*J. Agric. Food. Tech.*, 3(12)5-13, 2013

ISSN 2090 – 424X
**Journal of Agriculture and Food Technology**
*www.textroad.com*



# Effect of Heat Treatments on Physico-Chemical Characteristics of Skimmed Milk

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## ABSTRACT

Present study reports the influence of various heat treatments on physical (titratable acidity, pH values and specific gravity) and chemical (protein, fat, ash and moisture) characteristics of skimmed milk. The skimmed milk was obtained from buffalo milk through cream separator and divided into four groups viz. A, B, C and D. The group A was kept as control (untreated) whereas B, C and D were treated with thermization (60$^{o}$C for few sec), pasteurization (65$^{o}$C for 30 min), and sterilization (110$^{o}$C for 10 min), respectively. The results indicated that the pH value of sterilized skimmed milk was significantly higher ($P<0.05$) however the titratable acidity was reported lower, followed by pasteurized, thermizide and untreated skimmed milk. The specific gravity of pasteurized and sterilized skimmed milk was relatively similar and both of these were comparatively ($P<0.05$) higher than that of thermizide and control group. The fat content of thermizide and pasteurized skimmed milk was not significantly ($P>0.05$) different from that of control skimmed milk, and remained significantly ($P<0.05$) higher in sterilized skimmed milk. Protein content in pasteurized skimmed milk was remarkably ($P<0.05$) higher compared to that of sterilized, thermizide and control skimmed milk. Lactose and ash contents of skimmed milk were affected ($P<0.05$) with the use of pasteurization and sterilization processes.

## INTRODUCTION

Milk is known as a complete diet, providing the primary source of nutrition for young mammals before they become capable of digesting other types of food. Milk is composed of various essential components including protein, lactose, fat, minerals and vitamins etc. Milk can be used as whole milk or as milk products such as cheese, yoghurt, butter, ice cream, skimmed milk etc. Skimmed milk is the processed milk obtained by skimming of milk through cream separator. Skimmed milk contains low fat content as compared to whole milk. Each 100 grams of skimmed milk provides about 34 to 38 Kcal of energy to human body (Sangwan, 2008). Skimmed milk is used in many food products like cheese, flavored beverages, cakes and breads. Milk is not only an ideal and perishable product for humans, but it is also susceptible to spoilage microbes, which results in change of physico-chemical properties of milk and its products. In order to reduce the chances of spoilage it is necessary to apply various preservation methods to extend the shelf life of milk and make it safe for consumers. Heat treatment is one of the most common preservation methods applied to extend the shelf life as well as improve the quality of the products by reducing microbial load thus minimizing the risk of food contamination (McKinnon *et al*., 2009). The heat treatment is also applied to improve the keeping qualities by inactivating the enzymes. In addition to beneficial effects, heat treatment also results in changing the physical as well as chemical properties of the skimmed milk during production process. Keeping the above views in mind, present study was planned to observe the effects of various heat treatments; pasteurization, sterilization and thermization on skimmed milk, and to observe their effects on physical as well as chemical characteristics of treated skimmed milk.

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## MATERIALS AND METHODS

The whole buffalo milk was collected from Livestock Experiment Station, Department of Livestock Management, was brought to the Dairy processing laboratory, Department of Animal Products Technology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam. On arrival of sample in the laboratory, it was pre-heated to 40$^{o}$C and transferred to the supply tank of cream separator for skimming. After skimming, the milk was measured into four equal parts and accredited with codes A, B, C and D. The group A was kept as control (untreated) whereas B, C and D were treated with thermization (60$^{o}$C for few sec), pasteurization (65$^{o}$C for 30 min), and sterilization (110$^{o}$C for 10 min), respectively. A total of six trials each in duplicate batches were conducted and analyzed for physical (titratable acidity, pH values and specific gravity) and chemical (protein, fat, ash and moisture) characteristics.

### Analysis of pH values

The pH values of thermizide, pasteurized, sterilized and untreated (control) skimmed milk samples were determined by using pH meter (Model HI, Hanna Instruments, Italy). Electrodes and temperature probe of pH meter was dipped into the samples to be analyzed and the reading on the screen of pH meter was noted.

