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EFFECT OF DIFFERENT HEATING TEMPERATURES ON THE RHEOLOGICAL PROPERTIES OF LACTIC GEL MADE FROM BUFFALO MILK

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A B S T R A C T

Heat treatment and acidification are the fundamental processing technologies for the development of fermented dairy products with desired attributes. Before lactic acid gel formation, milk is heated to destroy the microorganisms, to increase shelf life, to improve texture and ultimately to improve the quality of final products. The main aim of this study was to evaluate the rheological properties particularly texture of buffalo milk lactic acid gel made from different heat treatments i.e. 80°C, 85°C, 90°C and 95°C. The products formed by the respective treatments was further subjected to physicochemical (pH and acidity), compositional (protein fractions (total protein, non-protein nitrogen, non-casein nitrogen), fat, ash, total solids, moisture, lactose and minerals), rheological (synersis, viscosity and texture profile analysis), microbiological (total viable count and coliform count) and sensory analysis during storage of 14 days at refrigeration condition with an interval of 7 days. The rheological and organoleptic properties of lactic gel samples were influenced by different heating temperatures and storage time. There was significant difference in rheological parameters between the samples and the storage time. Lactic gel prepared at 90°C showed highest sensory scores and has better texture at the end of storage period. The viable count increased during storage and the coliform count remained negative throughout the storage period; however a significant difference in compositional parameter were observed between the samples and storage time. Changes in titratable acidity and pH showed significant difference during storage. Results indicated that different heating temperatures significantly affected the overall quality of lactic gel during storage.

Keywords: Buffalo milk, lactic gel (LG), heating temperatures, yoghurt.

INTRODUCTION

Among buffalo-milk producing countries Pakistan ranks second in the world after India (Hussain *et al*., 2010). Buffalo milk is blessed with high concentration of major constituents. It has high level of fat, proteins, total solids and minerals (especially calcium and phosphorus) which makes it preferable for fermented dairy products (Ajit and Khan, 2006). During the manufacturing of products in dairy industry heat treatment is an important processing technology utilized not only to increase the shelf life and safety by doing pasteurization but also to produce dairy products with specific rheological properties. In dairy product such as yoghurt and cheese casein gel and heat treatment are responsible for its rheological properties. Mainly the rheological study for product is done to analyze the texture and quality of the final product in dairy industries (Lopez *et al.,* 2002). Texture is parameter of rheological characteristics that define the quality of yoghurt. It shows all the rheological and structural parameters that are noticeable by means of mechanical, tactile, visual and auditory receptors **(**Sodini *et al*., 2006). These properties play vital role in controlling quality, storage and to prognosticate the texture. Texture of curd is an important attribute that determine acceptability and identity of the final product (Shaker *et al.,* 2000). Performing heating step at appropriate temperature can be helpful in minimizing the textural and quality defects of the final products. One of the main changes that occur during heat treatment is whey protein denaturation. These denatured proteins are more digestible than their naturally occurring form because the structure of protein

