur due to pH of honey and cinnamon which act as preservatives. But, the acidity that does not affect the nutrient of milk since it increases the shelf life of milk.

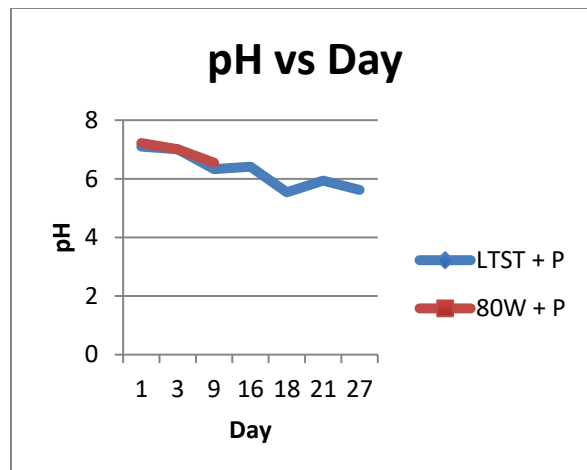


**Figure 5:** Comparison heated raw milk at 71.7C for 15s with the frequency 20Hz and the power 40W and 80W with added preservatives

Based on the Figure 5, it shows that the pH for 40W with preservatives indicate pH neutral while 80W with preservatives indicate pH low of alkaline on the 1<sup>st</sup> day. Then, the pH decreases on the 3<sup>rd</sup> day for both power (40W and 80W). On the 9<sup>th</sup> day, the pH of 80W with preservatives is deviates while the 40W with preservatives increases gradually although it was stale. But the deviation pH of 80W with preservatives, it still shows the optimum pH of milk.

Hence, according to the results above, the best heat treatment of pasteurization is LTST with

preservatives while for in-container sterilization is 80W with 20Hz with preservatives.



**Figure 6:** Comparison heated raw milk by LTST and 80W with added preservatives

From the Figure 6, the pH is compared from 1<sup>st</sup> day until 9<sup>th</sup> day due to 80W with preservatives was stale during the day. Hence it show that the LTST with preservatives is more acidic compared to 80W with preservatives. Although the LTST with preservatives more acidic but the pH of LTST is in the range of optimum pH of raw milk even it was stale on the 27<sup>th</sup> day.

From the results above, the acidity of milk is commonly happening to the presence of acid phosphate and casein. (Bosworth, 1914) Otherwise, the pH of milk in LTST is mostly affected due to acidity of the preservatives but the addition of preservatives does not changed the quality of raw milk instead it improved its quality.

### B. Microbial examination

Standard plate count, a known quantity of milk samples is diluted degree and equal portion of each dilution into nutrient agar medium and it also known as pour plate technique. Then, the nutrient agar medium is allowed to solidify after mixing with milk by gentle rotation of the plate or streaking. The microorganism present in the sample is expected to be appearing in the agar gel. The plates are incubated in the incubator shaker at 37°C for 48 hours.

During this method, each of organisms is expected to take up a separate position in the medium and grow in to a mass of cells of size sufficient enough to be counted by naked eye and being

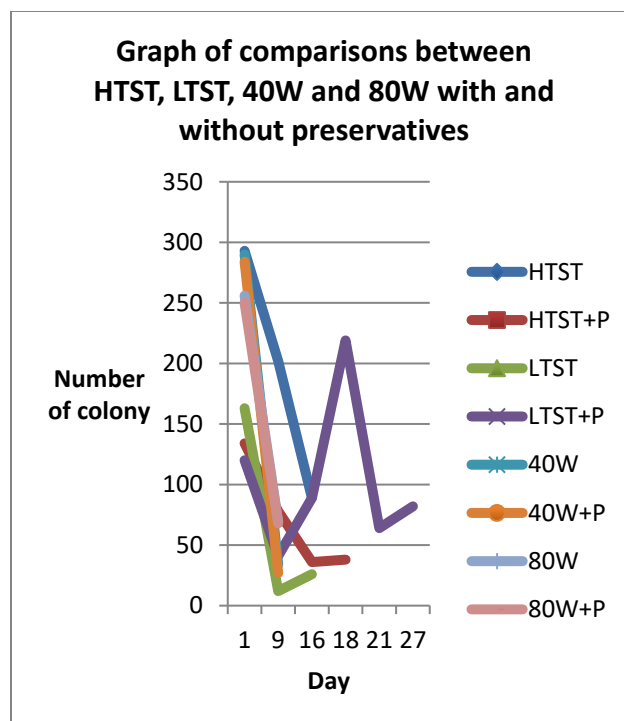
recognized as a colony forming unit (cfu). Number of colony present in the given volume of milk sample represents a colony count.

Microbial examination consist of some limitation such as the time consuming for those bacteria which are capable growing under given set of growth conditions and forming colonies can be counted.

