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EFFECT OF MICROWAVE HEAT TREATMENT ON THE QUALITY OF MILK

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ABSTRACT

Preservation is the prime concern for milk, which can be preserved for long time without any changes of nutritional values by applying new techniques rather than conventional methods. The objective of this study was to investigate the changes occur in physical and chemical qualities of milk after microwave heat treatment. Raw milk was collected from the local dairy farm and being kept in ice boxes directly taken to the laboratory. Six samples were prepared and among them five samples were tested at different temperatures and times in a microwave oven and one sample was used as raw milk sample. The physical parameters and chemical parameters were considered to evaluate the quality of milk samples. The colour, flavour, texture and specific gravity of raw and microwave heated milk samples were similar. But the acidity of raw milk (0.141 %) and microwave heated (S2, S3, S4, S5 and S6 were 0.128, 0.129, 0.130, 0.130 and 0.131 % respectively) milk differed to a great extent. Significant differences in the tested milk samples were also observed. The overall mean for carbohydrate, fat, protein, ash, solid-not-fat, total solid and water contents of milk samples were 5.09 %, 4.08%, 3.49%, 0.93%, 9.52%, 13.60%, and 86.39% respectively and excluding protein and ash content all microwave heated milk were significantly ($P < 0.05$) higher than those of unheated milk. It was found that despite of the same temperature, the mean values except ash and water content differed in case of time variation such as S2 (70°C for 2 min) gave the higher values than S4 (70°C for 1 min). Analysis of the tested samples showed that milk could be heated in a microwave not more than 90°C temperature and the milk does not get spoiled before one week.

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INTRODUCTION

Milk is a complete food item enriched with high nutritional ingredients but a fertile ground for the microbial growth. It is consumed directly or used in preparation of many food items such as pastries, pies, cakes and so on to provide specific functional properties (Meshref and Al-Rowaily, 2008). Generally, cow milk is composed of 87.7% water, 4.9% lactose (carbohydrate), 3.4% fat, 3.3% protein, and 0.7% ash (Constantin and Csatos, 2010). Milk fat is highly digestible and a rich source of energy containing different fatty acids in a larger scale than any other food items does. Milk protein contains all essential amino acids in well proportions. Lactose

is the only naturally available sugar found in milk that plays an important role for the nourishment of brain tissue. Moreover, it contains the available quantity of different vitamins and minerals for body growth (Kabir and Nazmul, 1998).

Milk is heated to remove pathogenic organisms as well as to increase its shelf life as an aid to further processing. Conventional methods used for preservation and sterilization of liquid food often cause a number of undesired changes in foods like loss of smell, colour, flavour, texture and losses of nutritional value. So, conventional preservation method applied in liquid food increases the safety and shelf life of the product but decreases the freshness and the final product quality (Sun, 2005). Pasteurisation is a conventional preservation method

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and those temperatures have a little effect on the nutritive value of milk. There is some loss of vitamin C and B group vitamins, but that is insignificant (O'Mahoney, 1988). Whereas, higher heat treatment methods may cause denaturation of β -lactoglobulin and may stimulate oxidation reactions and cause fat deterioration and off-flavors (Constantin and Csatlós, 2010). Despite the numerous methods employed by the dairy industries to carry out the heating of milk, other alternatives are being continuously investigated in order to improve the results obtained by the conventional methods. Such is the case for microwave energy used in milk processing. Continuous microwave treatment has been proved to be an effective system for pasteurizing milk, with the inclusion of a holding phase to maintain the time and temperature conditions required (Sierra, 1999). Cooking and reheating of foods by microwave ovens are widely used in food preparation (Meshref and Al-Rowaily, 2008). Microwave food heating results from the conversion of microwave energy into heat by friction of water molecules vibration due to rapid fluctuation in the electromagnetic field (Decareau, 1992; Potter and Hotchkiss, 1996).

Since several studies on microwave milk treatment, mainly focused on microbiological aspects, have been reported, it is very important to determine any modifications produced in the milk components as a result of the microwave processing. Hence, there are some reports directed at establishing the effects of microwave heating (pasteurization, sterilization, among others) on some of the milk nutrients.

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 and milk products (Mennane *et al.* 2007). A goal of food manufactures is to develop new methods to be used in processing technologies that retain or create desirable sensory qualities or reduce undesirable changes in food due to processing (Sun, 2005).