### Analysis of Titratable acidity

Acidity percentage was determined according to the method as described by Association of Official Analytical Chemists (AOAC, 2000). In brief 9 ml sample of each (thermizide, pasteurized and sterilized as well as untreated (control) skimmed milk was taken in a conical flask (each in duplicate batches), and 3 drops of phenolphthalein (Indicator) was added. Thereafter, it was titrated with N/10 NaOH solution using titration kit. The volume of alkali used was noted on the burette and calculation was made by putting it in following formula:

$$\text{Titratable acidity \%} = \frac{\text{Quantity of N/10 NaOH used} \times 0.009}{\text{Volume of milk sample used}} \times 100$$

### Analysis of Specific gravity

Specific gravity of heat treated and untreated (control) skimmed milk samples were determined by the method as described by AOAC (2000) using pycnometer. Briefly, the density of the milk was measured against the density of the water (standard). The empty weight of pycnometer was obtained and then filled with distilled water (at 20$^{o}$C) and weighed. Similarly, each milk sample was filled in the pycnometer and weighed at same temperature. Samples were analyzed in duplicate batches. Then specific gravity was calculated by using following formula:

$$\text{Specific gravity} = \frac{\text{Weight of milk sample}}{\text{Weigh of distilled water}}$$

### Analysis of Moisture content

Moisture content of sample was examined according to the method as described by (AOAC, 2000). After thorough mixing of sample, 5 gram of each sample was taken in a pre-weighed flat bottom dish and placed in hot air oven (101±1$^{o}$C for 3 hrs) (Memmert 854, Schawabch W. Germany) and then transferred to desiccators containing silica gel as a desiccant. After 1 hr, weight of the dish was taken and returned to hot air oven for further drying (30 min). It was again transferred to desiccators, cooled and weighed as before. The heating, cooling and weighing processes were repeated until constant weight. Each sample was analyzed in duplicate. The moisture content was calculated as:

$$\text{Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where,

$W_1 =$ weight of empty dish $W_2 =$ weight of dish + sample

$W_3 =$ weight of dish + dried samples

### Analysis of Fat content

Gerber method was applied for determination of fat content as described by James (1995). In brief, 11 ml of skimmed milk samples was taken in a butyrometer (1%) then 10 ml of sulfuric acid (90 %) and 1 ml of Amyl alcohol was added and closed with rubber cork and mixed well. The butyrometer was then placed in Gerber centrifuge machine (Funke Gerber, Model No. 12105 Germany) for 5 minutes @ 1100 rpm. Readings were obtained after adjustment of the fat through cork. The process was repeated twice for each sample.

**Analysis of Protein content**

Protein was determined by the method as described by British Standards Institution (BSI, 1990). Five gram skimmed milk sample was digested in micro Kjeldhal digester in the presence of catalyst (0.2 g of copper sulphate and 2.0 g of potassium sulphate) where sulfuric acid was used as an oxidizing agent. The digested sample was diluted up to 250 ml with distilled water. The diluted sample (5 ml) was distilled with 5 ml NaOH (40%) using micro Kjeldhal distillation unit, where steam was distilled over into 5 ml of boric acid (2%) containing an indicator for 3 minutes. The ammonia trapped in boric acid was determined by titration with 0.1N HCl. The nitrogen percentage was calculated using following formula:

$$N\,\% = \frac{1.4\,(V_2\text{-}V_1) \times \text{normality of HCl} \times 250}{\text{Weight of sample taken} \times \text{samples used for distillation}}$$

Where,

$V_1$ = titrable value $V_2$ = blank sample

While protein percentage was determined by conversion of nitrogen percentage to protein, assuming that all the nitrogen in skimmed milk was present as protein.

Protein percentage = N% × Conversion factor.

Where conversion factor = 100 / N% in protein of dairy products (i.e. 6.38).

