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REGULAR ARTICLE

FERMENTABILITY AND RHEOLOGICAL PROPERTIES OF LACTOPEROXIDASE ACTIVATED BUFFALO MILK YOGHURT

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ABSTRACT

Present study was planned to produce and evaluate the coagulum from buffalo milk preserved with the activation of its lactoperoxidase system (LPO-system). A total of 10 trials were conducted and in each trial milk base was equally divided into three parts, two of which were treated with 20 mg/L (A) and 30 mg/L (B) solution of Sodium thiocyanate + Hydrogen peroxide and third part was kept as control (C). All the samples were analyzed for fermentability trend, pH, acidity, viscosity, specific gravity, syneresis and organoleptic properties. The decreasing trend in pH during fermentation period was comparatively slow in LPO-system activated milk A and B (4h and 5h) as compared to control yoghurt (3h). The titratable acidity (% lactic acid) of A, B and C yoghurt was 0.86±0.022, 0.85±0.025 and 0.89±0.024, respectively. The viscosity and specific gravity of control yoghurt was significantly ($P<0.05$) higher than A and B yoghurt. Whey syneresis was recorded as 2.08±0.24 ml $2h^{-1}$ in control yoghurt, whereas, 2.32±0.26 and 2.5±0.27 ml $2h^{-1}$ in A and B yoghurts. No significant differences ($P>0.05$) were observed in the total solids, fat, ash,

lactose and protein contents among the control, A and B yoghurt. Two week stored samples of control yoghurt received lower sensory score for appearance, flavor, body/texture and overall acceptability as compared to fresh control yoghurt. Simultaneously, LPO-system treated A and B yoghurt received high score during storage period than the control yoghurt.

Keywords: Fermentability, rheological properties, lactoperoxidase, buffalo milk, yoghurt

INTRODUCTION

There are several methods used for retarding bacterial growth in raw milk. The lactoperoxidase (LPO) system is one such method that helps to minimize microbial proliferation and extend the shelf life of milk. The use of the LPO-system for preservation of raw milk has been reported by several workers from different countries **(Fonteh *et al.*, 2005)**. However, the effectiveness of the system depends on the conditions that prevail in a given area particularly the microbial load of the milk before treatment and the prevailing ambient temperature. Antimicrobial agents or compounds formed by the LP system exhibit reduced starter activity during the preparation of fermented milk products. The activation of the LP system delayed the coagulation time and reduced the activity of starter cultures used for yoghurt production as thermophilic lactic starter strains were found to be sensitive to the LP system inhibition, but they varied in their susceptibility (**Basaga and Dik, 1994**).

The LPO-system is a native antibacterial system in milk. The enzyme lactoperoxidase is present in buffalo milk in relatively high concentrations. It can oxidize thiocyanate ions in the presence of hydrogen peroxide. By this reaction, thiocyanate is converted into hypothiocyanous acid (HOSCN). At the normal pH of milk HOSCN is dissociated and exists mainly in the form of hypothiocyanate ions (OSCN-). This agent reacts specifically with free sulphydryl groups, thereby inactivating several vital metabolic bacterial enzymes, consequently blocking their metabolism and ability to multiply. As milk proteins contain very few sulphydryl groups and those that are present are relatively inaccessible to OSCN- (masked), the reaction of this compound in milk is quite specific and is directed against the bacteria present in the milk.

The LPO-system (LP-system) is an acceptable chemical method for raw milk preservation, especially in rural areas where adequate refrigeration facilities are not available. Milk production in Pakistan is dominated by small-scale traditional production systems from

local breeds. Therefore, milk processors operating in such situations must rely on small volumes of milk from many farmers. Application of the LP-system prolongs the shelf life of raw milk and also encourages grouping of farmers hence facilitating milk collection system. Therefore, the need arose for further studies on the influence of this method on milk processing as well as on the quality of fermented dairy products such as yoghurt.

Nevertheless, LPS-treated yoghurt has been produced in the past. It has been found that the increase in acidity of yoghurt during storage and delivery (after-acidification) has been a major problem, since excessive sourness is undesirable. LPS suppressed acid production in yoghurt during storage under refrigeration **(Nakada *et al.*, 1996)**. Not only suppression of after-acidification, but also a change in texture was observed in LPS-treated yoghurt. Such yoghurts had softer and smoother textures than untreated yoghurts. Texture is a critical aspect of consumer acceptability of yoghurt **(Muir and Hunter, 1992; Cobos *et al.*, 1995)**. Various factors, such as total solids, milk composition, homogenization, type of culture, acidity, degree of proteolysis and heat pretreatment of milk, influence the rheological properties of yoghurt.