There is no systematic research was carried out in Bangladesh to observe the effect of microwave heat treatment on the quality of milk. Considering the previous discussions the present experiment was undertaken with the aim of investigating physical and chemical parameters of raw and microwave heated milk which determine for the quality of milk. Moreover, the effect of microwave heat on the nutritional quality of milk, and its feasibility to implement in our daily life were also investigated.

MATERIALS AND METHODS

Location of the study

The experiments were conducted at different laboratories of Hajee Mohammed Danesh Science and Technology University, Dinajpur, Bangladesh and at the laboratory of Rangpur Dairy (RD), Mithapukur, Rangpur, Bangladesh.

Preparation and testing of the milk samples

The raw milk was collected just after milking the cows in a sterilized clean glass container and was transported directly from the dairy farm to the laboratory by keeping them in ice box in the morning. All the milk was brought together in one day and mixed to prepare a composite sample. Then 500 ml raw milk was taken in each of the 6 similar containers.

Different physical and chemical parameters were performed using one sample which was used as raw milk sample. Five samples were tested at different temperature and time in microwave oven. After immediate cooling at room temperature, the samples were poured into five similar glass containers and immediately placed in the refrigerator at 4-7°C.

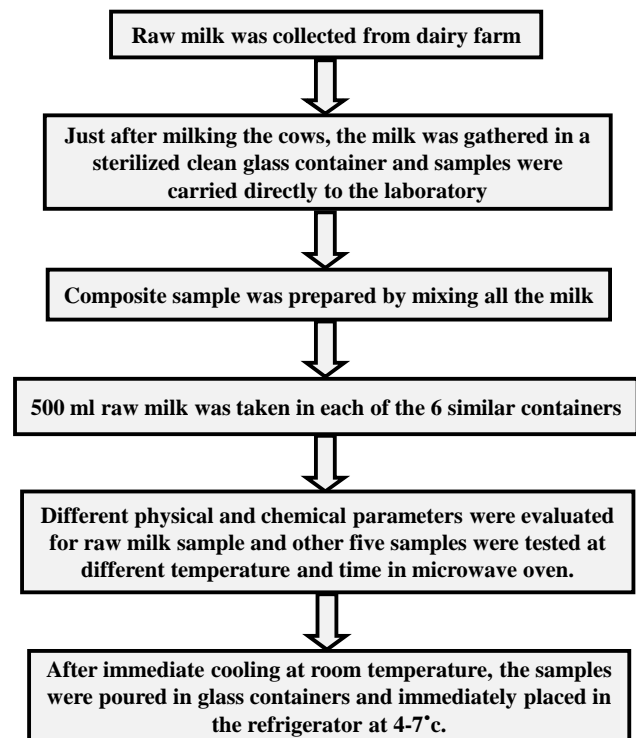


Figure 1 Flow diagram of milk sample preparation

Physico-Chemical Analysis

The following physical and chemical tests were carried out with each raw and microwave heated milk samples with having three replications of each test.

Physical tests

The physical parameters like organoleptic tests were performed visually and nasally to observe the colour, flavour and texture according to Nelson and Trout (1964).

Specific gravity

Specific gravity was determined according to O'Mahoney (1988). Fresh and microwave heated milk sample was filled sufficiently into a cylinder (100 ml capacity). Then lactometer was hold by the tip and inserted into the milk and awaited until it was almost in equilibrium. Then the lactometer reading was recorded at the top of the meniscus and meanwhile the temperature of the milk was recorded. The following formula was used to calculate the specific gravity of the milk.

$$\text{Specific gravity} = (L/1000) + 1$$

Where, L = the corrected lactometer reading at a given temperature, that is, for every degree above 15.56°C, 0.2 was added to the lactometer reading but for every degree below 15.56 °C, 0.2 was subtracted from the lactometer reading (O'Mahoney, 1988).

Acidity

The acidity, also called titratable acidity, is determined by titration. A 9 ml milk sample was taken in a beaker with the help of a pipette. Then 3 to 5 drops of 1% phenolphthalein indicator was added to it. After that, the sample was titrated with 0.1 N NaOH solution which was taken into the burette. When a faint but definite pink colour persisted, the end-point was reached. The acidity, expressed as % lactic acid, was finally calculated using the following formula. (O'Mahoney, 1988).

$$\% \text{ Lactic acid} = \frac{\frac{N}{10} \text{NaOH (ml)} \times 0.0009}{\text{Weight of milk sample (g)}} \times 100$$

Chemical tests

The total solid, protein, fat, ash, moisture, solid-not-fat and carbohydrate content were also determined for the raw and microwave heated milk. All the determinations were done in triplicate and the results were expressed as mean value.