**Analysis of Lactose content**

The lactose content of skimmed milk sample was determined by difference method using following formula: Lactose content =TS% - (fat% + protein% + ash%)

**Analysis of Ash content**

Ash content of skimmed milk sample was determined by Gravimetric method as described by AOAC (2000). In brief, 5 gram of skimmed milk sample was measured in pre-weighed crucibles, and transferred to muffle furnace (Newer Herm Model; L9/11/8KM, Germany) (550$^{o}$C) for 5 hrs. Ashed sample was transferred into desiccator having silica gel as desiccant for 1 hr and then weighed. The ash content was calculated using following formula:

$$\text{Ash}\,\% = \frac{\text{Weight of ashed sample}}{\text{Weight of sample taken}} \times 100$$

**Statistical Analysis**

The data was analyzed according to statistical procedure of analysis of variance (ANOVA), and in case of significant differences, the mean were further computed using least significant difference (LSD) at 5% level of probability through computerized statistical package i.e, Student Edition of Statistix (SXW), version 8.1 (Copyright 2005, Analytical software, USA).

## RESULTS

**Influence of heat treatments on pH of skimmed milk**

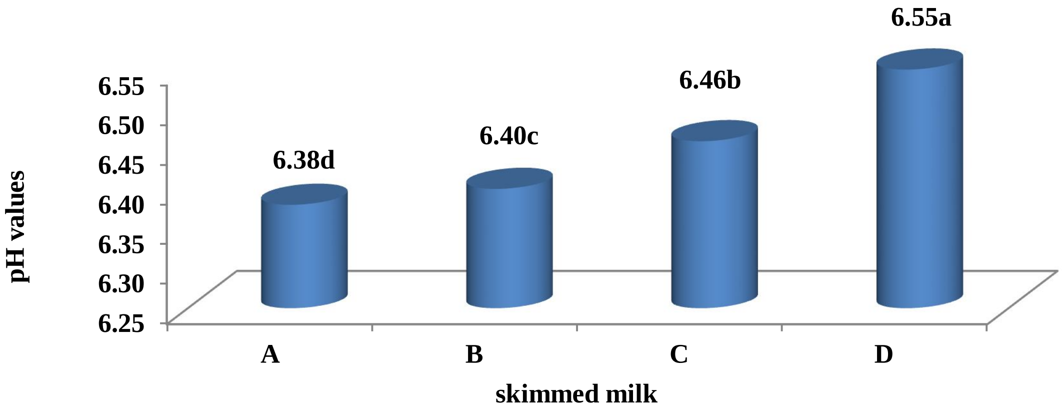
The pH values of heat treated and untreated (control) milk was analyzed, and results are shown in Figure-1. The results show that heat treatment had significant influence on pH values of skimmed milk, but it varied with type of heat treatments used in this study. The pH value was in a range between 6.37 to 6.39 in group A, while it was in a range of 6.40 to 6.41 in group B, 6.44 to 6.48 in group C and 6.53 to 6.57 in group D. pH values were comparatively ($P<0.05$) less affected with thermization process compared to that of pasteurization and sterilization processes. The pH values of sterilized skimmed milk (group D; 6.55±0.0001) were remarkably higher ($P<0.05$) compared to that of pasteurized skimmed milk (group C; 6.46±0.001) and thermizide skimmed milk (group B; 6.40±0.0002). The pH values of all heat treated skimmed milk samples were significantly ($P<0.05$) higher than that of un-treated or control skimmed milk sample (group A; 6.38±0.0002).

**Influence of heat treatments on titratable acidity of skimmed milk**

Results shown in Figure-2 reveals that the titratable acidity of skimmed milk was also remarkably affected by heat treatment applied to it. However, influence of heat treatments was inverse as observed for pH values in the present study. The titratable acidity of group B (0.17±0.0001%), was significantly ($P<0.05$) higher followed by C (0.16±0.0002%) and D (0.14±0.0001%). Moreover, the titratable acidity in all these groups (B, C and D) was remarkably lower ($P>0.05$) than that of control skimmed milk A (0.19±0.0002%). Furthermore, the variation in acidity within the same group was also observed which was in a range between 0.18 to 0.0.20% in group A, 0.17 to 0.18% in group B, 0.15 to 0.17% in group C and 0.135 to 0.14% in group D.