Therefore this study was designed to evaluate the effect of different concentrations of lactoperoxidase treated milk on fermentability and its influence on the physico-chemical and sensory properties of yoghurt.

MATERIALS AND METHODS

Collection and standardization of buffalo milk samples

Fresh buffalo milk samples were collected from Livestock Experiment Station, Department of Livestock Management, and brought to the Processing Laboratory, Department of Animal Products Technology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam to achieve the objective of the study. Raw milk was pre heated (37 to 40ºC) in a manual heating unit developed in the laboratory and the cream was separated at 40ºC with separation speed of 5000 to 6000 rpm in cream separator (Alfa Laval, Sweden). The skimmed milk obtained was standardized (3.5% fat) by remixing of an appropriate quantity of cream.

Preparation of activator 1 (NaSCN)

Sodium thiocyanate (1.0g) was dissolved in distilled water and transferred to volumetric flask (100ml). It was made up to volume with distilled water to make stock solution of activator 1.

Preparation of activator 2 (H_2O_2)

Thirty percent hydrogen peroxide solution (1ml) was dissolved in a 100 ml of distilled water to make stock solution of activator 2.

Preservation of buffalo milk

Standardized buffalo milk was divided into three equal parts and accredited with A, B and C codes. Milk sample under the code A was preserved by the activation of lactoperoxidase system (LPO-system) with 20 mg/L strength of each Activator 1 (Sodium thiocyanate) and activator II (Hydrogen peroxide) by adding 20 and 6.66 ml/L of stock solution, respectively. While sample under the code B was preserved by mixing 30 ml of stock solution of activator 1 and 9.99 ml of activator 2 per liter to obtain the final strength of 30 mg/L for the activation of LPO-system. Whereas, no any activator was added to milk sample under the code C and kept as control.

Yoghurt making

A total of 10 batches of yoghurt were prepared according to the method reported by **Yadav *et al.* (1993)** with slight modification and equal number of control samples with each batch. The standardized milk preserved by the activation of LPO-system was heated (60°C) and homogenized at 17.3 MPa pressure in homogenizer (Rannie-Homogenisator, Denmark). It was pasteurized in locally produced pasteurizer unit (Technology International, Pakistan) at 90°C for 10 min and cooled to 45°C. The artisan starter culture was prepared from skimmed buffalo milk and purified with repeated inoculation of a part of previous made culture in milk base for several days. The milk was heated at 90ºC for 10 min and cooled to 30ºC in running

tap water. Then it was inoculated (2%) with previous made culture and incubated at 42ºC for 3 hr. This culture was then maintained as mother culture during the study period.
The LPO activated and control samples of milk were inoculated with 3 % starter culture and incubated till pH decreased in the range of 4.5 to 4.7. Yoghurt was then transferred to refrigerator till further study. However, the trend of decrease in pH values was also recorded with an interval of 1hour during the fermentation period.

Fermentability trend of milk base

The pH values of milk base preserved by the activation of LPO-system were recorded with an interval of one hour during fermentation period. The observed values were compared with the values of milk base of controlled samples in order to observe the effect of LPO-system on the fermentability of milk base.

Analysis of yoghurt

The pH values of yoghurt samples were recorded using pH meter. Titratable acidity percentage, specific gravity, total solids content and ash was determined according to the method as described by **AOAC (2000)**. Fat content was determined by Gerber method as described by **James (1996)**. Protein content was determined according to the method of British Standards Institution **(BSI, 1990)**.

Viscosity of yoghurt was measured by using LFRA Texture Analyzer, (Brookfield Engineering Laboratories, Inc., USA). The analysis was carried out in the controlled stress made to measure the viscosity of the different types of yoghurt. Yoghurt sample (250 g) was placed into the stationary rheometer cup. Measurements were made on duplicate samples stored at 4-8^{o}C using spindle #4LV at rotational speed 10 rpm. The spindle factor was 600. The result was calculated by multiplying the viscometer readings made with the spindle/speed combination by 600 to obtain the viscosity in Pa.s.