**Table 2:** Standard colony number based on different types of methods

Methods/Day	1	9	16	18	21	27
<b>HTST</b>	293	202	89			
<b>HTST + Preservatives</b>	134 (brown colour)	78	36	38		
<b>LTST</b>	163 (brown colour)	12	16			
<b>LTST + Preservatives</b>	120	41	89	219	64	82
<b>40W</b>	290	34				
<b>40W + Preservatives</b>	284	27				
<b>80W</b>	256	9				
<b>80W + Preservatives</b>	250	8				

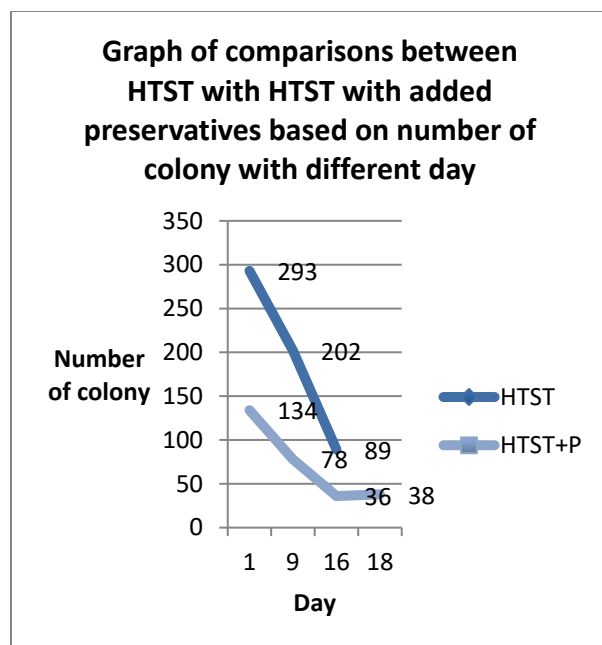
(-): Represent heated raw milk was stale



**Figure 7** Graph of comparisons between HTST, LTST, 40W and 80W with added preservatives based on number of colony

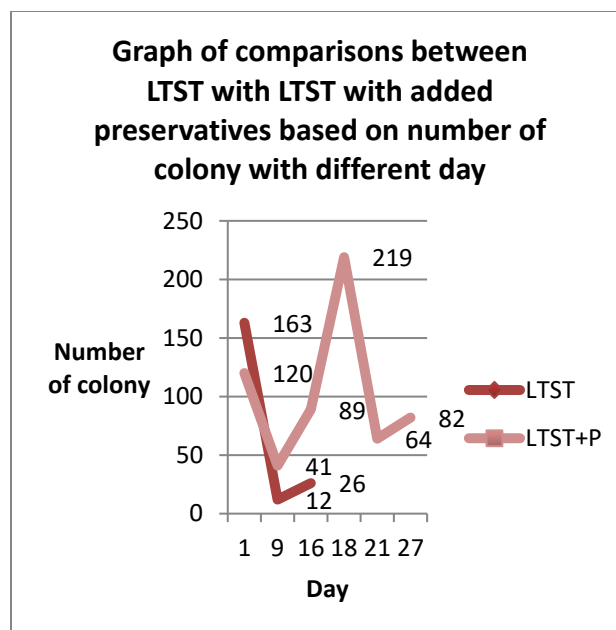
Based on Figure 7, the number of colony in different heat treatment methods were decline in line with days. The greater number of colony is HTST and the least number of colonies is LTST with added preservatives. The number of colony is affected due to high temperature during the heat treatment and low of power consumption during the homogenization. The low of heat and high power of ultrasonic homogenisation with added preservatives such as honey and cinnamon will remain the quality of the raw milk itself.





**Figure 8** Graph of comparisons between HTST with HTST with added preservatives based on number of colony with different day

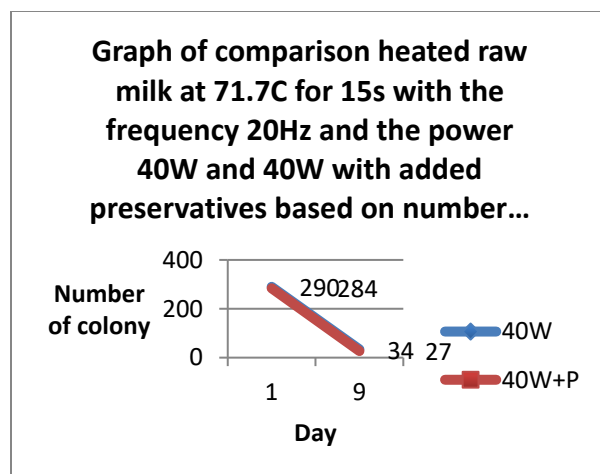
Change in pH is highly dependent on the microbial activity in the milk due to the fermentation of milk and hence increase the production of lactic acid. pH is decrease after a change in counts was observed. Moreover, the raw milk is considered spoilage when the number of colony is exceeded 250. According to the Figure 8 HTST with added preservatives is low number of colony and does not exceed 250 and it can be concluded that the raw milk is still remains the quality as fresh raw milk although the raw milk has been heated. The number of colony after the heat treatment is decreases in line with the day and it show that the heat treatment were applied to the raw milk will reduce the number of colony.



