Effect of Refrigerated Storage on Quality of Set Yoghurt Made from Ultrafiltered Milk

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ABSTRACT

Plain set yoghurts were manufactured by keeping milk solids level at 13.9% and fat at 3.3% in the yoghurt milk base, by the addition of calculated amount of 5 fold ultrafiltered cow skim milk retentate and cow milk cream, respectively. Storage quality of yoghurts was investigated at 4±1°C against control yoghurts made by milk standardized with skim milk powder. Significant ($p<0.05$) increase of acidity development and pH reduction of yoghurts was observed with advancing storage period. Whey syneresis appeared on day 17th of storage irrespective of the type of yoghurt and increased significantly ($p<0.05$) with increasing storage period. Experimental yoghurts had significantly ($p<0.05$) lower amount of whey syneresis compared to control yoghurts. Water holding capacity was significantly ($p<0.05$) higher in experimental compared to control yoghurts and increased significantly ($p<0.05$) with advancing storage period up to 9th day and thereafter decreased. Firmness increased significantly ($p<0.05$) with increasing storage period irrespective of the type of yoghurt and significantly ($p<0.05$) higher in experimental compared to control yoghurts. Lactic acid bacteria (LAB) count decreased significantly ($p<0.05$) and yeast and moulds increased significantly ($p<0.05$) with advancing storage period, irrespective of the type of yoghurt. No differences of LAB and yeast and mould count were found between experimental and control yoghurts. Coliforms were not detected in any of the yoghurts during storage. On the basis of increased yeast and mould count, shelf life of experimental yoghurts was observed to be 17 days at 4±1°C.

Keywords: Acetaldehyde concentration, Retentate, Textural attributes, Ultrafiltration, Water holding capacity

1. INTRODUCTION

Yoghurt suits all palates and meal occasions and is formed by slow fermentation of lactose to lactic acid (LA) by thermophillic yoghurt starter bacteria namely *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* [1]. Consumption of yoghurt has steadily increased over the last 30 years in many countries of the world [2]. Yoghurt is retailed mainly in one of the three physical states, namely set, stirred and fluid/drinking according to the method of production and the physical structure of the coagulum. The set yoghurt is produced by packaging the yoghurt mix into individual containers before fermentation. Whey syneresis is a major defect of set yoghurt. The formulation of yoghurt products with optimum consistency and stability to whey syneresis is of primary concern to the dairy industry [3]. Some of the methods adopted by manufacturers to address this problem include addition of skim milk powder, addition of natural or synthetic gums and stabilizers, enzymatic stimulation of protein interaction in milk etc. However, there is increased consumer demand towards more 'natural' products with no additives and stabilizers.

Increasing the total solids (TS) content in the milk base by the addition of ultrafiltered milk retentate is widely recognized and can be applied to manufacture various cultured dairy products including set yoghurt. Ultrafiltration (UF) offers several benefits to yoghurt formulations such as reduction of harshness caused by excessive acidity, reduction in lactose content, improvement of texture due to the increased protein content in base milk that minimize the need of stabilizer-like additives and production of a more natural product demanded by the current consumer. High nutritional image of yoghurt could be further expanded by increasing the protein, calcium and phosphorus content through UF process.

Several investigations into the shelf life of different yoghurts have been reported. Some researchers stated that the shelf life of yoghurt products is determined by the time the product remains safe to eat, the time its functional claims remain true to label or to regulatory requirements and the time its sensory properties remain acceptable to consumers [4]. Fresh yoghurt is at its best in the first few weeks of shelf life, after which there is a discernible reduction in sensory characteristics. The combination of severe heat treatment, low pH and a dense population of living starter bacteria (typically 10^7-10^9 cfu/ml) inhibit growth of spoilage bacteria. Nevertheless, yeast and mould may thrive under these conditions and can spoil the product [5]. Yeast and mould are the principal agents of microbial spoilage of yoghurt. In fresh yoghurt products yeast and mould may be present due to contamination in the processing operations, including from added fruit preparations, from the packaging materials and/or the filling operations [4]. Yeast and mould are little affected by low pH and may cause spoilage of yoghurt during storage [6].

Information on the behaviour of yoghurt manufactured using milk standardized with UF retentate during storage is lacking in the literature and important to study because its shelf life is based on whether the products display any of the physical, chemical or sensory characteristics that are unacceptable for consumption. Studies of the changes in these quality characteristics during storage would enable producers to predict the shelf life of the product more accurately. Hence, the current study was conducted to investigate the effect of refrigerated storage on changes of quality characteristics of yoghurt manufactured using milk standardized with ultrafiltered cow skim milk retentate.