Extent of serum separation (syneresis)

Serum separation of yoghurt was estimated using a drainage test according to the method as described by **Dannenberg and Kessler (1988)**. Yoghurt sample (20g) was placed

on mesh (0.4 mm) and transferred to refrigerator for two hours at 5-8ºC. The drained liquid was measured and expressed as ml/2h.

Sensory evaluation

Appearance, taste/flavor, body/texture and overall acceptability of different types of yoghurts were examined according to the hedonic scale as reported by **Muir and Hunter (1992)**.

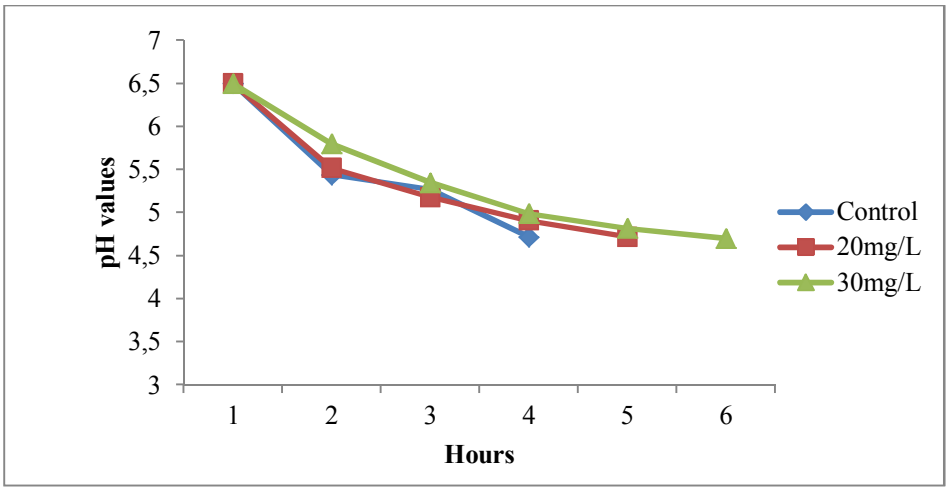
Statistical analysis

The data obtained was analyzed according to the statistical methods of analysis of variance (ANOVA) and the significant treatment means were further computed using least significance difference (LSD) at 5% level of probability by using computerized statistical package i.e. Student Edition of Statistics (SXW), Version 8.1 (copy right 2005, Analytical Software, USA).

RESULTS

Fermentability trend of milk base

The pH values of milk base treated with (LPO-system) were recorded with an interval of one hour during fermentation period (Figure 1). At the time of addition of starter culture the pH of control and treated milk (A and B) was recorded as 6.5. During the observation period of 1st hour, the pH value of control milk (C) was decreased to 5.44; while milk treated with 20mg/L (A) was at 5.52, whereas, milk treated with 30 mg/L (B) revealed 5.8. The decrease in pH during the observation period of 2nd hour in control milk base was 5.18, while A revealed 5.27 and 5.35in B. During the observation period of 3rd hour the control milk base acquired the required level of pH 4.72, while A revealed 4.91 and B at 4.99. Further, the desirable pH (4.70) of milk base A (20 mg/L) reached after 4th hour and B (30 mg/L) after 5th hour of fermentation period.



SE = ± 0.086, LSD at 5% = 0.187

Figure 1 pH trends of milk base/ during fermentation of yoghurt

Physical properties

The results of physical parameters are presented in Table 1.The mean pH value of B yoghurt (4.74±0.01) was comparatively higher than A yoghurt (4.72±0.009) and control yoghurt (4.69±0.01). Statistically, significant difference was observed between control and B yoghurt. There were no significant difference (P>0.05) in pH values of A and B yoghurt.

It was observed that the average acidity (% lactic acid) in control yoghurt (0.89±0.024 was slightly higher than by A yoghurt (0.86±0.022) and B yoghurt (0.85±0.025).The comparison of means regarding acidity (%lactic acid) in different yoghurt illustrated insignificant difference (P>0.05) among the control, A and B yoghurt.

The mean values of the viscosity (Pa.s) of control yoghurt (14.766±0.292), was remarkably higher followed by A (13.128±0.285) and B yoghurt (12.018±0.303).There were statistically highly significant differences (P<0.001) observed in the different treated and untreated (A, B and control) yoghurts.