**Figure 9** Graph of comparisons between LTST with LTST with added preservatives based on number of colony with different day

The low temperature and short time of heat applied to the raw milk will remain the quality of the raw milk. From Figure 9 it show that the number of colony from LTST with and without the preservatives is low than 250, hence it show both raw milk by those treatment was still in good condition. LTST without was stale on 16<sup>th</sup> day while LTST with preservatives was stale on 27<sup>th</sup> day.

Furthermore, the number of colony on day 16<sup>th</sup> was increases for both heat treatment methods due to low temperature during refrigerated. The use of LTST with the low of temperature and short time and added preservatives will enhance the inactivation of enzymes and bacteria present in milk. Thus, it increases the shelf life of raw milk and remain the quality in milk.



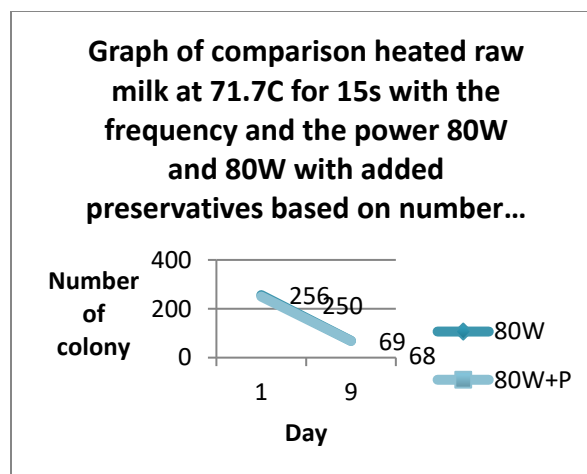
**Figure 10** Graph of comparison heated raw milk at 71.7°C for 15s with the frequency 20Hz and the power 40W and 40W with added preservatives based on number of colony

Heated of raw milk at 71.7°C for 15s with the frequency 20Hz and the power of 40W may further enhance the inactivation of enzymes and bacteria present in milk. By adding the preservatives to the milk itself, will reduce the number of colony compared to without any of preservatives.

A number of psychotropic organisms which can dominate the microflora of refrigerated milk produce heat stable lipases. Milk enzyme in raw milk which are plasmin, xanthine oxidase and lipolytic were reduced after heat and homogenisation treatment compared with the raw milk.

Finding from the experimental work, the heated raw milk at 71.7°C for 15s with the frequency without any preservatives at 40W are higher compared to the other heat treatment method thus enhancing the number of colony in milk. The low power during homogenisation will reduce the shelf life and consist of higher microbial number compared to milk with the preservatives.

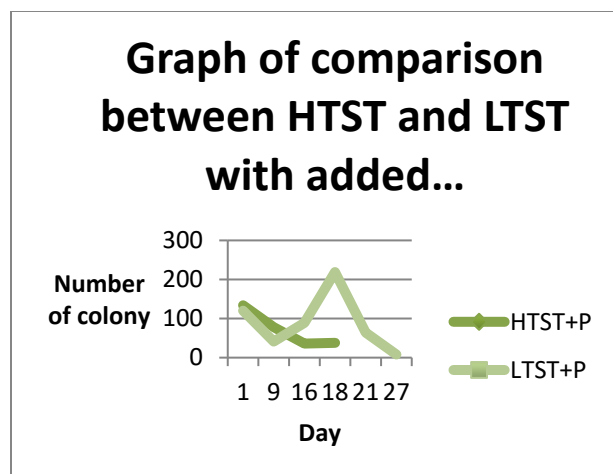
From Figure 10 the number of colony for the both heat treatment is higher than 250 and it show the quality of raw milk have been reduced and hence reduced the shelf life. Temperature during refrigerated also one of the reason why the number of colony in milk is low but it does not mean it will not affect the quality and flavour of the milk.



**Figure 11** Graph of comparison heated raw milk at 71.7°C for 15s with the frequency 20Hz and the power 80W and 80W with added preservatives based on number of colony

Heated of raw milk at 71.7°C for 15s with the frequency 20Hz and the power of 80W may further enhance the inactivation of enzymes and bacteria present in milk more compared to 40W. By adding the preservatives to the milk itself, will reduce the number of colony compared to without any of preservatives. The greater the power and time consumption during homogenisation with added preservatives is one of the reason in enhancing to reduce microbial number in milk.