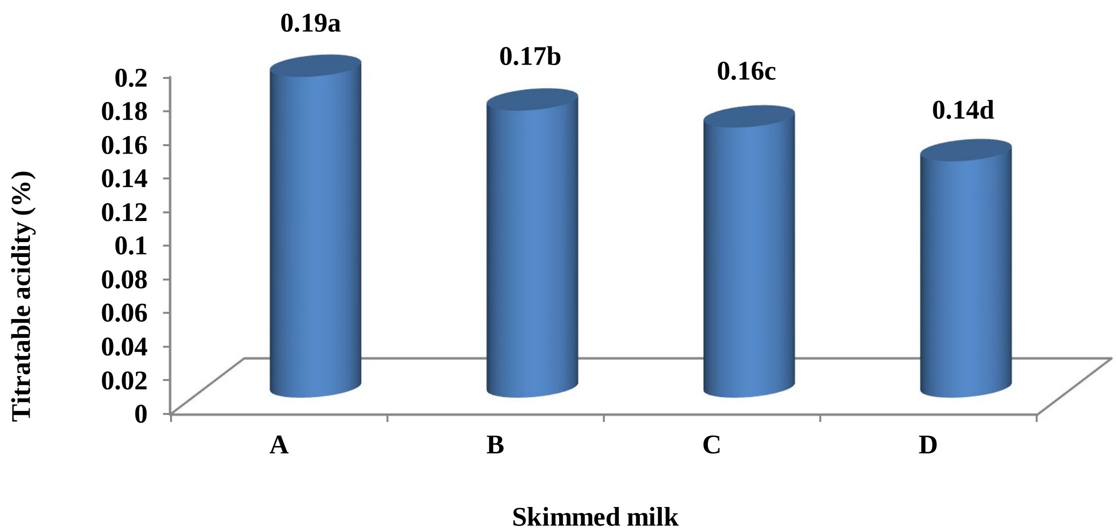
**Influence of heat treatments on specific gravity of skimmed milk**

Significant influence of heat treatment on specific gravity of skimmed milk was noticed (Figure-3). The specific gravity was recorded as 1.0342 to 1.0347 in group A, 1.0348 to 1.0354 in B, 1.0356 to 1.0360 in C and 1.0352 to 1.0359 in D. Least significant difference of mean (LSD; 0.05) test showed that there was no comparable variation ($P>0.05$) in the specific gravity of pasteurized (1.036±0.0001) and sterilized (1.036±0.00001) skimmed milk. Nevertheless, the thermization process showed significantly ($P<0.05$) lower effect on specific gravity of skimmed milk (1.035±0.0001) compared to that of pasteurization and sterilization processes. It was further observed that heat treated (Thermizide, pasteurized and sterilized) skimmed milk had comparatively ($P<0.05$) higher specific gravity compared to that of control skimmed milk (1.034±0.0001).