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is loosened and enzymes can act easily. β-Lactoglobulin (β-lg) and α-lactalbumin (α-la) are the most abundant whey proteins, and play an important role in determining the functional properties of the heated product (Oldfield *et al.,* 2000; Anema and Li, 2003). Heat treatment causes association of β-lg to the surface of casein micelles by disulphide bridging to k-casein thus introducing a further barrier to agglomeration. Aggregate that is formed by this cross linking is responsible for increase in micelle size after heating (Remeuf *et al.,* 2003; Livney *et al.,* 2003). The pH at which milk is heated is important in determining both the extent of casein dissociation from the micelle, and also whey protein association with the micelle. Milk after heating promotes aggregation, giving stronger gel with greater storage module and decreasing extent of acidification required to allow association to occur (Law and Leaver, 2000). In yoghurt manufacturing after heat treatment as preliminary necessary step, acidification will take place for the gel formation. As acidification process precedes the denatured whey protein interact with the casein micelles and internal structural properties of casein micelles changes as a result of colloidal calcium phosphate (CCP) solubilzation. Upon reaching the isoelectric point (pH 4.6) electrostatic repulsion between charged groups decreases and new hydrophobic interactions will form. Ultimately as a result of these entire changes gel formation take place (Lee and Lucey, 2010). The gel formed is basically cluster of aggregated spherical colloidal particles (majorly caseins) making a network in the form of a consistent structure throughout the enclosing volume (Phadungath, 2005; Penna *et al.,* 2007). Management of accurate time, rate of acidification and temperature at which milk is heated are very important for superior and homogenous quality of final product. Synersis and perceived viscosity are the two major quality defects with other defects i.e. improper texture, sourness, acidity and hardness which reduces the shelf life and affects the quality of yoghurt rendering it unacceptable (Lee and Lucey, 2010; Brabandere and Baerdemaeker, 2002). Conditions such as high heating temperatures, fast rate of acidification and high incubation temperatures all gave high level of whey separation (Lucey, 2001). There are various parameters by which we can control these quality defects one of them is the proper selection of heating temperature. Heating has a definite effect on viscosity and strength of the lactic gel during coagulation. It enhances the firmness and reduces the synersis. Main objective of the study was to compare different heating temperatures and their effects on rheological properties particularly texture of lactic gel made from buffalo milk.

MATERIALS AND METHODS

Procurement of Materials: Fresh raw whole buffalo milk was supplied from Dairy farm, University of Agriculture, Faisalabad. Commercially available freeze dried cultures (blend of *Streptococcus thermophilus*and *Lactobacillus delbrueckiisubspbulgaricus*) was provided by commercial suppliers (Rhodia).

Preparation of lactic gel samples: Raw milk was skimmed, standardized at 3.5 % fat and then was homogenized. The standardized milk was then heated at different heating temperatures i.e., 80°C, 85°C, 90°C and 95°C. The lactic gel prepared after heating milk at 90°C was used as a control (T_o) because most of the industries are preparing lactic gel at 90°C. The other lactic gel samples prepared by heating milk at 80°C, 85°C and 95°C were named asT_1, T_2 and T_3 respectively. All these samples were then inoculated with 2.5 % starter culture of *Streptococcus thermophillus*and *Lactobacillus bulgaricus.* After this incubation was done at 45°C for 4 hours when pH 4.6 was attained the incubation was stopped and the samples were cooled and stored at 4°C. The lactic gel samples were then analyzed for their physicochemical, compositional, rheological, microbiological and organoleptic properties during 14 days of storage at refrigeration condition with an interval of 7 days. The analysis was replicated thrice.

Physico-Chemical analysis: Lactic gel samples were analyzed for pH and acidity using standard Association of official Analytical Chemist procedure (AOAC, 2000). The pH of standardized milk and lactic gel samples was measured through electronic digital pH meter (Inolab WTW Series 720). Acidity of standardized milk sample and lactic gel was assayed by simple titration method.

Compositional analysis: Lactic gel samples were analyzed for moisture, ash, fat, protein fractions (total protein, non-protein nitrogen and non-casein nitrogen), total solids, and lactose and mineral contents using standard Association of official Analytical Chemist procedure (AOAC, 2000) whereas protein fractions were analyzed by using procedure provided by International dairy federation (IDF, 1993).

Rheological analysis: Synersis of lactic gel samples was determined by adopting the method as described by Rodarte *et al*. (2004). Brookfield DV-E viscometer was

used for the determination of viscosity of lactic gel following the methodology as detailed by Gassem and Frank (1991) and TA-XT plus texture analyzer was used to assess the firmness of the lactic gel samples according to Kumar and Mishra (2008).

Microbial analysis: Yousaf and Calstrom, (2003) method was used to conduct Total viable count and Coliform count.