Fat determination

The main tests used to determine the fat content of milk and milk products are the Gerber and Babcock tests. Here, the Gerber method was used to determine the fat content of milk. The milk sample was taken in a butyrometer with a pipette. Then, 10 ml having a specific gravity of 1.825 sulphuric acid was dispensed into that butyrometer and 1 ml of amyl alcohol was added to it. The butyrometer was tightly closed using a clean and dry stopper. It was shaken and inverted several times until all the milk was absorbed by the acid. Then the butyrometer was placed in a water bath at 65°C for 5 minutes. After that it was centrifuged in Garber centrifuge machine for 4 minutes at 1100 rpm. Finally the sample was returned to the water bath at 65°C for 5 minutes and fat percentage was recorded from the butyrometer reading (O'Mahony, 1988).

Protein content

Protein was determined using AOAC (1990) method. For digestion, 5 g of each sample was taken in three 250 ml Kjeldahl flask. 15 g potassium sulphate, 1.0 ml of copper sulphate solution and 25 ml of concentrated sulphuric acid (H₂SO₄) were added to the flask and mixed gently. The flasks were placed in inclined position on the stand in digestion chamber and were heated continuously. The digestion was carried out until the solutions became clear. After cooling, digestion flask was placed in the distillation equipment for distillation and 30 ml of distilled water and 75 ml of 50 % NaOH solutions were added into it and immediately connected to a stream-trap and condenser. Then ammonia was distilled and 50 ml of 40% boric acid solution, few drops of bromocresol green indicator solution were added until blue colour appeared. Finally, the contents of the receiver were titrated against 0.1 N hydrochloric acid solution, the end points were marked by a pink colour and the readings for blank sample were also determined and deducted from the titration. A protein conversion factor was used to calculate the percent protein from nitrogen determination. Percentage of nitrogen and protein was calculated by the following equation:

$$\% \text{ Nitrogen} = \frac{(A-B) \times \text{Normality of HCl} \times \text{volume made up of the digest} \times 100 \times 14}{\text{Aliquot of the digest taken} \times \text{weight of the sample} \times 1000} \times 100$$

Where, A = Titer volume of the sample (ml), B = Titer volume of the blank (ml), and % Protein = % Nitrogen × 6.38

Ash content

Total ash content was determined adopting AOAC method (1990). The dried milk samples were transferred into the muffle furnace and burnt at 550 °C temperature for 4-6 hours and ignited until light gray ash resulted (or to constant weight). The samples were then cooled in desiccators and weighed. The ash content was expressed as:

$$\% \text{ Ash} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

Moisture content

At first, 5 g milk sample was weighted into the cup. Then, the milk was heated over a low flame until it ceased foaming and a light-brown colour appeared. While the sample was being heated, the container was placed on the asbestos-centre wire gauze on a tripod to distribute the heat evenly across the bottom of the cup. After the moisture was driven from the milk, the sample was allowed to be cool and reweighed. Percentage moisture content of the milk was calculated as (O'Mahony, 1988):

$$\text{Moisture \%} = \frac{(\text{Original weight} - \text{final weight})}{\text{Original weight}} \times 100$$

Total Solids (TS) content

The total solid content was determined by using the following formula.

$$\text{TS} = \frac{L}{4} + (1.22 \times \text{fat \%}) + 0.72$$

Where, L = Corrected lactometer reading (O'Mahoney, 1988).

Solid-not-fat content

The solid-not-fat content of the milk samples was determined using the following formula (O'Mahoney, 1988).

$$\text{Solid-not-fat (SNF) content} = \text{Total solid (TS)} - \text{fat \%}$$

Carbohydrate content

The carbohydrate (Lactose) content was determined by subtracting the fat, protein and ash percentages from the percentage of the total solids (O'Mahoney, 1988).

Statistical analysis

Data obtained were analyzed by one way ANOVA using SPSS program (IBM Corporation, Inc, 2013, version 22). Duncan's Multiple Range Test (DMRT) was used to differentiate the mean values significantly.