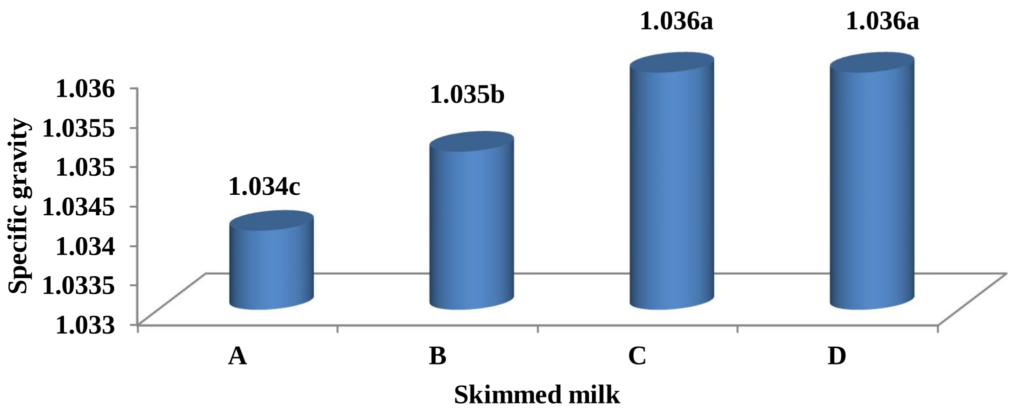

LSD (0.05) = 0.0120
SE± = 0.00057

**Figure 1 pH values of thermizide, pasteurized, sterilized and control skimmed milk**




LSD (0.05) = 0.00062
SE± = 0.00029

**Figure 2 Titratable acidity (%) of thermizide, pasteurized, sterilized and control skimmed milk**




LSD (0.05) = 0.000024
SE± = 0.000011

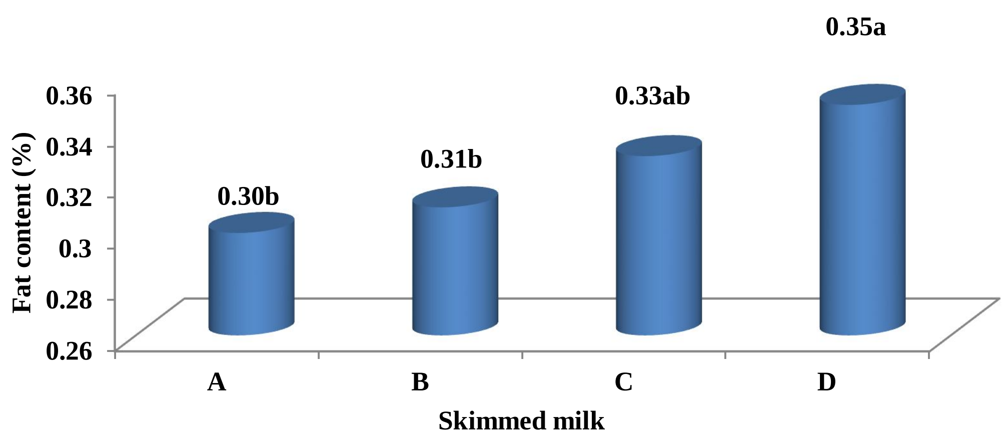
**Figure 3 Specific gravity of thermizide, pasteurized, sterilized and control skimmed milk**

**Influence of heat treatments on fat content of skimmed milk**

The results shown in figure-4 reveals that fat content of skimmed milk varied between 0.27 to 0.35% , 0.28 to 0.36%, 0.30 to 0.38% and 0.32 to 0.38% in group A, B, C and D, respectively. The analysis of variance (ANOVA) showed significant influence of heat treatment on skimmed milk), while LSD (0.05) comparison of mean showed that thermization and pasteurization processes had no significant (P>0.05) influence on fat content of skimmed milk. The fat content of thermizide (0.31±0.01%) and pasteurized (0.33±0.01%) skimmed milk was not comparatively different from that of control skimmed milk (0.30±0.01%). But sterilization process did show comparable (P<0.05) influence on skimmed milk as sterilized skimmed milk showed significantly highest fat content (P<0.05).

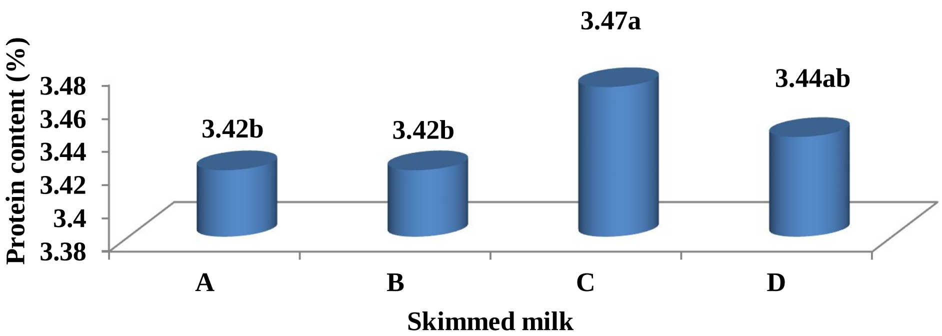
**Influence of heat treatments on protein content of skimmed milk**

Protein content of heat treated and untreated (control) milk was analyzed, and results are presented in Figure-5. These results revealed that the protein content of untreated (control) skimmed milk was in a range between 3.38 to 3.46%, 3.38 to 3.47% in thermized skimmed milk, 3.44 to 3.51% in pasteurized skimmed milk and 3.41 to 3.49% in sterilized skimmed milk. LSD (0.05) comparison of means revealed that there were non-significant differences (P>0.05) between heat treated (B and D) groups compared to control group (A). These results indicate that thermization and sterilization processes had no significant influence on protein content of skimmed milk. Moreover, pasteurization process showed remarkable effect on protein content of skimmed milk. The mean value of protein was highest in group C (3.47±0.01%), whereas, in group B, D and A, it was (3.42±0.01%), (3.44±0.01%) and (3.42±0.01%), respectively.