Organoleptic properties: The Organoleptic properties of lactic gel samples were evaluated by 7 panelists. In terms of intensity the evaluation was made on the basis of nine point hedonic scale by Meilgarrd *et al*. (1999).

Statistical analysis: Significant difference among the treatments final data obtained was subjected to statistical analysis using analysis of variance technique (ANOVA) under two factor factorial completely randomized design (CRD). The mean of all treatments were also compared by using LSD test adopting the method as described by Steel *et al,* (1997).

RESULTS & DISCUSSION

Physico-Chemical analysis: Mean values of pH and titratable throughout storage period are shown in Table 1. pH of the lactic gel samples decrease during storage. The interaction between storage time and samples were also significant (P<0.01). pH and acidity of all the samples was in the range of 4.14-4.17 and 0.85 respectively. The similar results are reported by Anjum *et al*. (2007) and Wofschoon *et al.* (1983). This decrease in pH is due to the consumption of lactose by microbial culture that ultimately results in the formation of lactic acid, formic acid and small quantity of CO_2 (Panesar and Shinde, 2011). Titratable acidity of all lactic gel samples heated at different temperatures increased significantly during storage. Lactic acid bacteria act on milk sugar (lactose) and convert it into lactic acid, this conversion cause an increase in acidity. These results were according to the findings of Bilal (1995) and Shin *et al*. (1991).

Compositional Analysis: Mean values for fat, lactose and ash are shown in Table 2. Heating temperature have non-significant effect on fat, lactose and ash of lactic gel samples and the results showing decrease in fat and lactose % during storage are in accordance with the study of Kauser *et al.* (2011) and Anjum *et al*. (2007). Ahmad (1999) concluded that acidic storage for longer periods of time or lipolytic activity of microflora caused the reduction of fat contents. There is no change in ash content of all the samples. Ash content was 0.8% and remained same with small variations as shown in Table 3. The results are in accordance with Aziznia *et el*. (2008).

Protein % of the lactic gel samples was significantly affected due to the different heating temperatures as shown in the Table 3. At high heating temperature the denaturation level is more so the protein content is less in samples prepared from high heat treated milk than that prepared from low heated milk. The results are according to the findings of Fetahagic *et al.* (2002) who reported different % of protein at different heating temperatures. The trend of the results are according to the research of Hassan and Amjad (2010), Qureshi *et al.* (2012) and Rashid *et al.* (2012) who also observed slight increase in protein content. Non-protein nitrogen % and Non-casein nitrogen % between different samples was in range 0.19-0.15% and 0.98-0.80% respectively.

Table 1: The pH and titratable acidity % changes of lactic gel samples.

Treatments		Storage days			Mean
		01	07	14	
pH	T_0	4.58±0.03	4.10±0.05	3.82±0.05	**4.17 A**
	T_1	4.60±0.01	3.99±0.08	3.84±0	**4.14A**
	T_2	4.59±0.01	4.09±0.02	3.79±0.05	**4.16A**
	T_3	4.60±0.02	4.08±0.01	3.83±0.05	**4.17A**
Mean		**4.59B**	**4.06A**	**3.8242A**	-
Acidity	T_0	0.99±0.5	1.08±0.05	1.12±0	**1.06 A**
	T_1	0.99±0.5	1.08±0.05	1.13±0.01	**1.07A**
	T_2	0.99±0.5	1.08±0	1.13±0.01	**1.07A**
	T_3	0.99±0.5	1.08±0.05	1.13±0.01	**1.07A**
Mean		**0.99B**	**1.08A**	**1.13A**	-

T0, control heat treated at 90°C; T1, heat treated at 80°C; T2, heat treated at 85°C; T3, heat treated at 95°C

ABCDE Letters indicate significant difference among storage time and lactic gel samples, P<0.01.