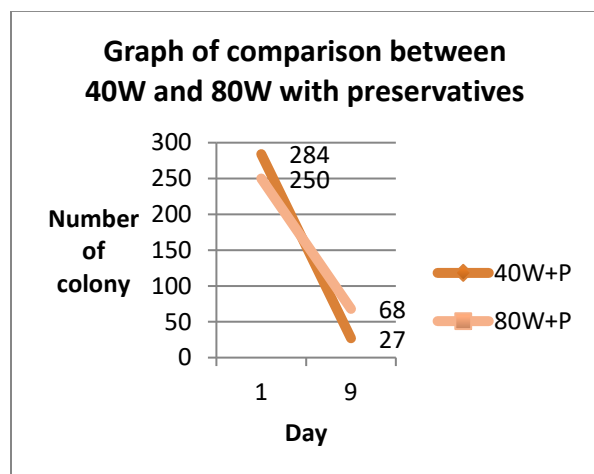
From Figure 11 the number of colony for the both heat treatment is higher than 250 and it show the quality of raw milk have been reduced and hence reduced the shelf life. Temperature during refrigerated also one of the reason why the number of colony in milk is low but it does not mean it will not affect the quality and flavour of the milk.



**Figure 11:** Graph of comparison between HTST and LTST with added preservatives based on number of colony

Preservatives are used in order to increase the shelf life of milk and reduce the activation of microbial activity.

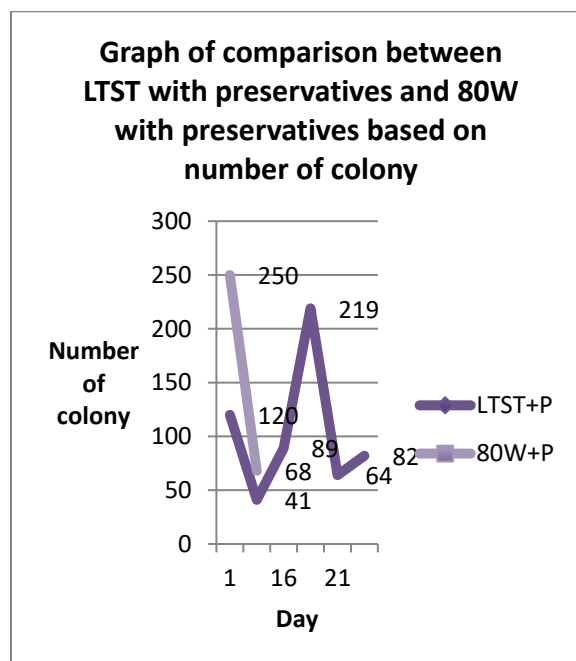
Based on the experimental data of number of colony, in line with the day, number of colony is reducing. This data is proven by data from Figure 4.2.6, the number of colony from LTST with added preservatives is lower than HTST due to lower temperature and short induce to the milk. This preservatives also is one of the factor in order to remain nutrient quality and enhance the flavour of the milk.



**Figure 12:** Graph of comparison between 40W and 80W based on number of colony

Preservatives and greater power induce to the milk will enhance the inactivation of microbial and enzyme of LPL in milk. This statement has been proven by looking from Figure 4.2.7. It show that

80W with added preservatives has lower number of colony compared to 40W. But, this kind of method have greater microbial colony compared to pasteurisation and will reduce the shelf life.



**Figure 13** Graph of comparison between LTST with preservatives and 80W with preservatives based on number of colony.

The severity of heat treatment methods are due to inactivation of LPL and a dampening effect on microbial numbers which directly impacts on the enzyme activity available in milk samples. The hydrolysis of triglycerides which constitute higher than 98% of milk fat and it was the principle of biochemical transformation of fat and lead to the production of FFAs. The LPL milk was catalysed the lipases and esterases in milk during hydrolysis reaction. In milk, LPL is not active normally due to the protection afforded the fat globule membrane and presence of lipolysis and then it inhibiting glycoproteins presence in skim fraction. In addition, the milk LPL is relatively unstable enzyme and being inactivated by heat, acid, oxidising agents, and prolonged freezing. Because of this reason, the LPL causes little or no lipolysis in pasteurised milk or product from pasteurisation.

Finding from the experimental work, by comparing the pasteurisation method such as High Temperature Short Time (HTST) and Low Temperature Short Time (LTST), it show that the number of colony from LTST is lower compared to HTST and it reducing the enhancing the activation of

enzyme in milk. By adding the preservatives to the milk, it will more reducing the enzyme and bacterial activation.

However, for in container sterilisation, the heated milk for 71.7°C for 15s with the power of homogenisation 80W and the frequency 20Hz show that the low number of colony thus it show the greater impact in inactivation of enzyme and bacterial in milk.

From the best result of LTST and 80W with the added preservatives, it has been comparing that LTST with preservatives is less number of colony and the number of colony does not achieved 250 which will gives the severity of the milk. 80W with preservatives is method that has been gives the severity impact that comes from the contribution of the microorganism and enzyme of LPL.

### C. The effects of protein denaturation

Heat treatment increased the degree of whey protein denaturation as the holding time increases for all temperatures. Whey proteins are the protein milk which is soluble in solution at pH 4.6. It can be separated from casein fraction of milk during coagulation processes of the casein, such as rennet or acid coagulated cheese. There four major whey proteins,  $\beta$  – Lactoglobulin,  $\alpha$  – Lactalbumin, Bovine Blood Serum Albumin (BSA) and Immunoglobulins.