RESULTS AND DISCUSSION

Physical and chemical quality of raw milk

The physical and chemical parameters of raw milk (S1) were analyzed before the milk was heat treated which were shown in the Table 1. The physical parameters of the raw milk samples were studied after collection. The colour of the sample was golden yellowish white and had normal flavour. Islam *et. al.* (1984) indicated that the flavour of the milk produced hygienically was normal. The milk sample collected from farm had normal texture (free flowing liquid).

Table 1 Physical and chemical parameters of raw milk (S1).

Parameters	Raw Milk	
Physical	Colour	Golden Yellowish White
	Flavour	Sweet aroma
	Texture	Free flowing
	Specific Gravity	1.030
	Acidity	0.141 %
Chemical	Carbohydrates (Lactose)	4.5 %
	Fat	3.6 %
	Protein	3.4 %
	Ash	0.9 %
	Solid-not-fat	8.8 %
	Total solid	12.4 %
	Water	87.6 %

The specific gravity of normal milk sample ranged from (1.027-1.035) g per ml with a mean value of 1.032 g per ml (Tamime, 2009). The found specific gravity of the raw milk sample was 1.030 which was in the range of Tamime (2009) findings. Normal fresh cow milk has an acidity varies on an average from 0.13-0.14 percent (Sukumar, 1980). The observed acidity was 0.141 % which was nearly equal to higher value of the range.

According to European Union quality standards for unprocessed whole milk, carbohydrate content should not be less than 4.2 % (Tamime, 2009). The observed carbohydrate content was 4.5% and that is greater than the standard. Again, European Union fixed that the standard fat content for unprocessed whole milk should not be less than 3.5 % (Tamime, 2009). The study found 3.6% fat content which was greater than the standard and lower than Mansson *et. al.* (2003) who reported a fat content of 4.3%. Further, in accordance with European Union quality standards for unprocessed whole milk, protein content should not be less than 2.9 % (Tamime, 2009). The study found 3.4% protein content which was greater than the standard and also greater than Debebe (2010) who reported 3.2±0.11 % and lower than Fikrineh *et. al.*(2012) who observed protein content of 3.46±0.04 %. The observed ash content of this study was 0.9 % which was higher than all of the mean values of ash content of raw milk samples by Gemechu *et. al.* (2015).

The observed total solid was 12.40 %. This is greater than the findings of Bille *et. al.* (2009) and lower than Mirzadeh *et. al.* (2010) who reported TS of 12.33 % and 12.57% respectively. According to European Union quality standards for unprocessed whole milk, solid-not-fat content should not be less than 8.5 % (Tamime, 2009). The observed solid-not-fat content was 8.8 % which was not only greater than the standard but also than the findings of Bille *et. al.* (2009)

Which was 8.7 % and lower than Fikrineh *et. al.* (2012) who reported a solid-not-fat content of 9.10 %. Quantitatively, the predominant component of milk is water (approx. 87.5%) and total dry matter (total dry extract) is approx. 12.5 % which is the nutritious milk (Constantin and Csatlos, 2010). The study found 87.6% water content which was nearly same with Constantin and Csatlos (2010).

Physical and chemical parameters of microwave heated milk

Raw milk was microwave heated at five different temperatures and times of S2=70°C for 2min, S3=88°C for 1 min, S4=70°C for 1min, S5=80°C for 1 min and S6=68°C for 3min. The physical parameters were mainly organoleptic (colour, flavour, texture), specific gravity and acidity. The obtained results about the colour, flavour, texture, specific gravity and acidity were presented in the Table 2.

Out of five samples three samples were yellowish white and the remaining samples were white. Among the five samples, 2 samples had normal flavour and others had sweet flavour. The milk samples had no odd flavour at the time of the experiment. This indicated that the milk was clean and fresh. All the milk samples had normal texture (free flowing liquid). The specific gravity of the milk samples S2, S3, S4, S5 and S6 were 1.031, 1.032, 1.0315, 1.0285 and 1.029 respectively. The highest specific gravity was obtained by sample 3. The acidity of the milk samples S2, S3, S4, S5 and S6 were 0.128, 0.129, 0.130, 0.130 and 0.131 % respectively. The specific gravity and acidity were not changing at a regular manner with the increase or decrease of temperature and time.

Statistical analysis was done with the six samples. First sample was collected from the initial milk sample which was not processed. Sample 2, 3, 4, 5 and 6 were collected after microwave heated at different temperature and time combinations.