Mean values for moisture and total solids are shown in Table 4. It is also revealed that total solids % and moisture % of all lactic gel samples heated at different temperatures is significantly affected as a function of storage time and is significant in relation to different heating temperature treatments. It is evident from the results that reduction in total solids throughout storage period might be due to change of lactose into lactic acid O' Neil *et al.* (1979) and Anjum *et al.* (2007) also observed variation in total solids % of yoghurt samples. The results of moisture are according to the finding of Dublin-Green and Ibe (2005) and Iwalokun and Shittu (2007) who concluded from there research that there is slight increase in moisture content during storage period.

Table 2: The fat, lactose and ash (%) changes of lactic gel samples.

Treatments		Storage days			Mean
		01	07	14	
Fat	T_0	3.56±0.05	3.46±0.01	3.36±0.02	**3.46^A**
	T_1	3.56±0.01	3.46±0.03	3.36±0.05	**3.46^A**
	T_2	3.56±0.05	3.43±0.05	3.36±0.05	**3.45^A**
	T_3	3.56±0.01	3.46±0.05	3.36±0.05	**3.46^A**
	Mean	**3.56^A**	**3.45^B**	**3.36^C**	-
Lactose	T_0	4.52±0.01	4.32±0	4.10±0.02	**4.31^A**
	T_1	4.51±0.01	4.32±0.02	4.10±0.02	**4.31^A**
	T_2	4.52±0.01	4.32±0.02	4.10±0.01	**4.31^A**
	T_3	4.52±0.01	4.32±0.01	4.10±0.03	**4.31^A**
	Mean	**4.51^A**	**4.32^B**	**4.10^C**	-
Ash	T_0	0.82±0.01	0.82±0.01	0.82±0.02	**0.82^A**
	T_1	0.82±0.05	0.82±0.05	0.82±0.05	**0.82^A**
	T_2	0.82±0.05	0.82±0.02	0.82±0.05	**0.82^A**
	T_3	0.82±0.01	0.82±0.01	0.82±0.01	**0.82^A**
	Mean	**0.82^A**	**0.82^A**	**0.82^A**	**0.82^A**

T0, control heat treated at 90^{o}C; T1, heat treated at 80^{o}C; T2, heat treated at 85^{o}C; T3, heat treated at 95^{o}C
ABCDE Letters indicate significant difference among storage time and lactic gel samples, P<0.01.

Table 3: The changes in protein and protein fractions (%) of lactic gel samples.

Treatments		Storage days			Mean
		01	07	14	
TP	T_0	4.47±0.1	4.48±0.01	4.54±0.05	**4.49^C**
	T_1	4.84±0.05	4.85±0.3	4.91±0.5	**4.86^A**
	T_2	4.68±0.005	4.69±0.1	4.76±0.01	**4.71^B**
	T_3	4.01±0.1	4.02±0.01	4.08±0.05	**4.04^D**
	Mean	**4.52^C**	**4.51^B**	**4.57^A**	-
NPN	T_0	0.17±0.02	0.18±0.01	0.19±0.06	**0.18^B**
	T_1	0.18±0.01	0.19±0.02	0.20±0.04	**0.19^A**
	T_2	0.18±0.01	0.19±0.01	0.20±0.03	**0.19^A**
	T_3	0.15±0.02	0.16±0.03	0.17±0.01	**0.16^C**
	Mean	**0.17^C**	**0.18^B**	**0.19^A**	-
NCN	T_0	0.88±0.06	0.89±0.01	0.90±0.01	**0.96^A**
	T_1	0.95±0.05	0.96±0.02	0.98±0.04	**0.96^A**
	T_2	0.93±0.04	0.94±0.01	0.96±0.05	**0.89^C**
	T_3	0.80±0.01	0.81±0.02	0.82±0.01	**0.80^D**
	Mean	**0.89^B**	**0.89^B**	**0.91^A**	-

T0, control heat treated at 90^{o}C; T1, heat treated at 80^{o}C; T2, heat treated at 85oC; T3, heat treated at 95^{o}C
ABCDE Letters indicate significant difference among storage time and lactic gel samples, P<0.01.TP: Total protein; NPN: Non-Protein Nitrogen; NCN: Non-Casein Nitrogen.

