Storage Changes And Shelf Life Of Strawberry Set Yogurt Made By Milk Standardized Using Ultrafiltered Skim Milk Retentate

Narayana Mudiyanselage Nayana Kumari Narayana, Vijay Kumar Gupta

Abstract: Strawberry set yogurt was manufactured by keeping milk solids level at 10.5% and fat at 1.5% in yogurt mix, by addition of calculated amount of 5 fold ultrafiltered cow skim milk retentate and cow milk cream, respectively. Strawberry pulp and sugar level was maintained at 6% and 8%, respectively. Physicochemical, physical, textural and microbiological quality of developed yogurt was investigated during storage at 4±1°C against control yogurt made by milk standardized with skim milk powder. Significant ($p<0.05$) increase of acidity development and pH reduction was observed with advancing storage period, irrespective of the type of yogurt. Whey syneresis appeared only in control yogurt on 13th day of storage. Water holding capacity was significantly ($p<0.05$) higher in developed compared to control yogurt. Acetaldehyde concentration significantly ($p<0.05$) decreased with advancing storage period and was significantly ($p<0.05$) higher in developed yogurt throughout the storage. Values of textural attributes increased significantly ($p<0.05$) with increasing storage period irrespective of the type of yogurt and significantly ($p<0.05$) higher in developed yogurt. Lactic acid bacteria count decreased significantly ($p<0.05$) and yeast and moulds increased significantly ($p<0.05$) with advancing storage period in both yogurts. After 13th day of storage, overall acceptability of the developed yogurt decreased significantly ($p<0.05$) due to flavor and acidity related changes. Developed strawberry yogurt had 1.30 times more protein and 1.24 times less lactose compared to control. On the basis of increased yeast and mould count and sensory defects, shelf life of developed yogurt was observed to be 13 days at 4±1°C.

Index Terms: acetaldehyde, retentate, textural attributes, ultrafiltration, water holding capacity

1 INTRODUCTION

Yogurt, originated in the Balkans and the Middle East, is a well-known dairy product with a worldwide distribution. It is obtained through milk fermentation with a specific starter culture consisting of a mixture of two species of lactic acid bacteria (LAB), Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus. Along with health claims and therapeutic values, the flavor of yogurt has an important role in increasing its consumer demand (Routray & Mishra, 2011). Plain yogurt is predominantly sour. Therefore, fruits, flavorings and sweeteners are added either to improve the flavor balance (Kagan, 1985) or to mask partially the acetaldehyde flavor characteristic (Bills et al., 1972). Further, addition of fruits increases nutrient content including minerals and vitamins (Kumar & Mishra, 2003). Apart from these, fruits contain important phytochemicals which are having positive health effects. According to the method of production and the physical structure of the coagulum, yogurt is classified mainly in to three types namely set, stirred and fluid/drinking. Among these, set yogurt is popular in some countries of the world and is produced by packaging the yogurt mix into individual containers before fermentation. Whey syneresis (spontaneous separation of whey on the surface of yogurt) and textural defects are major defects of set yogurt. Addition of fruits increases these defects further (Celik & Bakirci, 2003), due to the disturbance of the protein gel network of the yogurt.

- *Narayana Mudiyanselage Nayana Kumari Narayana - Senior Lecturer in the Department of Animal Science, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka PH +94412292387.*
  E-mail: nayananarayana1970@gmail.com
- *Vijay Kumar Gupta-Principal Scientist, National Dairy Research Institute, Karnal, Haryana, India. PH +911842280486. E-mail: vkg@ndri.res.in*