Heat treatment gives different chemical change in milk such as non – enzymatic browning reactions involving lactose and especially lysine residues in protein. Higher degree of denaturation for heat treatment occurs during the temperature below 100°C. From the journal by Singh, 2011 states that some important functions of whey proteins are water binding capacity, emulsification, foaming, whipping and gelation properties. Thus, increasing in heating temperature and holding time will lead to an increase in whey protein denature which lead have a negative effect on the coagulation. (Akkerman, 2014)

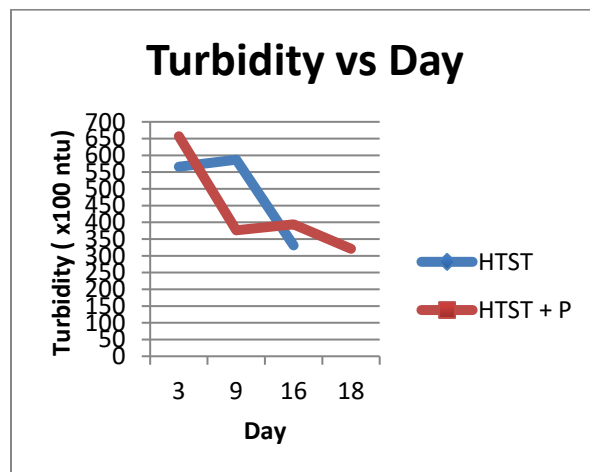
Moreover, heat treatment causes whey protein denaturation which is an irreversible process and also changes the mineral balance. (Akkerman, 2014)

Protein denaturation is measured by turbidity test. The turbidity test is a measure of serum protein denaturation, which depends on the precipitation of denatured serum proteins with casein in 20% ammonium sulphate solution. The turbidity for milk is greater than 4000 ntu. To measure the turbidity of

milk is not easy due to cloudiness of milk. Hence, 1ml of samples is added with 99ml of distilled water before being analyzed by turbidity meter.

**Table 3:** The different heat treatment methods with day based on turbidity (x 100 ntu) of milk

Method/ Day	3	9	16	18	21	27
HTST	566	587	331			
HTST+ P	657	376	394	321		
LTST	629	557	789			
LTST+ P	486	769	418	220	307	184
40W	681	650				
40W+P	410	363				
80W	560	453				
80W+P	243	284				

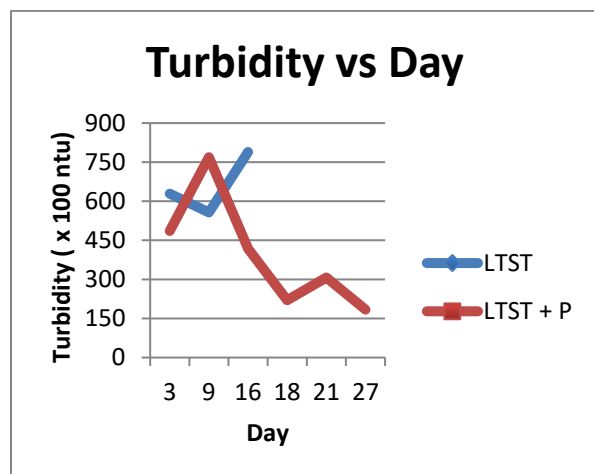


**Figure 14:** Comparison heated raw milk by HTST at 71.7°C for 15s with and without added preservatives based on turbidity

Based on Figure 14, the turbidity values were decreased within days. The value of turbidity for HTST with preservatives decrease gradually on the 9<sup>th</sup> day while turbidity value of HTST is increases. On the 16<sup>th</sup> day, the turbidity value for HTST with preservatives is increases a bit but for HTST is decreases and it became staled. Turbidity value for HTST + P is decreases on the 18<sup>th</sup> day.

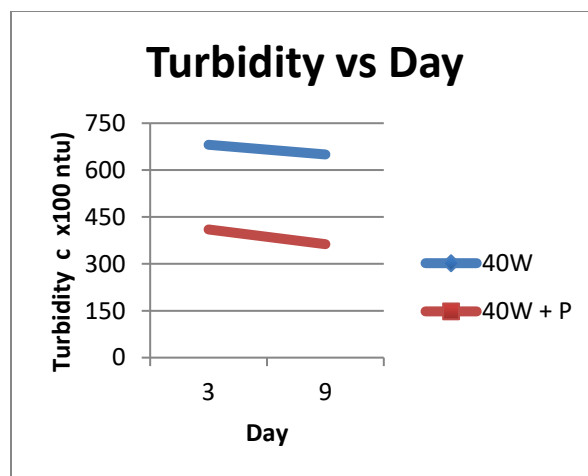
From Table 4 in Journal "Honey as a natural preservative of milk" by N S A Krushna , the result of turbidity with honey is lower than without honey. (N S A Krushna, 2007)

Due to milk by HTST was stale on the 16<sup>th</sup> day, the comparable with HTST with preservatives is measured during 3<sup>rd</sup> day until 16<sup>th</sup> day. Looking on the graph, the addition of preservatives to the milk were increase the turbidity but on the 9<sup>th</sup> day, the turbidity value for HTST with preservatives were ascending while for HTST without preservatives were descend. During 16<sup>th</sup> day, the HTST milk was stale hence make it more turbid more compared with HTST with preservatives. The higher the turbidity value of the raw milk, it represents the greater degradation of protein.