Table 4: The changes in moisture and total solids (%) of lactic gel samples.

Treatments		Storage days			Mean
		01	07	14	
Moisture	T_0	85.53±0.05	85.58±0.01	85.63±0.05	**85.58^{B}**
	T_1	85.18±0.01	85.30±0.01	85.60±0.01	**85.36^{D}**
	T_2	85.37±0.02	85.44±0.04	85.63±0.01	**85.48^{C}**
	T_3	85.66±0.01	85.68±0.01	85.73±0.01	**85.69^{A}**
	Mean	**85.43^{C}**	**85.50^{B}**	**85.64^{A}**	-
TS	T_0	14.43±0.02	14.41±0.01	14.37±0.01	**14.40^{C}**
	T_1	14.83±0.01	14.70±0.1	14.39±0.04	**14.63^{A}**
	T_2	14.62±0.02	14.55±0.04	14.38±0.01	**14.51^{B}**
	T_3	14.34±0.06	14.31±0.15	14.27±0.05	**14.30^{D}**
	Mean	**14.55^{A}**	**14.49^{B}**	**14.35^{C}**	-

T0, control heat treated at 90^{o}C; T1, heat treated at 80^{o}C; T2, heat treated at 85^{o}C; T3, heat treated at 95^{o}C
ABCDE Letters indicate significant difference among storage time and lactic gel samples, P<0.01.

Table 5: The changes in sensory parameters of lactic gel samples.

Treatments		Storage days			Mean
		1	07	14	
Surface Appearance	T_0	7.23±0.02	6.93±0.01	6.63±0.05	**6.93^{A}**
	T_1	6.43±0.02	6.37±0.002	5.86±0.01	**6.22^{D}**
	T_2	7.06±0.05	6.76±0.01	6.33±0.14	**6.72^{B}**
	T_3	6.28±0.01	6.53±0.05	6.43±0.01	**6.41^{C}**
	Mean	**6.75^{A}**	**6.65^{B}**	**6.31^{C}**	-
Body and Texture	T_0	8.16±0.28	7.56±0.05	7.38±0.05	**7.70^{A}**
	T_1	7.29±0.02	6.68±0.05	6.43±0.01	**6.80^{B}**
	T_2	7.08±0.14	6.89±0.02	6.53±0.05	**6.83^{B}**
	T_3	7.16±0.28	6.73±0.05	6.63±0.03	**6.84^{B}**
	Mean	**7.42^{A}**	**6.96^{B}**	**6.74^{C}**	-
Falvour	T_0	6.72±0.01	6.44±0.01	5.83±0.02	**6.33^{A}**
	T_1	6.75±0.05	6.02±0.02	5.72±0.05	**6.16^{B}**
	T_2	6.74±0.14	5.97±0.05	5.55±0.1	**6.09^{D}**
	T_3	6.42±0.01	6.17±0.1	5.72±0.02	**6.10^{C}**
	Mean	**6.66^{A}**	**6.15^{B}**	**5.70^{C}**	-
Taste	T_0	6.96±0.02	6.34±0.02	5.86±0.01	**6.39^{A}**
	T_1	6.81±0.1	6.16±0.2	5.76±0.01	**6.24^{AB}**
	T_2	6.74±0.01	6.08±0.02	5.64±0.01	**6.15^{B}**
	T_3	6.73±0.01	6.25±0.57	5.67±0.1	**6.22^{B}**
	Mean	**6.81^{A}**	**6.21^{B}**	**5.73^{C}**	-
Acidity	T_0	6.82±0.05	6.22±0.05	5.87±0.01	**6.30^{A}**
	T_1	6.25±0.05	5.80±0.01	5.35±0.02	**5.80^{D}**
	T_2	6.34±0.01	6.03±0.1	5.59±0.5	**5.98^{C}**
	T_3	6.9783±0.05	6.05±0.01	5.68± 0.02	**6.23^{B}**
	Mean	**6.59^{A}**	**6.02^{B}**	**5.62^{A}**	-
Surface Appearance	T_0	7.41±0.28	6.70±0.1	6.31±0.35	**6.81^{A}**
	T_1	6.70±0.01	6.23±0.02	5.83±0.05	**6.25^{B}**
	T_2	6.74±0.01	6.29±0.05	5.90±0.2	**6.31^{B}**
	T_3	6.60±0.02	6.30±0.5	6.04±0.02	**6.31^{B}**
	Mean	**6.86^{A}**	**6.38^{B}**	**6.02^{C}**	-