*Here, S1= Unheated milk, S2= Microwave Heating at 70°C for 2 min, S3= Microwave Heating at 88°C for 1 min, S4= Microwave Heating at 70°C for 1 min, S5= Microwave Heating at 80°C for 1 min, S6= Microwave Heating at 68°C for 3 min. From Table 3, the carbohydrate and fat content of all microwave heated milk is significantly (P 0.05) higher than unheated milk. Higher carbohydrate (5.25±0.03) and fat content (4.22±0.02) were obtained by S6 (68°C for 3 min) and S2 (70°C for 2 min) respectively. It was also observed that carbohydrate content was decreasing with the increase of temperature. But the fat content was not increasing in a similar way. Microwave heated milk samples were not significantly (P 0.05) different for carbohydrate and fat content.

Table 2 Physical Parameters of Microwave heated milk samples.

Physical Parameter	S2	S3	S4	S5	S6
Colour	Golden yellowish white	Yellowish white	Yellowish white	Normal white	Normal white
Flavour	Sweet aroma	Sweet aroma	Sweet aroma	Normal	Normal
Texture	Free flowing	Free flowing	Free flowing	Normal flowing	Normal flowing
Specific Gravity	1.031	1.032	1.0315	1.0285	1.029
Acidity (%)	0.128	0.129	0.130	0.130	0.131

[Here S2= Microwave Heating at 70°C for 2 min, S3= Microwave Heating at 88°C for 1 min, S4= Microwave Heating at 70°C for 1 min, S5= Microwave Heating at 80°C for 1 min, S6= Microwave Heating at 68°C for 3 min]

The difference took place in the carbohydrate content might be due to the hydrolyzing enzymes produced by microorganisms before the heat treatment. The protein content of unheated milk (3.40 ± 0.10) was also significantly ($P < 0.05$) lower than that of the microwave heated milk except S6 (68°C for 3 min).

S2 (70°C for 2 min) gave the higher values than S4 (70°C for 1 min) for all the variables except ash and water content. Only total solid content of S2 (70°C for 2 min) is significantly ($P < 0.05$) different from S4 (70°C for 1 min).

Table 3 Mean values for chemical qualities of raw milk and microwave heated milk.

Variables (%)	S1	S2	S3	S4	S5	S6	Overall mean
Carbohydrates (Lactose)	4.50 ± 0.20^b	5.24 ± 0.02^a	5.16 ± 0.01^a	5.20 ± 0.06^a	5.20 ± 0.03^a	5.25 ± 0.03^a	5.09 ± 0.28
Fat	3.60 ± 0.10^b	4.22 ± 0.02^a	4.18 ± 0.03^a	4.20 ± 0.03^a	4.14 ± 0.04^a	4.16 ± 0.01^a	4.08 ± 0.22
Protein	3.40 ± 0.10^c	3.57 ± 0.03^a	3.50 ± 0.01^{ab}	3.53 ± 0.06^{ab}	3.52 ± 0.02^{ab}	3.45 ± 0.03^{bc}	3.49 ± 0.07
Ash	0.90 ± 0.02^c	0.95 ± 0.03^{ab}	0.90 ± 0.02^c	0.99 ± 0.01^a	0.93 ± 0.03^{bc}	0.94 ± 0.02^{bc}	0.93 ± 0.03
Solid-not-fat	8.80 ± 0.03^d	9.76 ± 0.05^a	9.56 ± 0.03^c	9.73 ± 0.02^a	9.65 ± 0.05^b	9.64 ± 0.01^b	9.52 ± 0.34
TS	12.40 ± 0.02^c	13.98 ± 0.03^a	13.74 ± 0.04^d	13.93 ± 0.03^b	13.79 ± 0.01^c	13.80 ± 0.02^c	13.60 ± 0.56
Water	87.60 ± 0.05^a	86.02 ± 0.01^d	86.26 ± 0.02^b	86.07 ± 0.04^d	86.21 ± 0.01^{bc}	86.20 ± 0.03^c	86.39 ± 0.56