LSD 0.05 = 0.0318
SE± = 0.0153

**Figure 4 Fat content (%) of thermizide, pasteurized, sterilized and control skimmed milk**




LSD (0.05) = 0.0327
SE± = 0.0157

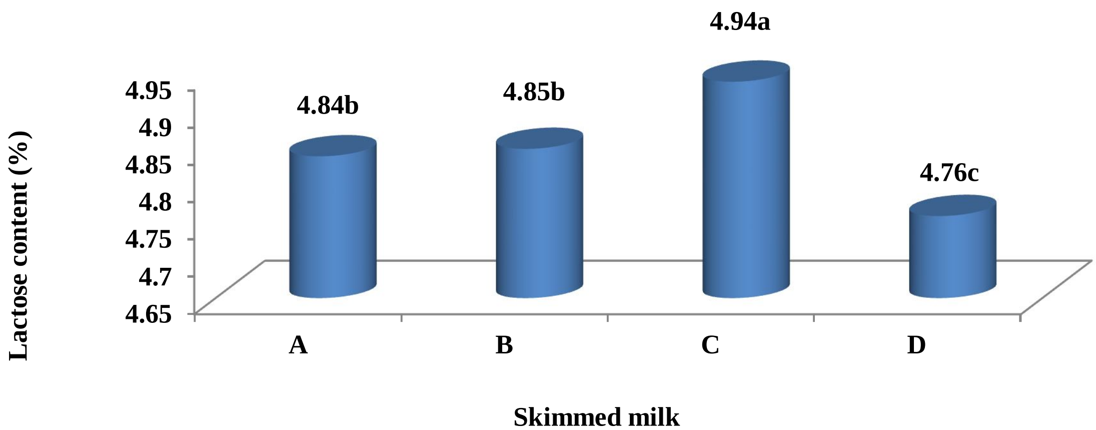
**Figure 5 Protein content (%) of thermizide, pasteurized, sterilized and control skimmed milk**

**Influence of heat treatments on lactose content of skimmed milk**

Lactose content in heat treated and untreated (control) milk was analyzed, and results are shown in Figure-6. The lactose content was in between 4.77 to 4.91% in group A, 4.78 to 4.92% in B, 4.88 to 5.12 in C and 4.73 to 4.79% in D. LSD (0.05) comparison of means showed that there was significant ($P<0.05$) influence of pasteurization and sterilization processes on lactose content of skimmed milk, while thermization process has no significant ($P>0.05$) effect on lactose content of skimmed milk. Lactose content in the thermized skimmed milk (4.85±0.02%) was relatively similar ($P>0.05$) to that of control skimmed milk. While pasteurized skimmed milk (4.94±0.04%) showed comparatively higher ($P<0.05$) and sterilized skimmed milk (4.76±0.01%) the lower than that of control skimmed milk (4.84±0.02%).

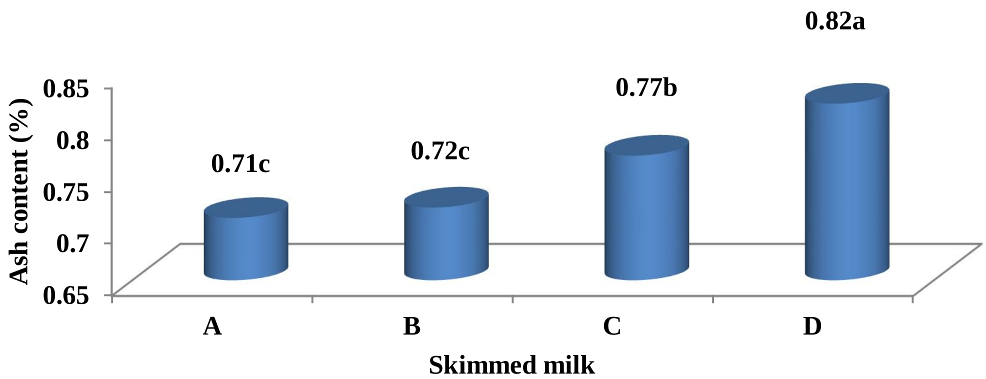
**Influence of heat treatments on ash content of skimmed milk**