T0, control heat treated at 90^{o}C; T1, heat treated at 80^{o}C; T2, heat treated at 85^{o}C; T3, heat treated at 95^{o}C
ABCDE Letters indicate significant difference among storage time and lactic gel samples, P<0.01.

Mineral Analysis: Calcium and phosphorous (ppm) of the lactic gel samples are affected significantly due to storage. And significant relation exists between storage time and samples. T_o have maximum value of calcium i.e., 1522.7ppm and T_2 has minimum value of calcium i.e., 1156.7ppm at the end of storage period. For phosphorous T_1 have maximum value of i.e., 1178ppm and T_3 has minimum value of i.e., 1132ppm at the end of storage period. Variation in calcium and phosphorous are shown in Figure 1 and Figure 2.

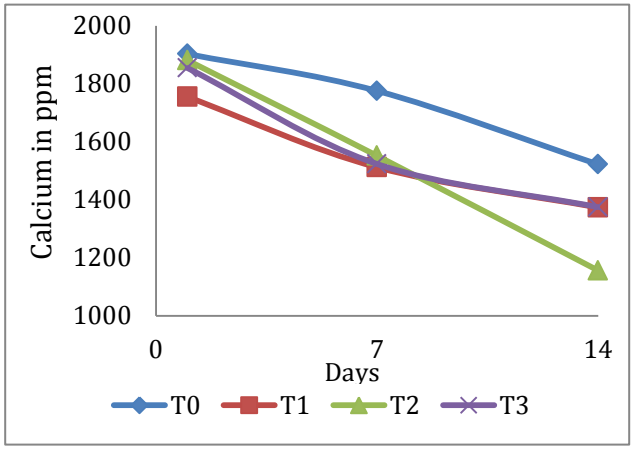

Figure 1: Changes in calcium (ppm) of lactic gel samples during storage.
T0, control heat treated at 90°C; T1, heat treated at 80°C; T2, heat treated at 85°C; T3, heat treated at 95°C

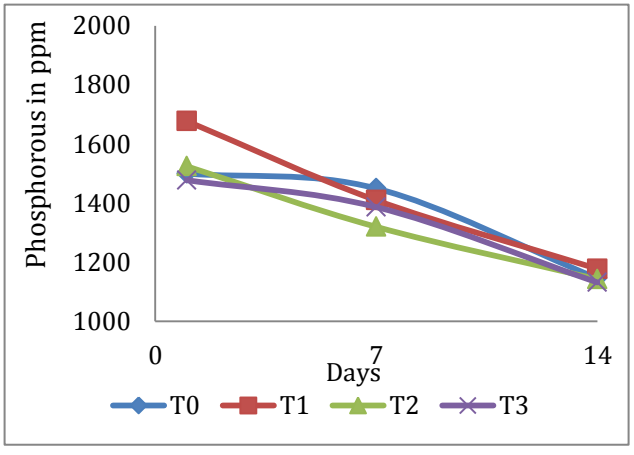

Figure 2: Changes in phosphorous (ppm) of lactic gel samples during storage.
T0, control heat treated at 90°C; T1, heat treated at 80°C; T2, heat treated at 85°C; T3, heat treated at 95°C