The average specific gravity (Kg/m^3) of control yoghurt was 1.036±0.00023; followed by A and B yoghurt 1.035±0.00063 and 1.034±0.00016, respectively. Data revealed significant differences (P<0.05) in specific gravity between B and control. However, there were no significant differences (P>0.05) among specific gravity of A and B yoghurt.

The yoghurt samples produced from buffalo milk activated with LPO-system was analyzed for whey syneresis and the results illustrated that whey syneresis in control yoghurt was 2.08±0.24 ml $2h^{-1}$, whereas, 2.32±0.26 ml $2h^{-1}$ and 2.54±0.27 ml $2h^{-1}$was observed in A and B

yoghurt, respectively. The comparison of means regarding syneresis in different yoghurt revealed no significant differences (P>0.05) between the control, A and B yoghurt.

Table 1 Mean physical properties of yoghurt prepared by activation of LPO-system A(20 mg/L)B (30 mg/L) and C (control).

Parameter	A	B	C
pH	4.72±0.009	4.74±0.010	4.69±0.010
Titratable Acidity (% lactic acid)	0.86±0.002	0.85±0.025	0.89±0.024
Viscosity (Pa.s)	13.128±0.285	12.018±0.303	14.766±0.292
Specific gravity (Kg/m^3)	1.035±0.0006	1.034±0.0001	1.036±0.0002
Syneresis (ml/2h)	2.32±0.26	2.54±0.27	2.08±0.24

Chemical analysis

The results of chemical analysis are presented in Table 2.The mean total solids content (% age) in the control yoghurt was (13.66±0.19), slightly lower than A (13.92±0.19) and B (14.09±0.16) yoghurt. There were no significant differences (P>0.05) among the control, A and B yoghurt.

The results illustrated that the average fat content of control, A and B yoghurt was 3.6%.

The average ash content was 0.95±0.02 in A and 0.93±0.02 in B yoghurt. There was no significant difference (P>0.05) among the control, A and B yoghurt.

The mean value of protein in control yoghurt was 4.02±0.14%, while 4.02±0.14% and 4.02±0.14% in A and B yoghurt. There was insignificant differences (P>0.05) among treated (A and B) and control yoghurt.

The mean value of lactose in control yoghurt was 5.09±0.19, while it was higher in A yoghurt 5.35±0.2 and B yoghurt 5.49±0.20. There were no significant differences (P>0.05) among the control, A and B yoghurt.

Table 2 Mean chemical properties of yoghurt prepared by activation of LPO-system A (20 mg/L)B (30 mg/L) and C (control).

Parameter	A	B	C
Total solids (%)	13.92±0.19	14.09±0.16	13.66±0.19
Fat (%)	3.6±0.00	3.6±0.00	3.6±0.00
Protein (%)	4.01±0.14	4.01±0.14	4.01±0.14
Lactose (%)	5.35±0.2	5.49±0.02	5.09±0.19
Ash (%)	0.95±0.02	0.93±0.02	0.98±0.03

Sensory evaluation of fresh yoghurt

The results of sensory evaluation of fresh yoghurt are presented in Table 3.Appearance of fresh sample of yoghurt prepared from buffalo milk preserved with LPO-system was evaluated and the scores rated by panel of judges for appearance of control yoghurt was 82.46±0.30. While the score rated for A yoghurt was 72.64±0.22 and B yoghurt was mean, 65.33±0.17.

It was observed that the flavor in control yoghurt was higher 82.44±0.2 than the flavor of A 74.26±0.21 and B yoghurt 65.16±0.25.

The mean score for fresh body/texture was 82.93±0.27 in control, whereas, A and B yoghurts showed 74.28±0.53 and 64.59±0.30, respectively.

The overall acceptability of control yoghurt was 82.59±0.33 followed by A 76.96±0.45 and B 66.23±0.38 yoghurt. There was highly significant differences ($P<0.001$) observed among the control, A and B yoghurts.

Table 3 Mean Sensory analysis of fresh yoghurt prepared by activation of LPO-system A(20mg/L)B (30mg/L) and C (control).