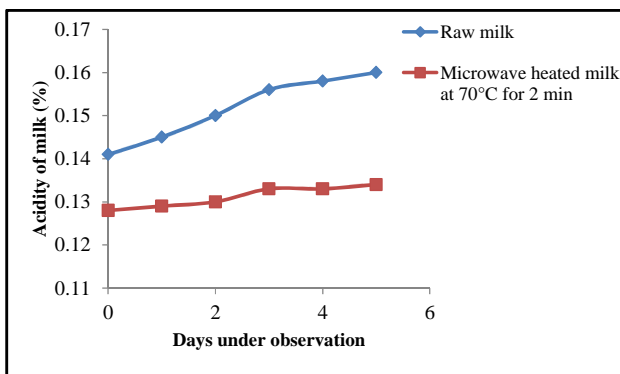
*Mean values \pm standard deviation (SD)

*Means followed by different superscript letters with a row are significantly different ($P < 0.05$)

The reason behind the variation may be due to the change of protein pattern in the heated milk samples. Protein content of microwave heated milk was not increasing at a regular way. S2 (70°C for 2 min) gave the higher protein content. Remarkable ($P < 0.05$) difference was found between S2 (70°C for 2 min) and S1 (unheated milk). Ash content of unheated milk was not significantly ($P < 0.05$) different from heated milk samples except S2 (70°C for 2 min) and S4 (70°C for 1 min) from Table 3. Unheated milk gave the same value (0.90 ± 0.02) with S3 (88°C for 1 min). S4 (70°C for 1 min) gave the highest value (0.99 ± 0.01) for ash content.

For solid-not-fat content (SNF) of all microwave heated milk is significantly ($P < 0.05$) higher than unheated milk (8.8 ± 0.03). S2 (70°C for 2 min) and S4 (70°C for 1 min) were significantly ($P < 0.05$) higher than other microwave treated milk. All the samples were significantly ($P < 0.05$) different for total solid content. But S5 (80°C for 1 min) and S6 (68°C for 3 min) gave nearly same results. The higher total solid content observed in S2 (70°C for 2 min) and it was (13.98 ± 0.03). European Union established quality standards for total solid content of cow milk not to be less than 12.5% (FAO/WHO, 2007). The mean values of all the microwave heated milk samples were higher than the recommended standards.

Table 3 also showed that water content of unheated milk (87.60 ± 0.05) is significantly ($P < 0.05$) higher than the microwave heated milk samples. Marked difference found among the sample S2 (70°C for 2 min), S3 (88°C for 1 min) and S6 (68°C for 3 min). From table 3, an important observation was found that at same temperature the mean values were different for two different times.



Graph 1 Average acidity percentage of raw and microwave heated milk

Acidity percentage

The production of acid in milk is normally termed "souring" and the sour taste of such milk is due to lactic acid. The percentage of acid present in dairy products at any time is a rough indication of the age of the milk and the manner in which it has been handled. Fresh milk has an initial acidity due to its buffering capacity (O'Mahoney, 1988).

Acidity of raw and microwave heated milk (70°C for 2 min) sample were observed upto 5 days. The percentage of acidity of the raw milk and that of the microwave heated milk samples were increasing day by day. The microwave heated milk samples showed comparatively lower percentage of acidity. From Sukumar (1980), it was known that cow milk has an acidity that varies on an average from 0.13-0.14 percent which was previously mentioned. The acidity of the microwave heated milk samples were within this limit. But the acidity of the raw milk samples was higher than this range. Fresh milk showed an acidity of above 0.141% which was due to the presence of citrate, phosphate, carbon-di-oxide (CO_2) and milk casein. If the sample was kept for several hours or days without pasteurization or cooling or any kind of heat treatment, the lactose would undergo fermentation and produce lactic acid in milk. This additional acidity was known as developed acidity which was reasonable for quick spoilage of milk. Acidity of the microwave heated milk samples indicated that there was no developed acidity in the samples and the quality of the milk was good.

CONCLUSION

Recently many experiments achieved negative phosphatase tests and low bacterial counts in raw cow's milk heated by microwave in a continuous flow unit under several temperature and time combinations ($73.170\text{C}-96.70\text{C}$) (Sierra, 1999). Considering the physical and chemical parameters of raw milk, it could be concluded that the milk samples of HSTU farm were acceptable. For the production of "better quality milk" under farm condition, it is necessary to train up the farmers about hygienic aspects of milk production. That may help to reduce the incidence of decreasing quality of milk. Finally it was recommended that microwave heat treatment could be taken as a processing method for milk because major nutrient contents were not lost during heating. Milk can be heated at high temperature (not above 90°C) by microwave and

microwave heat treated milk remains safe for nearly one week. After one week, milk is not suitable for human consumption.

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