Ash content of heat treated and untreated (control) skimmed milk was analyzed, and results are shown in Figure-7. The ash content was in a range between 0.69 to 0.73% in group A, 0.71 to 0.73% in group B, 0.76 to 0.77% in group C and 0.80 to 0.85% in group D. Statistical analysis showed that mean ash content was higher in group D (0.82±0.01%), followed by group C (0.77±0.0002%), B (0.72±0.0003%) and A (0.71±0.01%). LSD (0.05) comparison of means revealed that group B was statistically similar ($P>0.05$) to A, while significant difference ($P<0.05$) was observed in group D in comparison to A, B and C. These results indicate that thermization process has no significant ($P>0.05$) influence, while pasteurization and sterilization processes had remarkable ($P<0.05$) influence on ash content of skimmed milk.




LSD (0.05) = 0.0686
SE± = 0.0329

**Figure 6 Lactose content (%) of thermizide, pasteurized, sterilized and control skimmed milk**

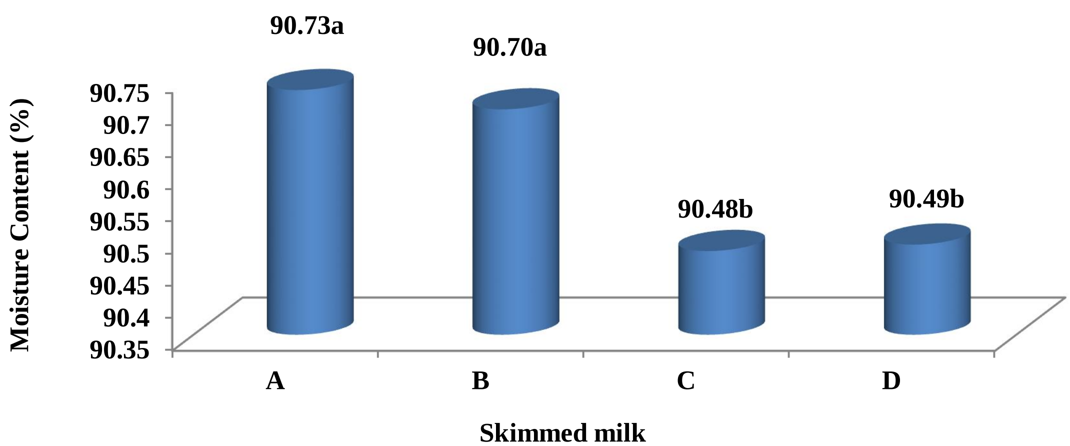



LSD (0.05) = 0.0155
SE± = 0.00074

**Figure 7 Ash content (%) of thermizide, pasteurized, sterilized and control skimmed milk**

**Influence of heat treatments on moisture content of skimmed milk**

The percent of moisture content of heat treated and untreated (control) skimmed milk was analyzed, and results are shown in figure-8. The moisture content was in a range between 90.68 to 90.80% in group A, 90.66 to 90.79% in group B, 90.37 to 90.54% in group C and of 90.40 to 90.53% in group D. ANOVA resulted significant influence of heat treatment on moisture content of skimmed milk. LSD (0.05) comparison of means revealed that the difference was statistically non-significant (P>0.05) between group (A and B) as well as in group (C and D). While, the moisture content in both (A and B) was significantly higher (P<0.05) as compared to group C and D. These results indicate that thermization process has no significant influence, while pasteurization and sterilization processes has shown remarkable effect on moisture content of skimmed milk.




LSD (0.05) = 0.0619
SE± = 0.0297

**Figure 8Moisture content (%) of thermizide, pasteurized, sterilized and control skimmed milk**

## DISCUSSIONS

In present findings, results showed that increase in temperature significantly increases (P<0.05) the pH of milk. These finding are in agreement with results reported by Molina *et al.* (2005), Petrus *et al.* (2011), Hassan *et al.* (2009), and Hussain (2011), who also found that increase in temperature results in increasing the pH of milk samples. Whereas, Walstra *et al.* (2006), Erdam and Yuksel (2005) and Hussain *et al.* (2003), reported that heat treatment causes some possible physico-chemical changes that results in decreasing the pH of milk due to increase in concentration of lactic acid produced from degradation of lactose content. They also reported that changes in Calcium phosphate is also responsible for decreasing the pH and thus increases the acidity of milk samples.