Parameter	A	B	C
Appearance	72.64±0.22	65.33±0.17	82.46±0.3
Flavor	74.26±0.21	65.16±0.25	82.44±0.2
Texture	74.28±0.53	64.59±0.30	82.93±0.27
Overall acceptability	79.96±0.45	66.23±0.38	82.59±0.33

Sensory evaluation of stored yoghurt

The results of sensory evaluation of stored yoghurt are presented in Table 4.The mean of one-week stored yoghurt appearance was 70.93±0.4 in control yoghurt whereas, A and B yoghurt showed 66.72±0.16 and 65.36±0.37, respectively. Statistical analysis (ANOVA) revealed highly significant differences ($P<0.001$) among the control, A and B yoghurts. Further results of LSD (0.05) comparison of means revealed that the one week stored yoghurt treated (A and B) and control are significantly different ($P<0.001$) from each other. However, the appearance of two-week control yoghurt was 55.46±0.86, while A yoghurt revealed 63.14±0.34 and B yoghurt 58.36±0.27. The comparison means regarding to appearance in different types of yoghurt revealed that highly significant difference ($P<0.001$) observed in between the control, A and B yoghurts.

The average flavor score of control yoghurt was lower 63.98±0.47 than A 73.46±0.0.36 and B 71.29±0.21. Statistical analysis of means regarding flavor in different yoghurts were highly significant differences ($P<0.001$) occurs among the control, A and B yoghurts. The average flavor of two week stored control yoghurt was 41.54±0.23, followed by A (56.16±0.33) and B (63.10±0.32) yoghurts. Statistical analysis (ANOVA) revealed highly significant differences ($P<0.001$) among the control, A and B Further results of LSD (0.05) comparison of means reveals that the fresh sample yoghurt of different samples (control, A and B) are significantly different ($P<0.001$) from each other.

Body/texture of control yoghurt was 68.58±0.66, while A yoghurt revealed 66.61±0.2 and B yoghurt 63.71±0.3. The comparison of means reading of body/texture in different types of yoghurt were highly significant difference ($P<0.001$) observed among the control, A and B yoghurts. The mean body/texture score of two week stored yoghurt was 39.60±0.12 in control, 47.96±0.34 A yoghurt and 56.46±0.45 B yoghurt. The comparison of means regarding in body/texture in different yoghurt were significant differences ($P<0.05$) among the control, A and B yoghurts.

The average overall acceptability of 1st week control yoghurt was 63.62±0.3, followed by A yoghurt 65.42±0.24 and B yoghurt 64.81±0.36. The comparison of means regarding one week stored overall acceptability in different yoghurts showed non significant difference ($P>0.05$) among A and B yoghurts, while significant difference ($P<0.05$) was observed among control and A yoghurts. The overall acceptability score of two week stored control yoghurt was 39.58±0.43 followed by A 49.86±0.45 and B 59.81±0.52. Statistical analysis of mean

overall acceptability in different yoghurts revealed significant differences ($P<0.05$) among the control, A and B yoghurts.

Table 4 Mean Sensory analysis of stored yoghurt prepared by activation of LPO-system A(20mg/L)B (30mg/L) and C (control).

Parameter	A	B	C
Appearance	63.14±0.34	58.36±0.27	55.46±0.86
Flavor	56.16±0.33	63.10±0.32	41.54±0.23
Texture	47.96±0.34	56.46±0.45	39.6±0.12
Overall acceptability	49.86±0.45	59.81±0.52	39.58±0.43

DISCUSSION

The fermentability trend of milk base was significantly different in LPO-system treated yoghurt from control yoghurt. The decreasing trend in pH was comparatively slower in LPO-system treated milk (A and B) as compared to control yoghurt. These results are in line with the findings of **Hirano *et al.* (1998)**; they investigated that the metabolism of lactic acid bacteria in LPO-system treated yoghurt may be restricted by the activated LPO-system. However, with passage of time the bacteriostatic effect of LPO-system on Gram positive bacteria progressively slows down and gelation pH was achieved up to 5th hour. **Mehanna and Moussa (1999)** also expressed the similar views.

The pH values of control, A and B yoghurts were significantly different from each other. **Sadia and Tariq (2004)** also reported that pH of yoghurt samples treated with different concentrations of hydrogen peroxide and thiocyanate in contrast to control yoghurt was significantly different from each other. It was observed that pH values of yoghurt were higher in control yoghurt and lower in B yoghurt. The results are in agreement with findings of **Hirano *et al.* (1999)**, who also observed that higher concentration of LPO-system increased the gelation pH of yoghurt.