The level of total solids and protein plays a significant role in the development of desirable consistency of yogurt. Overall quality of fruit yogurt could be improved by using milk standardized with ultrafiltered milk retentate. Ultrafiltration (UF) is a sieving process that employs a semi-permeable membrane with definite pore sizes, which allows small species to pass through as permeate and larger species to retain and concentrate as retentate, when a pressure is applied to a fluid. In UF of milk, non-protein nitrogen and soluble components such as lactose, salts and some vitamins pass through the membrane, whereas milk fat, protein and insoluble salts are retained by the membrane (Glover, 1985). Since Ultrafiltered milk retentate has higher protein content especially caseins, protein matrix density of yogurt will be reinforced and improve the firmness and water holding capacity (WHC) leading to less whey syneresis. This will be important to reduce or eliminate the addition of stabilizers, which otherwise widely used in the fruit yogurt manufacture. Several investigations into the shelf life of different yogurts and related products have been reported in the literature. Salvador and Fiszman (2004) studied the textural and sensory characteristics of whole and skimmed flavored set type yogurt during long storage at 10°C. MacBean (2010) stated that the shelf life of yogurt 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. Fresh yogurt is at its best in the first few weeks of shelf life, after which there is a noticeable reduction in sensory characteristics. Yeast and mold are the principal agents of microbial spoilage of yogurt. In fresh yogurt products yeast and mold may be present due to contamination in the processing operations, including from added fruit preparations, from the packaging materials and/or the filling operations (MacBean, 2010). Yeast and molds are little affected by low pH and may cause spoilage of yogurt during storage (Al-Ashmawy & Ibrahim, 2009). Information on the behavior of strawberry set yogurt manufactured from milk

standardized using ultrafiltered cow skim milk 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. Hence, the current study was conducted to investigate the effect of refrigerated storage on the changes of quality characteristics of strawberry set yogurt manufactured from milk standardized using ultrafiltered cow skim milk retentate.

2 MATERIALS AND METHODS

Raw cow skim milk, cow milk cream (about 50-55% fat), grade A sugar and Polystyrene cups of 100 ml with lids were obtained from Experimental Dairy of the National Dairy Research Institute, Karnal, Haryana, India. Commercial yogurt culture (IFB Vita Plus, Sri Lanka) was used in the manufacture of yogurt. Individually Quick Frozen (IQF) Strawberries (variety 'Winter down') were procured from Beniwal Strawberry Farm, GT Road, New Delhi. Erythrosine (E127) food color was purchased from a food ingredient supplier at Karnal. Spray dried low heat skim milk powder (SMP) was procured from Modern Dairies Ltd., Karnal.

2.1 Ultra filtration of Cow Skim Milk

Cow skim milk was ultrafiltered to 5 fold UF concentration as mentioned elsewhere (Narayana & Gupta, 2013).

2.2 Production of Experimental Yogurts

Cow skim milk was standardized to 10.5% (best level determined by previous studies) total milk solids (TMS) and 1.5% fat by adding calculated amount of 5 fold ultafiltered cow skim milk retentate and cow milk cream, respectively. Needed fruit pulp (6%) and sugar (8%) was also considered in standardization equations. Resultant standardized milk was used for the manufacturing of strawberry yogurt by pre-treating similarly as mentioned by Narayana & Gupta (2013) for mango yogurt. Previously pasteurized (85°C/10 min) and cooled (42°C) strawberry pulp [6% (w/w)] and sugar [8% (w/w)] mixture along with 0.01% (w/w) strawberry color (E127) was added in the preparation of yogurt. Control strawberry yogurt was prepared using yogurt mix standardized to 10.5% (w/w) TMS by the addition of SMP. Experiment was repeated 2 times.

2.3 Compositional and Physicochemical Analysis

Fat content of skim milk and ultrafiltered cow skim milk retentate was determined as per the method given in BIS (1981a), whereas in cream and in yogurt as per the methods given in BIS (1977) and in BIS (1981b), respectively. Protein content of yogurt was determined by semi-micro kjeldhal method (Menefee & Overman, 1940). Sucrose content was determined as per the procedure given in BIS (1981c). Lactose content was determined by taking the titration value before inversion and standardizing the Fehling solution with 0.5% lactose solution. Ash content was determined as per the method given in BIS (1981b), while acetaldehyde concentration was determined using enzymatic (aldehyde dehydrogenase)-based acetaldehyde determination kit (Megazyme International Ireland Ltd, Wicklow, Ireland). A pH meter (PHAN LABINDIA Model; Labindia Analytical Instruments Pvt. Ltd., Maharashtra, India) was used to determine pH of yogurt during incubation and storage. Titratable acidity (TA) was determined by potentiometric method as per the procedure given in ISO (1997).