Rheological Analysis: Variation in texture is shown in Figure 3. Texture of lactic gel samples was significantly affected with respect to storage time and heating temperatures. Decrease in texture of all the samples during storage was 3.99 to 3.46, 3.40 to 3.30, 3.44 to 3.39 and 3.90 to 3.55 g for T_0 to T_3 respectively. It indicates that the texture of the entire samples decline from 1st to 7th day study during storage. The study of Lucey *et al.* (1999) support the results who concluded that lactic gel prepared by high heat treatment will have structure, having more dense and branched network of protein. There is highly significant effect of storage and heating temperatures on synersis and viscosity of all lactic gel samples. Variation in synersis is shown in Figure 4 whereas variation in viscosity of lactic gel during storage is shown in Figure 5. To has minimum synersis 3.23 ml and maximum viscosity 4086 cps at the end of storage period. The results of synersis are according to the findings of Guven *et al.* (2005). Results of viscosity are supported by the study of Isolauri *et al.* (2001).

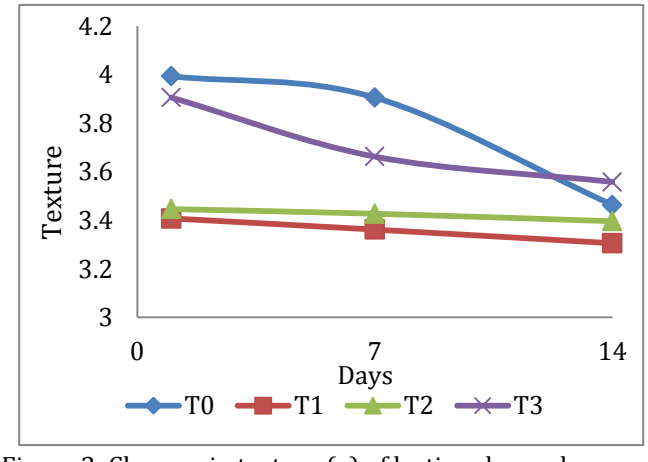

Figure 3: Changes in texture (g) of lactic gel samples during storage.
T0, control heat treated at 90°C; T1, heat treated at 80°C; T2, heat treated at 85°C; T3, heat treated at 95°C

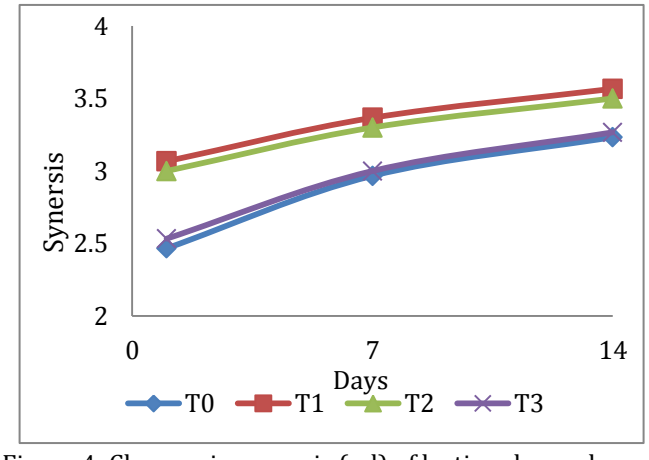

Figure 4: Changes in synersis (ml) of lactic gel samples during storage.
T0, control heat treated at 90°C; T1, heat treated at 80°C; T2, heat treated at 85°C; T3, heat treated at 95°C

Microbial Analysis: Total viable count of the lactic gel samples is significantly affected by different heating temperatures and due to storage as shown in Figure 6. Total viable count of all lactic gel samples increase during storage interval. Minimum total viable count was observed in T_3 i.e., 520.77 cfu/ml at the end of storage time. Results regarding the coliform count indicated that proper packaging and good quality of raw material results in no coliform till 14th day of storage. It may be due to acidity production in yoghurt, good quality of raw material and plus good storage conditions. Results of microbial analysis are in the agreement with Younus *et al*. (2000), Al-Hadethi *et al*. (1992) and Dave *et al*. (1992).