2. MATERIALS AND METHODS

Raw cow skim milk and cream (about 50-55% fat) was obtained from Experimental Dairy of National Dairy Research Institute, Karnal, Haryana, India. 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. Spray dried low heat skim milk powder was procured from Modern Dairies Ltd., Karnal, Haryana, India.

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 ZrO_2 membrane (membrane surface area, 1.68 m^2 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 until desired titratable acidity (TA) of ≥0.8% LA was achieved. Control yoghurts were prepared using yoghurt mix standardized to 13.9% (w/w) TS by the addition of SMP instead of UF cow skim milk retentate. Yoghurts were then immediately transferred and stored in a refrigerator maintained at 4±1°C. Experiment was repeated 2 times. Periodically drawn samples were analyzed to ascertain the changes in physicochemical, physical and microbiological properties against control yoghurt during the storage period. Composition of the yoghurts was also determined.

Physicochemical And Compositional Analysis

A pH meter (PHAN LABINDIA Model; Labindia Analytical Instruments Pvt. Ltd., Maharashtra, India) was used to determine pH of yoghurt during incubation and storage. Titratable acidity of yoghurts was determined using procedure recommended in BIS (1981a) [7]. Fat contents of skim milk and UF cow skim milk retentates were determined as per the method given in BIS (1981a) [7], whereas in cream and in yoghurt as per the methods given in BIS (1997) [8] and in BIS (1981b) [9], respectively. Total solids of yoghurts were determined according to a standard method [10]. Protein content of yoghurt was determined by semi-micro kjeldhal method [11]. Lactose content was determined by Lane-Eynon method as described in BIS (1981b) [9]. Ash content of yoghurt was determined as per the method given in BIS (1981b) [9], while acetaldehyde concentration was determined using enzymatic (aldehyde dehydrogenase)-based acetaldehyde determination kit (Megazyme International Ireland Ltd, Wicklow, Ireland).

Spontaneous Whey Syneresis (Sws)

Siphon method [12] 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 [13]. Within 12 h of the production of yogurt and thereafter every 4 days intervals until 21 days, a 10 g sample of yoghurt 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.

WHC (%) = {Pellet (g)/Sample (g)} ×100 (1)

Firmness

Firmness was determined according to a previous method [14] 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 firmness, 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 firmness 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 was calculated using the Texture Expert Exceed software (version 2.55) supplied by the manufacturer along with the instrument.

Microbiological Analysis

Microbiological quality of plain yoghurts in terms of LAB, yeast & mould and coliforms were determined in regular intervals during the storage of yoghurts using standard methods mentioned in BIS (1969) [15] and BIS (1999) [16].

Statistical Analysis

SPSS version 16.0 for Windows software (SPSS South Asia (P) Ltd., Bangalore, India) was used to analyze the data. Two-way ANOVA was used to check significant differences between developed product and control with time. Mean separation was performed by LSD. Significant differences were considered at $P < 0.05$. For the comparison of optimum product composition with control, t-test was used. Mean±SE (Standard Error) was calculated for compositional data using MS-Excel software (version 2007).

3.RESULTS AND DISCUSSION

Chemical Composition Of Yoghurts

Table 01 shows the chemical composition of experimental vis-à-vis control yoghurt. In experimental and control yoghurts milk solids level was observed to be 13.74±0.03 and 13.75±0.01%, respectively. It was observed that protein and ash percentages were significantly ($p<0.05$) higher in experimental than control yoghurt. This was due to the addition of UF cow skim milk retentate, which had more proteins and ash.

Table 1 Chemical composition* of experimental vis-à-vis control yoghurt

Component	Experimental	Control
TS (%)	13.74±0.03^a	13.75±0.01^a
Fat (%)	3.31±0.01^a	3.32±0.01^a
Protein (%)	5.44±0.02^b	4.24±0.04^a
Lactose (%)	4.15±0.01^a	5.46±0.02^b
Ash (%)	0.84±0.03^b	0.74±0.03^a
pH	4.55±0.01^a	4.53±0.01^a
TA (% LA)	0.859±0.005^a	0.864±0.005^a

*Mean of 2 trials

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

In early studies [17] it was mentioned that, due to removal of the soluble constituents with permeate, the composition of solid non fat (SNF) in UF retentate changes in favour of proteins and this also resulted in a moderate increase of calcium and phosphorus. Experimental yoghurt had, on average, 1.28 times more protein than control yoghurt. So, it can be considered as a high protein product. Further, lactose content in experimental yoghurt was significantly ($p<0.05$) lower than that of control yoghurt indicating the passage of lactose during the UF process

In addition to the product having high protein, less lactose is also an advantage for lactose intolerant individuals. Hence, developed yoghurt employing UF technique has many advantages compared to control yoghurt