2.4 Spontaneous Whey Syneresis (SWS)

Spontaneous whey syneresis of undisturbed strawberry set yogurt was determined by siphon method described by Amatayakul et al. (2006).

2.5 Water Holding Capacity

The WHC of strawberry yogurt was measured by a centrifuge method proposed by Supavititpatana et al. (2009).

2.6 Textural Attributes

Textural attributes of strawberry yogurt were determined according to the method given by Kumar and Mishra (2003) with slight modifications as mentioned by Narayana & Guptha (2013) using a TA-XT2i Texture analyser (M/s Stable Micro Systems, UK).

2.7 Microbiological Analysis

Microbiological quality of strawberry yogurt in terms of LAB, yeast & mold and coliforms were determined in regular intervals during the storage using standard methods mentioned in BIS (1969) and BIS (1999).

2.8 Sensory Evaluation

Sensory evaluation was only conducted for developed strawberry set yogurt using a 100 point score card suggested by Ranganadham & Gupta (1987). Sensory evaluation was carried out by a panel consisting 8 trained judges from NDRI, Karnal, India.

2.9 Statistical Analysis

SPSS version 16.0 for Windows software (SPSS South Asia (P) Ltd., Bangalore, India) was used to analyse 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 composition of optimum product 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

3.1 Chemical Composition of Strawberry Yogurt

Table 1 shows the chemical composition of developed strawberry yogurt made by milk standardized using ultrafiltered cow skim milk retentate vis-à-vis control strawberry yogurt made by milk standardized with skim milk powder.

TABLE 1
CHEMICAL COMPOSITION OF DEVELOPED VIS-À-VIS CONTROL STRAWBERRY YOGURT*

Component	Developed	Control
TS (%)	19.07±0.06[a]	19.05±0.04[a]
Fat (%)	1.52±0.01[a]	1.54±0.01[a]
Protein (%)	4.64±0.07[b]	3.58±0.06[a]
Lactose (%)	3.74±0.02[a]	4.65±0.09[b]
Sucrose (%)	8.19±0.09[a]	8.25±0.04[a]
Ash (%)	0.74±0.02[b]	0.71±0.03[a]
pH	4.43±0.01[b]	4.39±0.01[a]
TA (% LA)	0.851±0.005[a]	0.851±0.005[a]

**Mean of 2 trials*
[a, b] Means with different superscripts within each row differ significantly (p<0.05)

Milk solids level was observed to be 19.07±0.06 in developed and 19.05±0.04% in control yogurts. It was observed that protein content was 4.64±0.07% and 3.58±0.06% whereas lactose content was 3.74±0.02% and 4.65±0.09%, respectively in developed and control strawberry yogurts. Accordingly, developed strawberry yogurt had 1.30 times more protein and 1.24 times less lactose than control strawberry yogurt. Therefore, developed yogurt is a high protein and low lactose product, which suits current consumer needs. Less lactose is also an advantage for lactose intolerant individuals. Hence, developed strawberry yogurt employing UF technique has many advantages compared to control yogurt. Other constituents except ash (0.74±0.02 in developed vs. 71±0.03 in control) were not significantly different between two products. According to Becker & Puhan (1989), 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. Those can be the reasons for above observations. pH was observed to be higher in developed strawberry yogurt even though, TA was not different compared to control. Higher protein content in developed yogurt resists pH change due to higher buffering capacity than control yogurt.

