
N. M. N. K. Narayana⁽¹⁾ and V. K. Gupta⁽²⁾

Physical, textural and sensory attributes of plain set yoghurt made employing ultrafiltration technique as affected by titratable acidity during incubation

(1) Department of Animal Science, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka. (e-mail: nayanarayanan1970@gmail.com)

(2) Division of Dairy Technology, National Dairy Research Institute, Karnal, 132001, Haryana, India.

Abstract: Effect of stopping incubation at different titratable acidity levels {0.738±0.01% lactic acid (LA) (T₁), 0.815±0.005% LA (T₂) and 0.927±0.01% LA (T₃)} of plain set yoghurt made employing ultrafiltration technique was investigated on physical, textural and sensory properties. Water holding capacity was observed to be significantly ($p<0.05$) higher in T₂ compared to T₁ and T₃. Textural attributes increased significantly ($p<0.05$) with increasing yoghurt acidity level. Treatment T₁ had significantly ($p<0.05$) lower flavour and acidity scores. Body & texture and overall acceptability scores were observed to be significantly ($P<0.05$) higher in T₂ treatment. Hence, maintaining yoghurt acidity of around 0.815±0.005% LA during incubation was observed to be optimum.

Key words: ultrafiltration, retentate, whey syneresis, water holding capacity, textural attributes

Introduction

Yoghurt is a popular fermented dairy product consumed all over the world. It is formed by slow fermentation of lactose to LA by thermophilic yoghurt starter bacteria namely *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Lucey, 2002). Horiuchi *et al.* (2009) reported that the global sales of yoghurt in year 2006 were approximately US\$ 40 billion. According to a recent research conducted by Global Industry Analysts Inc., it was predicted that by year 2015, global yoghurt consumption will reach

20.6 million tons, equaling US\$ 67 billion in sales. Asia presents a huge opportunity due to the rising incidence of lifestyle-related health concerns, such as diabetes and obesity, brought on by rapid economic development and rising income levels, (Anon, 2010).

The set yoghurt is produced by packaging the yoghurt mix into individual containers before fermentation. As a commercial product, it is important that the set yoghurt has curd with sufficient hardness to stand up to the impact caused by shaking during transportation (Horiuchi *et al.* 2009). Nielsen (1975) suggested that the texture of set yogurt should be firm enough to remove it from the container with a spoon. According to Lewis and Dale (1994), set yoghurt should have a glossy surface appearance without excessive whey. Whey Syneresis is a major defect of set-style yoghurt (Lucey 2001). The formulation of yoghurt products with optimum consistency and stability to whey syneresis is of primary concern to the dairy industry (Biliaderis *et al.* 1992). Factors influencing yoghurt texture and whey syneresis include total solids (TS) content especially proteins, homogenization, type of culture, acidity resulting from growth of bacterial cultures and heat treatment of milk (Harwalkar and Kalab 1986).

Acidity of yoghurt is a consequence of lactic acidification obtained at the end of the incubation period and post acidification during the storage of yoghurt (Beal *et al.*, 1999). According to the prevailing

standards of yoghurt, final acidity vary between 0.7% (IDE,1992) to 0.9% LA (FDA,1996). FSSA (2006), India requirement is to have 0.85% to 1.2% LA during the shelf life of yoghurt. Acidity influences the quality attributes of yoghurt such as flavour, texture, whey syneresis, shelf life etc. Therefore, an attempt was made to improve quality of yoghurt made employing ultrafiltration (UF) technique by stopping incubation at various titratable acidity (TA) levels and made recommendations thereof.

Materials and Methods

Materials

Raw cow skim milk and cream (about 50-55% fat) was obtained from Experimental Dairy of National Dairy Research Institute, Karnal. Well reputed brand (Nestle') of commercial yoghurt containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* was used as the starter culture for the production of yoghurt.