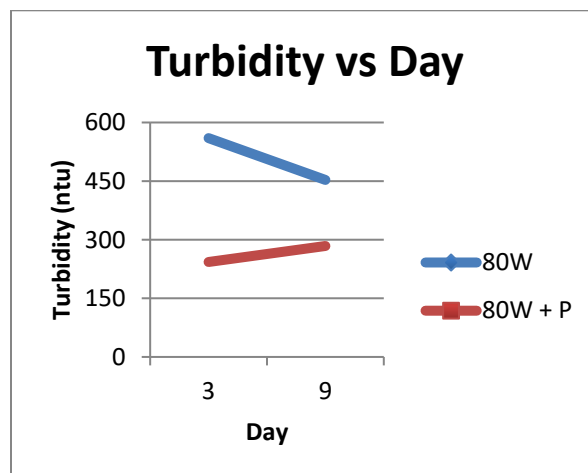
**Figure 15** Comparison heated raw milk by LTST at 61.7C for 0.05s with and without added preservatives

LTST with and without preservative are less turbidity compared to HTST due to longer time and higher temperature, hence it gives less chemical change. Based on Figure 8, it shows that the turbidity value for LTST with preservatives is lower than LTST without any preservatives on the 1st day. The lower the temperature is applied, the lower the degradation of protein. On the 16<sup>th</sup> day, when the heated milk was stale, the turbidity value is increased due to degradation of protein. LTST with preservatives indicates the lower turbidity value and lower the degradation on protein compared with without preservatives.



**Figure 16:** Comparison heated raw milk at 71.7°C for 15s and homogenized with the frequency 20Hz and the power 40W with and without added preservatives based on turbidity

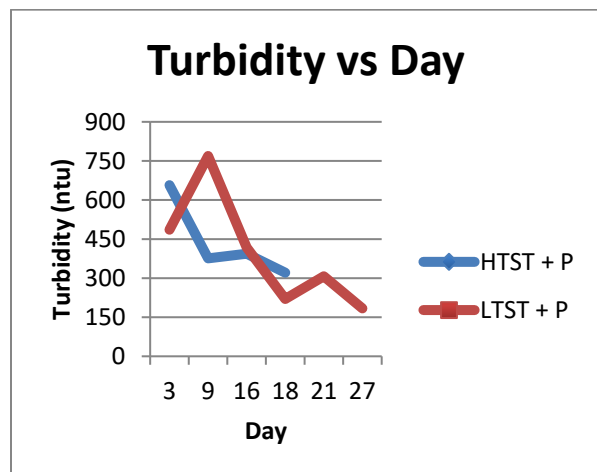
From Figure 16, it shows that the heated milk at 71.7°C for 15s and homogenized with the frequency 20Hz and the power 40W with preservatives indicates lower value of turbidity compared to without preservatives. Hence, it can be concluded that the protein denaturation of 40W with preservatives is lesser.



**Figure 17:** Comparison heated raw milk at 71.7C for 15s with the frequency 20Hz and the power 80W with and without added preservatives based on turbidity

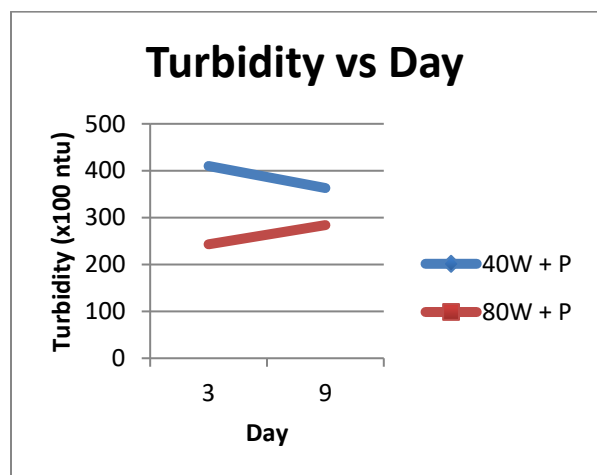
From the Figure 17, it shows that the value of turbidity in heated and homogenized milk at 20Hz and with the power of 80W with preservatives indicates lower value of turbidity compared to 80W without preservatives. Preservatives help to reduce

the physical chemical changes in milk, so that decrease the turbidity and less of whey protein is denatured.