3.2 Effect of storage period on physicochemical properties of yogurt pH and Titratable acidity

A significant (p<0.05) decrease of pH was observed in strawberry yogurt, irrespective of the type, with advancing storage period (Table 02). Initial pH of the developed and control yogurt was observed to be 4.43±0.01 and 4.39±0.01, respectively. They were significantly (p<0.05) different compared to each other and higher pH was observed in developed strawberry yogurt. This higher pH might be caused by the buffering action of higher protein and minerals present in developed yogurt as reported by previous authors (Premaratne & Cousin,1991) due to the addition of ultrafiltered milk retentate for the standardization of the yogurt mix. However, statistically no significant differences were observed in pH of developed and control strawberry yogurts after the first day of storage. With advancing storage period, pH decreased to 4.05±0.09 and 4.11±0.01 in developed and control strawberry yogurts, respectively. Early reports (Hassan & Amjad, 2010) also showed that pH of yogurt decreased with advancing storage period. Persistent metabolic activity of LAB during cooling and at refrigeration temperature is responsible for this decrease of pH in yogurts during storage. With advancing storage period a significant (p<0.05) increase of TA was observed in strawberry yogurts, irrespective of the type (Table 02). However, it was observed that the rate of acid production is decreased with advancing storage period, except in few instances. Several authors reported that TA of yogurt stored under refrigeration conditions increased significantly (p<0.05) with increasing storage period (Singh & Muthukumarappan, 2008; Hassan & Amjad, 2010). This might be due to the continuation of post-acidification by thermophilic yogurt bacteria even at refrigeration temperature. Titratable acidity of developed yogurt was observed to be non-significant compared to control yogurt. However, Ainaz & Ehsani (2008) reported that acidity of probiotic yogurt made using ultrafiltered milk showed greater values than the control probiotic yogurt during refrigerated storage. In developed yogurt, initial TA was observed to be 0.851±0.006% LA and at the end of the storage period it was 1.121±0.019% LA, whereas, in control yogurt corresponding values were 0.851±0.006 and 1.098±0.013% LA, respectively. Further, it was noted that the TA was within the acceptable range prescribed by FSSA regulations (FSSA, 2006) for yogurt during storage. The pH and titratable acidity variation observed during the storage of strawberry yogurt is considered normal, since refrigeration or chilling did not completely stop the metabolism of yogurt bacteria.

TABLE 2
PHYSICOCHEMICAL PROPERTIES OF STRAWBERRY SET YOGURT DURING STORAGE AT 4±1°C*

Parameter	Sample	Days of storage					
		1	4	7	10	13	16
pH	Developed	4.43±0.01^{dB}	4.34±0.01^{cdA}	4.25±0.01^{bcA}	4.19±0.02^{abcA}	4.12±0.02^{abA}	4.05±0.09^{aA}
	Control	4.39±0.01^{dA}	4.31±0.04^{cA}	4.22±0.01^{bA}	4.18±0.01^{bA}	4.15±0.01^{abA}	4.11±0.01^{aA}
TA (% LA)	Developed	0.851±0.006^{aA}	0.869±0.006^{aA}	0.945±0.013^{bA}	1.062±0.013^{cA}	1.112±0.019^{cdA}	1.121±0.019^{dA}
	Control	0.851±0.006^{aA}	0.864±0.000^{aA}	0.941±0.006^{abA}	1.008±0.051^{bcA}	1.026±0.051^{bcA}	1.098±0.013^{cA}
SWS (%)	Developed	0	0	0	0	0^{A}	0^{A}
	Control	0^{a}	0^{a}	0^{a}	0^{a}	0.15±0.07^{abA}	0.25±0.07^{bB}
WHC (%)	Developed	70.57±0.16^{bB}	69.15±0.66^{bB}	60.67±1.56^{aB}	68.35±0.13^{bB}	70.00±0.17^{bB}	72.94±0.11^{cB}
	Control	61.59±0.20^{eA}	58.99±0.48^{cdA}	54.49±0.35^{aA}	56.72±0.23^{bA}	57.81±0.24^{bcA}	59.53±0.42^{dA}
[Acet.] ppm	Developed	24.02±0.14^{cB}	ND	16.11±0.33^{bB}	ND	13.83±0.12^{aB}	ND
	Control	21.41±0.37^{cA}	ND	13.95±0.24^{bA}	ND	11.62±0.08^{aA}	ND