Methods

Ultrafiltration of cow skim milk and production of experimental yoghurts

Cow skim milk was heated to 80°C, cooled to 55-60°C and transferred to the balance tank of pilot UF plant {Tech-Sep, France with tubular module (channel diameter, 6 mm) having ZrO₂ membrane (membrane surface area, 1.68 m² and membrane molecular weight cut off, 50,000 Dalton)} and ultrafiltered at 50-55°C to 5 fold UF concentration. Cow skim milk was standardized to 13.9% TS and 3.3% fat by adding calculated amount of 5 fold UF cow skim milk retentate and cow milk cream, respectively. Resultant standardized milk was pre-heated to 65-70°C; homogenized in a two-stage homogenizer (M/s Goma Engineers, Mumbai) at 2000 and 500 psi at 1st and 2nd stages, respectively; heat treated at 85°C/30 min in a thermostatically controlled water bath (NAVYUG, India); cooled immediately in an ice water tub to 42-45°C; inoculated with 2% of yoghurt culture; mixed; filled in clean polystyrene cups; covered with lids and incubated at 42±1°C. Incubation was stopped at different TA levels viz. 0.738±0.01% LA (T₁), 0.815±0.005% LA (T₂) and 0.927±0.01% LA (T₃).

Yoghurts were then immediately transferred to a refrigerator maintained at 4±1°C. Respective pH of the samples were observed to be 4.77±0.02, 4.58±0.01 and 4.51±0.02. Quality of yoghurt was evaluated in terms of sensory and physical parameters including textural attributes. Experiment was repeated 3 times.

Physicochemical analysis

A pH meter (PHAN LABINDIA Model, Labtek Eng. Pvt. Ltd. India) was used to determine pH of yoghurt during incubation. Titratable acidity was determined using procedure recommended in BIS (1981a). Fat content of skim milk and UF cow skim milk retentates were determined as per the method given in BIS (1981a), whereas, in cream as per the methods given in BIS (1977)

Spontaneous whey syneresis (SWS)

Siphon method described by Amatayakul *et al.* (2006) was used with slight modifications to determine the SWS. A cup of yogurt (100 ml) was tilted immediately after removing from the refrigerator at an angle of 45° to collect the surface whey. Collected whey was siphoned out with a graduated syringe with a needle. The siphoning was performed within 10 s to avoid forced leakage of whey from the curd. The value was taken directly as the percent SWS.

Water Holding Capacity (WHC)

The WHC was measured by a centrifuge method given by Supavititpatana *et al.* (2009). Within 12 h of the production of yogurt, a 10 g sample was centrifuged at 2,000 g for 60 min at 10±1°C. The supernatant was removed within less than 10 min and the wet weight of the pellet was recorded. The WHC was expressed as follows.

$$\text{WHC (\%)} = \frac{\text{Pellet (g)}}{\text{Sample (g)}} \times 100$$

Textural attributes

Texture analysis was carried out according to the method given by Kumar and Mishra (2003) with slight modifications, using a TA-XT2i Texture analyser (M/s Stable Micro Systems, UK) fitted with a 25 kg load cell and was calibrated with a 5 kg standard dead weight prior to use. For determining the textural attributes,

the pasteurized and cooled standardized milk was filled up to 80 ml in 100 ml clean glass beaker and incubation was carried out. Experiments were carried out by compression tests that generated plot of force (N) versus time (s). A 25 mm perplex cylindrical probe was used to measure texture of yoghurt samples at a temperature of 10±0.5°C performing four repetitions. During analysis the samples were compressed up to 20 mm of their original depth. The speed of the probe was 0.5 mm/s during the compression, 2 mm/s during pre-test and relaxation. From the resulting force-time curves, firmness, stickiness, work of shear (WoS) and work of adhesion (WoA) were calculated using the Texture Expert Exceed software (version 2.55) supplied by the manufacturer along with the instrument.

Sensory evaluation

On the basis of desirable attributes for good quality yoghurt, the 100 point score card suggested by Ranganadham and Gupta (1987) was used for the sensory evaluation of yoghurt. The values of 100 point score were divided for flavour, body & texture, acidity, colour & appearance and container and closure viz., 45, 30, 10, 10 and 5, respectively. Yoghurts were sensory evaluated at 10±1°C by a panel of 8 trained judges at National Dairy Research Institute, Karnal.

Statistical analysis

The results obtained in the present study were subjected to one-way analysis of variance (ANOVA) using SPSS Version 16. LSD was used for mean comparisons. Critical difference (CD) was calculated according to the method described by Rangaswamy (1995). Significant differences were determined at 95% level of confidence.