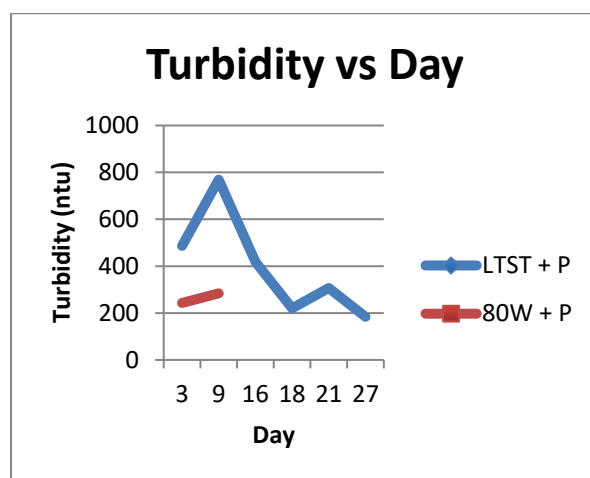
**Figure 18:** Comparison heated raw milk by HTST and LTST with added preservatives based on turbidity

Based on Figure 18, it shows that the comparable between HTST and LTST with the addition of preservatives based on the turbidity value. The value of LTST with preservatives contributes less value of turbidity compared to HTST with preservatives then it gives lesser chemical change. HTST with preservatives also more cloudiness compared to LTST with preservatives. Hence, it can be concluded that the lower the temperature heat to the milk, the lower the degradation of protein. The preservatives in milk will make the lesser protein degradation.



**Figure 19:** Comparison heated raw milk at 71.7°C for 15s with the frequency 20Hz and the power 40W and 80W with added preservatives based on turbidity

Based on Figure 19, it shows that the comparable between 40W and 80W with the same frequency 20Hz and with the addition of preservatives based on the turbidity value. Larger power induced to the milk will smaller the fat globules in milk. The value of 80W with preservatives contributes less value of turbidity compared to 40W with preservatives. Hence, it can be concluded that the higher value of power to the milk, the lower the degradation of protein. The preservatives in milk will also make the lesser protein degradation.



**Figure 13:** Comparison heated raw milk by LTST and 80W with added preservatives based on turbidity

Based on Figure 13, it shows that the value of turbidity in LTST with preservatives decreases gradually when it starts to become staled. But, compared to the milk that is heated by microwave oven at 71.7°C for 15s and then being homogenized using ultrasonic homogenizer at 80W and 20Hz with preservatives shows low turbidity value compared to LTST with preservatives during 3<sup>rd</sup> until 9<sup>th</sup> day. The higher value of turbidity at early day is due to higher temperature during analyze and when the milk was stale, the protein is less degraded. The lower the temperature and shorter time of heat treatment gives less chemical change, so that less whey protein is denatured.

Moreover, preservatives which consist of hydrogen peroxide will slow down the denaturation of whey protein during heat treatment.



#### D. Sensory evaluation

Sensory evaluation of the milk is being done by tasting the sweetness, observing the appearances and the smell the aroma until the milk is stale and rank it based on the ranking provided.

**Table 3:** Ranking of sensory evaluation of different types of heat treatment from 1<sup>st</sup> day until the milk was stale.

Methods/ Day	1	3	9	16	18	21	27
HTST	S: 2 AP: 4 AR: 4	S: 2 AP: 4 AR: 6	S: 8 AP: 7 AR: 8				
HTST + P	S: 2 AP: 2 AR: 2	S: 2 AP: 3 AR: 2	S: 3 AP: 4 AR: 3	S: 5 AP: 6 AR: 6	S: 8 AP: 7 AR: 8		
LTST	S: 3 AP: 3 AR: 3	S: 5 AP: 4 AR: 5	S: 7 AP: 5 AR: 7	S: 8 AP: 6 AR: 8			
LTST + P	S: 1 AP: 1 AR: 1	S: 1 AP: 1 AR: 1	S: 1 AP: 1 AR: 1	S: 2 AP: 1 AR: 1	S: 3 AP: 4 AR: 3	S: 3 AP: 4 AR: 3	S: 8 AP: 8 AR: 8
40W	S: 4 AP: 4 AR: 4	S: 6 AP: 7 AR: 7	S: 8 AP: 7 AR: 8				
40W + P	S: 3 AP: 4 AR: 5	S: 6 AP: 4 AR: 6	S: 8 AP: 5 AR: 8				
80W	S: 4 AP: 5 AR: 6	S: 7 AP: 6 AR: 7	S: 8 AP: 8 AR: 8				
80W + P	S: 3 AP: 4 AR: 2	S: 5 AP: 4 AR: 4	S: 8 AP: 6 AR: 7				

Pasteurisation causes little change to the colour, flavor and appearance of the milk. The low and shorter time of heat treatment is induced to the milk, there are no or little change to the appearances, aroma and smell of the milk.