3.3 Spontaneous whey syneresis and WHC

Whey syneresis is one of the most important factors that influence the consumer acceptance of set yogurt. Free whey was not observed in any of the strawberry yogurt samples until 10th day of storage. On 13th day of storage, 0.15±0.07% of whey was observed in control yogurt and increased significantly ($p<0.05$) during the storage whereas, it was not observed in developed strawberry yogurt throughout the storage period of 16 days. Yogurt gel contraction causes the production of free whey during storage (Salvador & Fiszman, 2004). Further, Afoakwa et al. (2014) mentioned that intrinsic instability of the protein gel is the reason for whey syneresis. Whey retention of strawberry yogurt made using milk standardized with ultrafiltered cow skim milk retentate was observed to be superior to that of control. This might be due to the higher amount of proteins and hence more dense gel structure and more stability of the protein gel in developed yogurt. Therefore, rearrangements and contraction of the gel network during storage (Lucey & Singh, 1998) might be limited resulting in less whey syneresis. Afoakwa et al. (2014) mentioned that WHC of a protein gel such as set yogurt is a critical parameter since it is related to whey syneresis. Changes of WHC of developed and control strawberry yogurt during storage at 4±1°C are shown in Table 02. It was observed that WHC of yogurts were significantly ($p<0.05$) affected by the storage period. Initial WHC of developed and control yogurts were 70.57±0.16 and 61.59±0.20%, respectively. It was observed to be decreased up to 7th day of storage and then started to increase gradually during the storage period irrespective of the type of yogurt. Similar observations were made by Sichani et al. (2014) in low fat set yogurt made using cress seed gum and locust bean gum. Kücükcetin et al. (2011) observed that WHC of cow milk stirred yogurt decreased with increase in storage period. In contrast, Singh and Muthukumarappan (2008) studied the WHC of control and calcium enriched fruit yogurt during the storage period of 14 days and observed that the WHC increased significantly ($p<0.05$) with storage period up to 7th day and then it was remained constant. From the above observations it can be stated that a clear trend of WHC doesn't exist in yogurt during the storage period and therefore, it is not directly related to whey syneresis. Water holding capacity measures the amount of water absorbed in the protein structure of the yogurt. Increased micelle size and increased whey-casein and casein-casein interactions lead to a more porous gel, which could retain more water (Parnell-Clunies et al., 1986). Further, WHC was observed to be significantly ($p<0.05$) higher in developed yogurt and exhibited greater ability to bind water compared to control yogurt throughout the storage period. Early studies showed that the reduction in whey separation (Saint-Eve et al., 2008) and increase in WHC (Kristo et al., 2003) of yogurt with increase of total solids level. In the present study by adding ultrafiltered cow skim milk retentate, without increasing total solids, resistance to syneresis and WHC were increased in strawberry set yogurt. This might be due to the increased level of proteins and thereby development of a denser yogurt structure capable of holding more water.