Results and Discussion

Effect of TA of yoghurt during incubation on whey syneresis and WHC

Whey syneresis was observed only in T₃ treatment (Tab 1). However, it was not significantly different between treatments. When yoghurts were kept in the incubator for more time (to develop acidity further), it was observed that whey syneresis started to appear. Water holding capacity was observed to be significantly (*p*<0.05) higher in T₂ compared to T₁ and

T₃. Further, WHC was significantly (*p*<0.05) higher in T₃ compared to T₁. Water holding capacity was observed to be highest in T₂ followed by T₃ and T₁. Corresponding values were 64.68, 63.60 and 62.78%, respectively (Tab 1). Sodini *et al.* (2004) mentioned that the yoghurt pH had a significant effect on WHC. There is a relationship between TA and pH and it affects WHC. According to the current study, low acidity/high pH (T₁) and high acidity/low pH (T₃) treatments had significantly (*p*<0.05) low WHC than the moderate treatment (T₂).

According to Aguilera and Kessler (1989) curds with high pH had a poor WHC in GDL-acidified gels. Harwalkar and Kalab (1986) noticed that, within the range of common final pH encountered for yoghurt manufacture, reduction in the pH, slightly decreased the WHC of the yoghurt. They reported WHC of 67% and 65% for yoghurt having pH 4.50 and 3.85, respectively. Findings of the current study also agreed with earlier reports.

Table 1:
Physical and textural parameters* of plain yoghurt as affected by TA during incubation

TA level (% LA)	Whey syneresis (%)	WHC (%)	Firmness (N)	Stickiness (N)	WoS (N.s.)	WoA (N.s.)
0.738±0.01	0	62.78 ^a	1.61 ^a	-0.33 ^a	52.27 ^a	-1.94 ^a
0.815±0.005	0	64.68 ^c	1.92 ^b	-0.43 ^b	54.62 ^b	-2.34 ^b
0.927±0.01	0.08	63.60 ^b	2.10 ^c	-0.50 ^c	57.43 ^c	-2.78 ^c
CD _{0.05}	NS	0.66	0.09	0.02	0.98	0.28

^{a,b,c} Means with different superscripts within each column differ significantly (*p*<0.05)

Effect of titratable acidity of yoghurt during incubation on textural attributes

Firmness, stickiness, WoS and WoA increased significantly (*p*<0.05) with increasing yoghurt acidity level (Tab 1). Rönnegard and Dejmek (1993) studied the linear viscoelastic properties of yoghurt fermented

to different pH values. They observed a higher complex viscosity and a lower angle shift when the pH was decreased from 4.50 to 4.25. Beal *et al.* (1999) showed that there was a significant effect of final pH on viscosity of yoghurt, and with decreasing pH, viscosity was reported to be increased. Harwalkar and Kalab (1986) reported an increase of 20% of gel firmness when the final pH was decreased from 4.50 to 3.85.

Effect of TA of yoghurt during incubation on sensory attributes

Effect of TA during incubation on sensory attributes of plain yoghurt is presented in Tab 2. It was observed that all the sensory scores significantly ($p < 0.05$) different between treatments (Tab 2). Flavour score was highest (40.63 out of maximum possible 45) in T_2 and it was not different from the flavour score obtained by T_3 . Treatment T_1 , which was having lowest acidity level during incubation had lowest flavour score (38.94 out of maximum possible 45) and it was significantly ($p < 0.05$) lower than T_2 and T_3 treatments. One of the flavour compounds that impart distinctive flavour to yoghurt is lactic acid (Beshkova *et al.*, 1998; Chaves *et al.*, 2002) among others. Yoghurts were served to sensory panel nearly after 24 hours of storage. When the TA of yoghurt is low during incubation (T_1), TA at the time of consumption is also less. Lactic acid production may be insufficient to give a distinctive flavour characteristic to final product and this may be the reason to have significantly ($p < 0.05$) lower flavour scores of the yoghurts of T_1 treatment compared to other treatments. On the other hand, T_3 treatment had lower flavour score than the treatment T_2 indicating that the higher acidity is also not favourable. Hence, it can be concluded that, treatment T_2 having $0.815 \pm 0.005\%$ LA/ 4.58 ± 0.01 acidity/pH value during incubation of yoghurt, is the best among tested treatments. This product had acidity/pH level of $0.860 \pm 0.005\%$ LA/ 4.56 ± 0.01 after 24 h of refrigeration, which agrees with the current FSSAI (2006) regulations of India for final TA of yoghurt.