Effect Of Storage Period On Physicochemi Cal And Physical Properties Of Yoghurt

Titratable Acidity And Ph

There was a significant (p<0.05) increase of TA and significant (p<0.05) decrease of pH of experimental and control yoghurts with advancing storage period. It was observed that, even at 4±1°C, acidity development (post-acidification) by thermophilic yoghurt culture was continued, but with a lower rate of acid production with advancing storage period. Several authors reported that TA was increased significantly (p<0.05) with increasing storage period even at refrigeration temperature [18, 19, 20, 21]. Titratable acidity of experimental yoghurt was observed to be non significant when compared with control yoghurt. In experimental yoghurt, initial TA was 0.859% LA and at the end of the storage period it was 1.184% LA, whereas, in control yoghurt corresponding values were 0.864 and 1.193% LA, respectively. Hence, it was noted that the TA was within the acceptable range prescribed by FSSA regulations [22] for yoghurt during storage.

Initial pH of the experimental and control yoghurt was 4.55 and 4.53, with advancing storage period, it was decreased to 4.43 and 4.4, respectively. Early reports [19, 21] also showed that pH of yoghurt decreased with advancing storage period. This decrease of pH of yoghurt is due to the persistent metabolic activity of LAB during cooling at refrigeration temperature [23]. Statistical analysis revealed that there was a significantly (p<0.05) higher overall pH in experimental yoghurt compared to control yoghurt. This higher pH might be caused by the buffering action of higher protein and minerals as reported in earlier studies [24, 25] in experimental yoghurt.

Acetaldehyde Concentration

The typical yoghurt flavor is caused by LA, which imparts an acidic and refreshing taste, and a mixture of various carbonyl compounds like acetone, diacetyl and acetaldehyde. Acetaldehyde is considered the major flavor component of yoghurt [26, 27].

Fig. 1 shows the changes of acetaldehyde concentration of experimental and control yoghurt during the storage period of 14 days at 4±1°C. With advancing storage period, acetaldehyde concentration decreased significantly (p<0.05)

confirming the findings of previous authors [3, 18, 28] Dehydrogenase activity by some lactic streptococci at low temperatures reduces acetaldehyde to some other compounds [18]. This might be the reason to have decreased acetaldehyde concentration with advancing storage period. Acetaldehyde concentration was observed

Fig.1: Acetaldehyde concentration of yoghurt as affected by storage period

to be significantly (p<0.05) higher in experimental compared to control yoghurts. On the first day of

storage period, experimental and control yoghurt had 34.15 and 32.04 ppm of acetaldehyde concentration, respectively. Thus, despite the lower

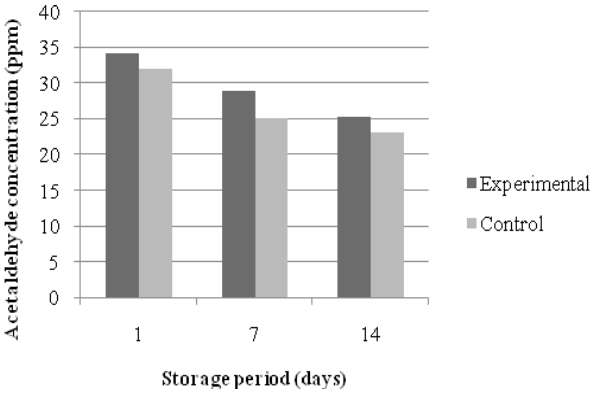

Fig.1: Acetaldehyde concentration of yoghurt as affected by storage period

lactose content of UF yoghurt, acetaldehyde production by starter bacteria was not impaired confirming the earlier findings [3]. Acetaldehyde and other carbonyl flavour compounds can be produced by more than one metabolic pathway and from various precursors including lactose, valine, pyruvate, threonine and acetyl phosphate [27]. Experimental yoghurt contained more proteins than control yoghurt and therefore, more amino acids such as valine and threonine which are precursors of acetaldehyde. This might be one of the reasons to have higher amount of acetaldehyde in experimental yoghurt made employing UF technique.

Spontaneous Whey Syneresis And Whc

Spontaneous whey syneresis was not observed in any of the yoghurt samples until 13th day of storage. On 17th day of storage, whey syneresis was observed in both experimental as well as control yoghurts. However, it was noted that experimental yoghurt had significantly (p<0.05) lower whey syneresis compared to control yoghurts. In control yoghurt, it was observed that with advancing storage period beyond day 13, whey syneresis was significantly (p<0.05) increased. Whey syneresis during storage is due to yoghurt gel contraction [29]. Experimental yoghurt made utilizing UF technique had more proteins and hence, more dense gel structure [30]. Therefore, rearrangements and contraction of the gel network during storage [31] might be limited resulting in less whey syneresis.