3.4 Acetaldehyde concentration

Flavor of soured milk products such as yogurt are characterized by numerous volatile bacterial metabolites, some of which are by-products of lactic acid fermentation or are produced by other reaction mechanisms (Routray & Mishra, 2011). Lactic acid itself is a major flavor compound responsible for yogurt flavor which imparts an acidic and refreshing taste while, a mixture of various carbonyl compounds like acetone, diacetyl and acetaldehyde produced by yogurt starter microorgamisms, Streptococcus thermophilus and Lactobacillus delbrueckii ssp. bulgaricus are also important contributors of yogurt flavor. Among them, acetaldehyde is considered the major and most important compound responsible for typical yogurt flavor (Tamime & Deeth, 1980). Changes of acetaldehyde concentration in both developed and control strawberry set yogurt during the storage period of 16 days at 4±1°C is presented in Table 02. With advancing storage period, acetaldehyde concentration decreased significantly ($p<0.05$), irrespective of the type of yogurt. According to Hamdan et al. (1971) higher concentration of acetaldehyde (in the range of 5 to 21 ppm) is necessary to produce a desirable yogurt flavor. In the current study, higher acetaldehyde concentrations were reported in both developed and control yogurts throughout the storage period. In developed strawberry yogurt the detected acetaldehyde concentrations was 24.02±0.14 at the first day and 13.83±0.12 at 13th day of storage period, which was well above the lowest value needed to give a good flavor to the yogurt. It was shown that the yogurt bacteria could reduce the acetaldehyde to ethanol over time due to dehydrogenase activity (Biliaderis et al., 1992). Acetaldehyde concentration was observed to be significantly ($p<0.05$) higher in developed compared to control yogurt. Developed yogurt showed >12 ppm higher acetaldehyde concentration compared to control yogurt throughout the storage period. Thus, even with lower lactose content of the yogurt fortified with ultrafiltered cow skim milk retentate, acetaldehyde production by yogurt starter bacteria was not affected, confirming the findings of Biliaderis et al. (1992). Acetaldehyde and other carbonyl flavor compounds responsible for yogurt flavor can be produced by more than one metabolic pathway and from various precursors including lactose, valine, pyruvate, threonine and acetyl phosphate (Tamime & Deeth, 1980). The most important path way is from break down of an amino acid threonine as reported by Routray & Mishra (2011). Developed yogurt with higher amount of protein due to the incorporation of ultrafiltered cow skim milk retentate, could contain more amino acids such as threonine and valine which are precursors of acetaldehyde. This may be one of the reasons to have higher amount of acetaldehyde in developed yogurt made employing UF technique.

3.5 Textural attributes

Table 03 shows the changes of the textural attributes namely firmness (the force necessary to attain a given deformation), stickiness (the negative force representing the work necessary to pull the compressing plunger away

TABLE 03

TEXTURAL ATTRIBUTES OF STRAWBERRY SET YOGURT DURING STORAGE AT 4±1°C*

Parameter	Sample	Days of storage					
		1	4	7	10	13	16
Firmness	Developed	2.743±0.044^{aB}	2.762±0.019^{aB}	2.800±0.015^{aB}	2.797±0.050^{aB}	2.870±0.025^{bB}	2.907±0.009^{bB}
	Control	1.372±0.044^{aA}	1.379±0.020^{aA}	1.408±0.011^{abA}	1.447±0.018^{bcA}	1.476±0.017^{cA}	1.497±0.014^{cA}
Stickiness	Developed	-0.634±0.024^{dA}	-.640±0.008^{cdA}	-0.669±0.009^{cA}	-.690±0.008^{bcA}	-0.711±0.019^{bA}	-0.684±0.046^{aA}
	Control	-0.276±0.004^{eB}	-0.294±0.005^{eB}	-0.325±0.013^{dB}	-0.354±0.014^{cB}	-0.391±0.003^{bB}	-0.421±0.004^{aB}
WOS	Developed	81.765±0.318^{aB}	82.118±0.123^{aB}	82.604±0.245^{bB}	83.026±0.071^{bB}	83.540±0.259^{cB}	83.991±0.142^{cB}
	Control	39.012±0.057^{aA}	40.427±0.170^{bA}	40.616±0.139^{bA}	41.241±0.246^{cA}	41.805±0.317^{dA}	42.743±0.126^{eA}
WOA	Developed	-3.544±0.114^{dA}	-3.772±0.243^{cdA}	-4.067±0.053^{cA}	-4.556±0.082^{bA}	-4.749±0.092^{abA}	-4.953±0.184^{aA}
	Control	-1.351±0.050^{fB}	-1.541±0.028^{eB}	-1.713±0.053^{dB}	-1.999±0.048^{cB}	-2.535±0.108^{bB}	-2.949±0.045^{aB}