In container sterilization gives a rich creamy appearances, and helped by Maillard browning component but it causes more loss of nutrient than pasteurization. (Deeth, 2008)

Preservatives such as honey and cinnamon will maintaining the quality of milk, prolong the shelf life and reduce the spoilage potential during their shelf life.

According to the ranking above, it shows that in-container sterilization methods were stale during day 9. The smell of the milk unappetizing at all, sour taste and the milk contain bubble. During 9<sup>th</sup> day also, HTST was started to staled when it gives sour taste and moderately attractive. LTST was stale during 16<sup>th</sup> day.

Based on pasteurization method, HTST was start to stale first due higher temperature and longer time heat the milk compared to LTST which is only takes 0.05s and 61.7°C. The higher the temperature and shorter time will reduce the shelf life hence it makes the milk was stale faster compared to the lower temperature, short time. The reducing of shelf life will denature the whey of protein and spoil the appearance and lastly gives unappetizing smell.

In container sterilization with 80W gives better appearances, smell and aroma compared to 40W. So that, it can be concluded the higher power will homogenized the milk efficiency.

Higher acidity or higher alkalinity will contribute to loss of nutrient in milk itself.

#### IV. CONCLUSION

As a conclusion, the best method is low- temperature-short- time (LTST) pasteurization with preservatives due to longer shelf life, optimum pH, a smaller amount of microbial count and the less turbidity. it can maintaining its flavor and nutrient quality, low cost and can fulfill the customer demands.

#### ACKNOWLEDGMENT

This work was carried out by Siti Nur Hamraa' Binti Mohd Isa with the support of references. The author wish to thank Dr. Siti Noor Suzila Binti Maqsood Ul-Haque from Bioprocess Engineering Department, Faculty of Chemical Engineering, Universiti Teknologi MARA (UiTM), for the excellent guidance. The author also want to thank Prof. Dato' Dr. Mohd Yusof Hj. Othman, Professor from Physic Programme, Universiti Kebangsaan Malaysia (UKM).

#### REFERENCES

- Harding, F. (1999). Processed milk . In F. Harding, Milk Quality (p. 114). Maryland : Aspen Publishers, Inc. .
- N S A Krushna, A. K. (2007). Honey as a natural preservative of milk . Journal of Experimental Biology , 459 - 464.
- Deeth, M. L. (2008). Heat Treatment of Milk. BLBK061-Tamime , 168-225.
- Catherine M. McAuley, T. K.-M. (2016). Microbiological and

- physiochemical stability of raw, pasteurized or pulsed electric field treated milk . *Innovative Food Science and Emerging Technologies*
- Phillip R. Myer, K. R. (2016). The effect of a novel low temperature short time (LTST) pasteurisation to extend the shelf life of fluid milk . Myer et.al SpringerPlus, 1 - 12.
  - Asaad Rehman Saeed Al- Hilphy, A. K.-T. (2012). Effect of Ultrasonic Treatment on Buffalo Milk Homogenization and Numbers of Bacteria. *International Journal of Food Science and Nutrition Engineering*, 113 - 118.
  - Steven C. Murphy, N. H. (2016). "Influence of raw milk quality on processed dairy products: How do raw milk quality test results relate to product quality yield? *Journal of Dairy Science*, 10128 - 10149.
  - *Making A Difference for Dairy* . (2017). Retrieved 6 16, 2017, from International Dairy Foods Association : <http://www.idfa.org/news-views/media-kits/milk/pasteurization>
  - Asaad Rehman Saeed Al- Hilphy, A. K.-T. (2012). Effect of Ultrasonic Treatment on Buffalo Milk Homogenization and Numbers of Bacteria. *International Journal of Food Science and Nutrition Engineering*, 113 - 118.
  - Azaquar. (2011). *Technology of drinking milk: pasteurized milk, sterilized and UHT*. Retrieved 6 16, 2017, from Food science and technology: <http://www.azaquar.com/en/doc/technology-of-drinking-milk-pasteurized-milk-sterilized-and-uht>
  - Fellows, P. (2017). Pasteurisation . *Food Processing Technology (Fourth Edition)* , 563 - 580.
  - Khanitta Ruangwittayanusorn, D. P. (2016). Monitoring the Hygiene of Raw Milk from Farms to Milk Retailers . *Agriculture and Agricultural Science Procedia* 11 , 95 - 99.
  - Mohammad Kamal, R. K. (2017). Monitoring of mild heat treatment of camel milk by front face fluorescence spectroscopy . *LWT- Food Science and Technology* , 586 - 593.
  - Phillip R. Myer, K. R. (2016). The effect of a novel low temperature-short time (LTST) process to extend the shelf-life of fluid milk. *SpringerPlus*, 1 - 12 .
  - Teshome Gemechu, F. B. (2015). Physical and chemical quality of raw cow's milk produced and marketed in Shashemene Town, Southern Ethiopia . *ISABB - Journal of Food and Agricultural Science* , 7 -13.