In present study results revealed that increase in temperature significantly decreases ($P<0.05$) the titratable acidity of milk. These finding are in agreement with results reported by Hassan *et al.* (2009), Kang *et al.* (2007) and Hussain (2011); that increase in temperature results in decreasing the acidity of milk. While findings disagreed with findings by Walstra *et al.* (2006), Erdam and Yuksel (2005) and Hussain *et al.* (2003), who reported that heat treatment results in increasing the acidity of milk due to increase in concentration of lactic acid produced from degradation of lactose content.

In present findings, results showed that increase in temperature significantly ($P<0.05$) increases the specific gravity of milk samples. The present results are in line with findings by Sharma (2006) and Nangraj (2011), who concluded that increase in temperature causes evaporation of some moisture level during the heat treatment process. This resulted increased total solid contents in treated samples and in turn increased the specific gravity of treated milk samples.

The results showed that increase in temperature slightly increased the fat content of milk samples. These findings are in agreement to the results by Nangraj (2011) and Abu Bakar *et al.* (2001), who reported that increase in temperature, resulted in slight increase in fat content. This is further supported by Petrus *et al.* (2011) and Winarso *et al.* (2011), who reported that changes in chemical composition of fat could occur at higher temperatures under which a level of milk fat slightly reduced from that of fresh milk due to evaporation of some components of milk during heating process.

It was observed that the protein contents increased with increase in temperature in group B (thermization) and C (pasteurization). These results are in favor of the findings of Nangraj (2011), who also observed an increase in protein content of pasteurized milk due to loss of moisture and increase in total solids. Moreover, in group D (sterilization) decrease in protein content was observed compared to group B and C. This could be attributed with sterilization process under which denaturation of protein could occur due to higher temperature ($110^{o}C$). These findings resembles with the results of Fetahagic *et al.* (2002), who found that nitrogen content decreased with increase in temperature due to denaturation of protein that resulted decreased protein content of treated milk samples. These findings also agreeing with work of Miyamoto *et al.* (2009), who stated that temperature above the level of $60^{o}C$ results in denaturation of protein content thus decreases the level of protein in treated samples.

It was resulted in present study that increase in temperature slightly increased the lactose content in thermizide and pasteurized milk samples. These findings agreed with the results of Nangraj (2011) and Hussain (2011), who stated that lactose content in milk samples pasteurized at various temperatures was slightly higher than that of control. But it started to decrease in sterilized milk because of lactose degradation, resulting in decreasing the lactose content, which agreed with work reported by Siddiqui *et al.* (2010) and Cattaneo *et al.* (2008), who found that more lactulose (an intermediate product of lactose break down) is formed in heat treated milk, resulted in lowering of the lactose content of milk samples treated at higher temperatures. Walstra (1999) also concluded that the milk subjected to highest temperature treatments yielded minimum lactose content, which might be due to maillard reaction as compared to lower processing temperatures.

The present findings showed that increase in temperature resulted in decreasing the moisture level of milk samples. These results are in accordance with the observations by Nangraj (2011), who resulted that increasing temperature caused some of the moisture to evaporate and thus resulted in decreasing the moisture content of treated milk as compared to control group. The ash was slightly increased as temperature was increased in the present study. The results supports the findings by Hussain (2011), who found that heating of skimmed milk samples resulted in increasing the ash content. These findings are in cross with the results by Siddiqui *et al*. (2010), stated that there was no significant difference in milk samples treated at various temperatures.

It was concluded that thermization, pasteurization and sterilization processes revealed significant ($P<0.05$) influence on pH, titratable acidity, specific gravity, lactose and ash content of skimmed milk. Pasteurization and sterilization treatments significantly affected the protein and fat contents of the skimmed milk. Thermization process did not affect the macro-nutrients of skimmed milk.

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