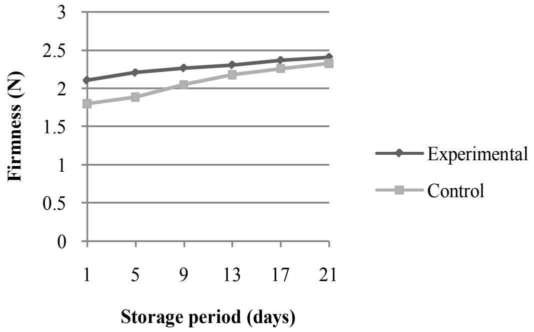

Fig.3: Changes of firmness of yoghurts during storage at 4±1°C

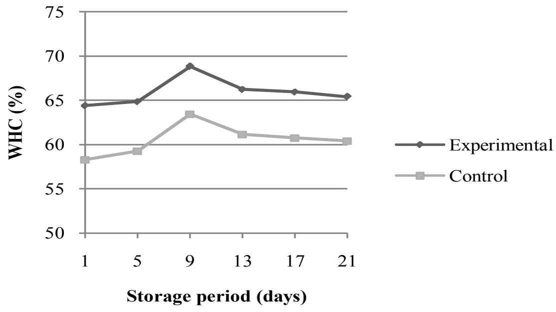

Fig.2: Changes of WHC of yoghurts during storage at 4±1°C

Changes of WHC of experimental and control yoghurt during storage at 4±1°C are shown in Fig. 2. It was observed that WHC was significantly (p<0.05) affected by storage period. Further, WHC was significantly (p<0.05) lower in control yoghurt when compared to experimental yoghurt. Initial WHC of experimental and control yoghurts were 64.4 and 58.3%, respectively. It was increased by 6.88 and 9.31%, respectively in experimental and control yoghurts with advancing storage period up to 9 days and then gradually decreased (Fig. 2).

Water holding capacity of control and calcium enriched fruit yoghurt during the storage period of 14 days was reported [20]. In that study, the WHC increased significantly (p<0.05) with storage period up to 7th day and then it was remained constant. Another group of researchers [32] also observed higher WHC on day 42, compared to day 1 in cow milk plain yoghurt made employing vat heat treatment. Further, increase of WHC of soy milk yoghurt made employing ultra high pressure homogenization was observed, with increasing storage period. In current study also a similar trend was observed. However, in contrast, some earlier findings [33] showed that WHC of cow milk stirred yoghurt decreased with increase in storage period.

Firmness

Firmness of yoghurt showed significantly (p<0.05) increasing trend with advancing storage period (Fig.3). This increase of texture possibly might be due to increased acidity during the storage period due to post acidification by residual microbial activity and increase in the hydration of casein [34, 35]. Residual activity of microorganisms in the product leads to a reinforcement of the strength of the protein network [35]. Further, it was observed that firmness was significantly (p<0.05) higher in experimental yoghurt manufactured utilizing UF process compared to control yoghurt.

Experimental yoghurt had higher amount of protein than that of control. This is in turn increases the gel strength of the yoghurt which might be the reason to have higher firmness value in experimental yoghurt made employing UF technique.

Microbiological Quality

Lactic acid bacteria count decreased significantly (p<0.05) with advancing storage period while, significant differences were not found between experimental and control yoghurts. On the first day of storage, experimental and control yoghurt had 8.151 and 8.812 log cfu/g of LAB, respectively. At the end of the storage period, it was observed that LAB was reduced by 1.3 and 2 log cycles in experimental and control yoghurts, respectively. Nevertheless, even after 21 days of storage period, LAB continued to give high number of viable cells and the value exceeded the requirement according to FSSA regulations [22]. Early reports indicated that LAB count decreases with increasing storage period [36].

Yeast and moulds were not observed in any of the yoghurts up to 13th day of storage. However, after 17th day, yeast and moulds were detected in both type of yoghurts and the values were within the acceptable limit prescribed in FSSA regulations [22]. After 21st day of storage, yeast and mould count did not meet the FSSA standards [22] in both experimental and control yoghurts. Coliforms were not detected throughout the storage period in any of the yoghurts, indicating proper hygienic measures practiced during the production, packaging and storage of yoghurts.

4. CONCLUSIONS

Experimental yoghurt prepared using milk standardized with UF cow skim milk retentate had lesser amount of whey syneresis, higher amount of acetaldehyde, better WHC and firmness during the storage at 4±1°C compared to control yoghurts. Whey syneresis increased and WHC increased and then decreased with advancing storage period irrespective of the type of yoghurt. Acetaldehyde concentration progressively decreased and firmness increased with advancing storage period. Experimental yoghurts had on average, 1.28 times more protein and 1.32 times lesser lactose than control yoghurt. On the basis of increased yeast & mould count, shelf life of experimental yoghurts was estimated to be 17 days at 4±1°C.

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