The average titratable acidity (% lactic acid) of control yoghurt was higher than the LPO-treated yoghurts (A and B). These results confirmed the findings of **Ndambi *et al.* (2008)**; they observed lower acid development in the treated yoghurt samples during incubation and showed that LPO-system has an inhibitory effect on lactic acid bacteria which retarded acid formation in the treated yoghurt. However, **Nakada *et al.* (1996)** investigated that the LPO-system suppressed acid production of yoghurt.

The result of viscosity (Pa.s) of control yoghurt was higher than A and B yoghurts. The LPO-system affects the texture and increased the softness of the yoghurt. The study conducted by **Hirano *et al.* (1998)** found that the yoghurt treated with LPO-system had softer and smoother texture than untreated yoghurts. The apparent viscosity of yoghurt was also reduced by addition of LPO-system. However, **Tamime and Robinson (2007)** suggested that the LPO-system treated yoghurt had weak consistency, low viscosity and also affected the rheological properties of yoghurt. Similarly, the average specific gravity of control yoghurt was higher than A and B yoghurts. These results confirmed the findings of **Hirano *et al.* (1998)**, who observed that the LPO-system treated yoghurt had low hardness and soft, smooth texture. Other possible reasons for rheological changes would be the effects of radicals on rheological properties. In the present study the whey syneresis of treated yoghurt (A and B) was higher than untreated (control) yoghurt. These results are consistent with the findings of **Tamime and Robinson (2007)**, who reported that the yoghurt made from milk treated with LPO-system, was of weak consistency with high whey separation.

The total solids content of control yoghurt was lower than the LPO-system activated yoghurt A and B. These results are in agreement with the findings of **Ndambi *et al.* (2008)**, who reported that the total solids content of treated yoghurt was higher than control yoghurt. The higher total solids content probably resulted from the inhibitory effects of the LPO-system on bacteria that degrade milk solutes. The fat content of control yoghurt was 3.6, while LPS-system treated with A and B yoghurts also showed similar results as control. These results are also supported by **Ndambi *et al.* (2008)**, who also reported that the fat content was similar to that of the treated and control yoghurts. The average protein content of control, LPO-system treated with A and B yoghurt was not significantly different from each other. The results are consistent with the findings of **Ndambi *et al.* (2008)**, who noted that the percentage of protein was not significantly different between yoghurt samples of treated LPO-system and control. The average ash content of control yoghurt was higher than treated (A and B) yoghurts. These results are consistent with the results of other reported work i.e. in between 0.72 to 0.92% **(Mohammad *et al.*, 2007)**. Whereas, the average lactose content of control yoghurt was lower than A and B yoghurts. The results are consistent with the findings of **Ndambi *et al.* (2008)**, who suggested that the lower acid development in the treated yoghurt sample during incubation showed that LPO- system has inhibitory effects on lactic acid bacteria which convert the lactose into the lactic acid and retard acid formation in the treated yoghurt during fermentation.

In the present study the fresh control yoghurt perceived higher score in appearance, flavor, body/texture and overall acceptability than LPO-system treated yoghurt A and B. Similarly, **Ndambi *et al.* (2008)** indicated that the judge's preference was more for control yoghurt than that from treated yoghurt. However, the result of the present study are not in agreement with the findings of **Sadia and Tariq (2004)** who reported that the overall acceptability was better in 10ppm concentration with that of control, while higher concentration of LPO-system treated yoghurt gave negative results.

Moreover, stored samples of control yoghurt received lower scores, whereas, A yoghurt and B yoghurt received high scores during storage period than the control yoghurt**.** **Ndambi *et al.* (2008)** studied that at the end of the second week of storage nine out of twelve (75%) control yoghurt samples produced off-flavors and gasses indicating spoilage. However, **Nakada *et al.* (1996)** reported that the addition of LPO-System treated milk produced new type of yoghurt which retains acceptable quality during storage for at least two weeks.

CONCLUSION

It was concluded that fermentability was slow in LPO-system activated milk base compared to control yoghurt. Whereas, viscosity and specific gravity of yoghurt prepared from milk base was reduced in contrast to control yoghurt. Fresh control yoghurt perceived higher sensory score than yoghurt produced from LPO-system activated milk base. However, sensory attributes of yoghurt prepared from LPO-system activated milk base were improved with the passage of time compared to control yoghurt.

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