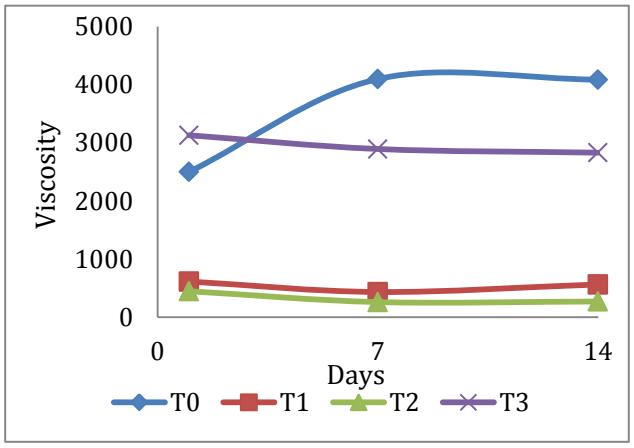

Figure 5: Changes in viscosity (cps) of lactic gel samples during storage.
T0, control heat treated at 90°C; T1, heat treated at 80°C; T2, heat treated at 85°C; T3, heat treated at 95°C

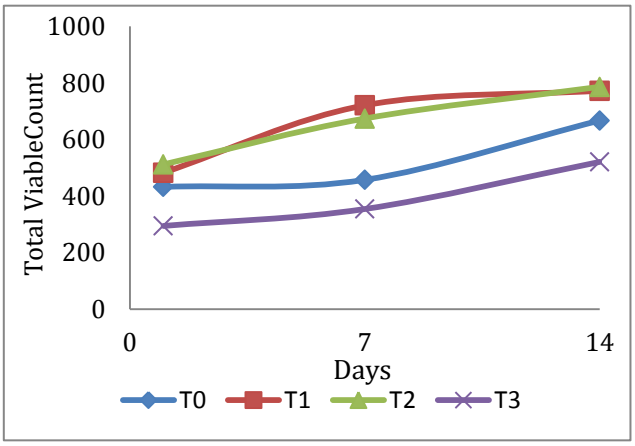

Figure 6: Changes in total viable count (cfu/ml) of lactic gel samples during storage.
T0, control heat treated at 90°C; T1, heat treated at 80°C; T2, heat treated at 85°C; T3, heat treated at 95°C

Sensory Analysis: This technique is utilized to develop and market the food products efficiently and meet the consumer demand. Surface appearance is important parameter of sensory to judge the quality (Meilgaard *et al*., 1999). Scores of different sensory parameters of lactic gel samples during storage is shown in Table 5. Surface appearance, body and texture and overall acceptability of all the lactic gel samples heated at different temperatures are affected significantly ($p<0.01$) due to different heating temperatures and storage. These changes are might be due to increase in acidity and declines in these sensory parameters score are supported by Bilal (1995), Kauser *et al.* (2011). Taste, flavor and acidity have significant relation with storage time, scores for these parameters also decrease with increase in time this is may be due to activity of microbes. These results are in accordance with Kamruzzaman *et al.* (2002) and Chawala *et al.* (1993). It was estimated from the statistical data that T_0 got highest scores as compared to other sample i.e. T_1, T_2 and T_3.

CONCLUSION

It is concluded at the end from all results that lactic gel prepared after heating milk at 90°C treatment named as T_o give better results. Less synersis more viscosity and better texture profile was observed for T_o. In sensory analysis overall acceptability is also high for T_o. The results of T_3 are also close to control but during storage better results are shown by control while other two treatments T_1 and T_2 does not full fill the requirement of storage It is also observed that lactic gel can be stored up to 14 days when kept at 4°C by giving proper storage and especially packaging condition.

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