Table 2:
Sensory scores* of plain yoghurt as affected by TA during incubation

TA level (% LA)	Flavour	Body & texture	Acidity	Colour & appearance	Overall acceptability
0.738 ± 0.01	38.94 ^b	26.19 ^b	7.13 ^b	8.59 ^a	85.84 ^c
0.815 ± 0.005	40.63 ^a	27.22 ^a	8.13 ^a	8.25 ^a	89.22 ^a
0.927 ± 0.01	40.31 ^a	26.25 ^b	7.88 ^a	7.81 ^b	87.25 ^b
CD _{0.05}	0.939	0.560	0.577	0.491	1.589

^{a,b,c}Means with different superscripts within each column differ significantly ($p < 0.05$)

Yoghurt acidity score also followed a similar trend as flavour scores between treatments indicating that acidity is a distinctive characteristic of flavour of yoghurt. In manufacturing yoghurt, fermentation is stopped at a pH inferior to 4.6. It could vary, depending on the process conditions from 4.6 to 4. It has a significant effect on sensory properties such as acidity, flavour, and texture (Lucey and Singh, 1998; Sodini, *et al.*, 2004).

Body and texture score was observed to be significantly ($P < 0.05$) higher in T_2 treatment followed by T_3 and T_1 . When acidity was less and pH was high (T_1), the curd was loose and obtained lower body and texture score. The acidification process results in the formation of three-dimensional network consisting of clusters and chains of caseins (Mulvihill and Grufferty, 1995). This completes at pH 4.6 which was the IEP of casein. Hence, pH above 4.6 is not favourable to have yoghurt having a good body and texture and current study further confirmed it. On the other hand, T_3 treatment which had highest TA and lowest pH combination, also obtained significantly ($p < 0.05$) lower body and texture score than T_2 treatment. With the increase of TA, whey syneresis was noted on the top of the curd and the yoghurt body was observed to be little shrunk. This might be the reason to have lower score for body and texture of yoghurts in treatment T_3 . This

shrinkage is due to the rearrangement of the three dimensional protein network of the yoghurt. Martin *et al.* (1999) reported that, stirred yoghurt obtained at a pH between 4.4-4.2 is more thick-in-mouth and consistent than those obtained at a pH between 4.8-4.7. However, in the current study, the pH of the best treatment that obtained highest body & texture score was 4.58 ± 0.01 which was higher than the value reported by Martin *et al.* (1999). Colour and appearance score was significantly ($p < 0.05$) higher in yoghurt made from T₁ compared to T₃ treatment. T₁ had the highest score of 8.59 out of maximum possible 10 followed by 8.25 in T₂. T₃ treatment obtained lowest score for colour and appearance (7.81) and this is due to the appearance of whey on the surface of the yoghurt. Overall acceptability score reflected the scores obtained by all sensory parameters and it was significantly ($p < 0.05$) higher in T₂ compared to T₁ and T₃. Hence, maintaining yoghurt acidity of around $0.815 \pm 0.005\%$ LA during incubation was observed to be optimum.

Correlations between some important parameters

Pearson's correlation coefficients were determined for selected parameters to check whether there is any correlation and to determine the strength of the correlation. It was observed that the acidity/pH level during incubation of yoghurt had a significant ($p < 0.05$) positive correlation with firmness of the yoghurt ($r = 0.958$). Further, it had a significant ($p < 0.05$) positive correlation with flavour score of yoghurts. This indicates that with increasing acidity/pH level, the flavour score was also increased in tested acidity/pH levels. However, it is important to note that the highest flavour score was obtained by T₂ treatment even though, it was not different compared to the flavour score obtained by T₃ treatment. Other sensory attributes such as body & texture, acidity and overall acceptability scores were not significantly correlated with acidity/pH levels. Apart from that, acidity score was significantly ($p < 0.05$) correlated with flavour score ($r = 0.821$) and overall acceptability score ($r = 0.825$). Further, flavour score was significantly ($p < 0.05$) correlated with overall acceptability score ($r = 0.896$).

Conclusion

Physical, textural and sensory quality of plain set yoghurt made employing UF technique could be improved by stopping incubation at $0.815 \pm 0.005\%$ LA. The optimum quality yoghurt had 64.68% WHC, 1.924 N firmness, -0.434 N stickiness, 54.616 N.s. WoS and -2.339 N.s. WoA with no whey syneresis. Further increase of acidity has adverse effect on quality of the product and hence, stopping incubation at $0.815 \pm 0.005\%$ LA would be recommended for the production of good quality plain set yoghurt.

Acknowledgement

The first author acknowledges the Sri Lanka Council for Agricultural Research Policy for awarding a scholarship to carry out this research.

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