**Mean of 2 trials*

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

^{A,B} *Means with different superscripts within each column for each parameter differ significantly (p<0.05)*

TABLE 04

MICROBIOLOGICAL QUALITY OF STRAWBERRY SET YOGURT DURING STORAGE AT 4±1°C*

Parameter	Sample	Days of storage					
		1	4	7	10	13	16
LAB	Developed	8.651±0.069^{d}	8.239±0.337^{cd}	7.845±0.000^{bc}	7.588±0.157^{bc}	7.151±0.231^{ab}	6.739±0.056^{a}
	Control	8.841±0.088^{e}	8.602±0.000^{d}	7.954±0.000^{c}	7.778±0.000^{c}	7.540±0.088^{b}	6.812±0.047^{a}
Yeast and Mould	Developed	0^{a}	0^{a}	0^{a}	0.50±0.70^{abA}	1.30±0.43^{abA}	1.78±0.25^{bA}
	Control	0^{a}	0^{a}	0^{a}	0.65±0.92^{aAB}	1.30±0.43^{aA}	1.66±0.26^{aA}

**Mean of 2 trials*

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

$^{A, B}$ *Means with different superscripts within each column for each parameter differ significantly (p<0.05)*

TABLE 5

SENSORY ATTRIBUTES OF STRAWBERRY SET YOGURT DURING STORAGE AT 4±1°C*

Attribute	Days of storage					
	1	4	7	10	13	16
FLV	41.86±0.90^{b}	41.86±1.07^{b}	41.71±0.95^{b}	41.57±0.98^{b}	40.43±1.13^{ab}	39.29±0.95^{a}
BT	28.00±0.58^{a}	27.79±0.81^{a}	27.86±0.69^{a}	27.57±0.98^{a}	27.36±0.75^{a}	26.71±1.11^{a}
ACI	8.43±0.79^{b}	8.71±0.39^{b}	8.36±0.48^{b}	8.14±0.63^{b}	7.79±0.57^{ab}	7.14±0.69^{a}
CA	8.93±0.19^{a}	8.71±0.39^{a}	8.79±0.39^{a}	9.00±0.50^{a}	8.79±0.70^{a}	8.43±0.53^{a}
OA	92.21±1.87^{b}	92.07±1.92^{b}	91.71±0.91^{b}	91.29±2.00^{b}	89.36±1.70^{ab}	86.57±2.07^{a}

**Mean of 2 trials*

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

From the sample) work of shear (area under the curve of positive peak, i.e. firmness) and work of adhesion (area under the curve of negative peak, i.e. stickiness) of developed and control strawberry set yogurt during the storage period of 16 days at 4±1°C. In determining the texture of set-type yogurt, firmness mainly and reported other parameters for less extent are commonly used. It was observed that the firmness and other textural attributes increased significantly (p<0.05) during the storage period at refrigeration temperature, in both developed and control yogurts. This increase of firmness and other textural attributes might be due to the increased acidity and decrease of pH during the storage period caused by post acidification of the residual microbial activity and increase in the hydration of casein as reported by previous authors (Dave & Shah, 1988; Saint-Eve et al., 2008). Residual activity of microorganisms in the product leads to a reinforcement of the strength of the protein network (Saint-Eve et al., 2008). Further, it was clear that the incorporation of ultrafiltered cow skim milk retentate to standardize the yogurt milk, significantly (p<0.05) increases the firmness and other textural attributes of the yogurt. The yogurt gel

structure is a network of the milk proteins, caseins, and whey proteins formed during acid gelation. Addition of ultrafiltered cow skim milk retentate increases the addition of proteins to the yogurt mix compared to the yogurt mix added with skim milk powder and enhances their aggregation resulting in a rigid acid gel affecting both the microstructure and the physical characteristics of yogurt. Elevated protein content gives higher firmness values as reported by previous authors (Abrahamsen and Holmen, 1980; Abd El-Khair, 2009; Becker & Puhan, 1989).) Therefore, it is possible to manufacture yogurt with improved stability throughout the storage period by utilizing ultrafiltered cow skim milk retentate without adding commercially available stabilizers which otherwise widely used especially in fruit yogurt industry. So addition of ultrafiltered cow skim milk retentate appears to be beneficial in fruit yogurt manufacturing.

3.6 Microbiological quality

Yogurt consumption is believed to be beneficial for human health due to the bacteria present in it. It is generally accepted that the yogurt should contain 10^7 cfu/ml of viable bacteria (Streptococcus thermophilus and Lactobacillus bulgaricus) (Salvador and Fiszman, 2004) even though, quantitative standards are different in different countries (Tamime & Deeth, 1980). Table 04 shows the LAB and yeast & mould count of strawberry set yogurt during refrigerated storage of 16 days at 4±1°C. Lactic acid bacteria count decreased significantly ($p<0.05$) with advancing storage period while, significant differences were not observed between developed and control yogurts. During the acceptable storage period of 13 days, LAB count reduced by 1.5 and 1.3 log cycles in developed and control strawberry yogurts, respectively. Even though considerable reduction was observed during the storage period, LAB continued to give high number of viable cells that comply with FSSA (2006) regulations. Several authors reported that LAB count in yogurt and related products decreases with increasing storage period (Kristo et al., 2003; Salvador & Fiszman, 2004). Yeast and moulds were not observed in any of the yogurts up to 7^{th} day of storage. However, on day 10^{th} yeasts and moulds were appeared and number increased with advancing storage period, irrespective of the type of yogurt (Table 04). At the end of the acceptable storage period of 13 days, yeast and mould count was observed to be acceptable according to FSSA (2006) regulations. On 16^{th} day of storage, yeast and mould count did not meet the FSSA (2006) standards in both developed and control yogurts. Coliforms were not detected throughout the storage period in any of the yogurts, indicating proper hygienic measures practiced during the production, packaging and storage of yogurts.

3.7 Sensory attributes

Table 05 shows the changes of the sensory quality of strawberry set yogurt made using milk standardized with ultrafiltered cow skim milk retentate during the refrigerated storage at 4±1°C. Even though there was a reduction in sensory scores in all the aspects checked with the advancement of the storage period, significant change was not noted until 13^{th} day of storage. However after 13^{th} day, there was a remarkable reduction in overall acceptability score due to the reduction of flavor and acidity scores of the developed yogurt. No significant differences were observed in body & texture and color & appearance scores throughout the storage period. This might be due to the firm texture of yogurt given by the addition of ultrafiltered skim milk retentate which is having high protein.

4 CONCLUSION

Developed strawberry set yogurt prepared using milk standardized with ultrafiltered cow skim milk retentate had no whey syneresis, better WHC and textural quality with higher amount of acetaldehyde during the studied storage period of 16 days at 4±1°C compared to control yogurt prepared using milk standardized with skim milk powder. Water Holding Capacity increased and then decreased with advancing storage period irrespective of the type of yogurt. Further, acetaldehyde concentration progressively decreased and firmness and other textural attributes increased with advancing storage period. Developed strawberry yogurt had on average, 1.30 times more protein and 1.24 times lesser lactose compared to control yogurt. On the basis of increased yeast & mould count and organoleptic scores, shelf life of developed strawberry set yogurt was estimated to be 13 days at 4±1°C. Addition of ultrafilterd cow skim milk retentate in fruit yogurt formulations has a significant positive impact on texture improvement with added acetaldehyde flavor note and thereby giving more natural product which satisfies the current consumer.

REFERENCES

[1] Abd El-Khair, A. A. Production and Evaluation of a High Protein Version of Non-fat Yogurt. Res. J. Agric. Biol. Sci. 5: 310-316, 2009

[2] Abrahamsen, R. K. and T. B. Holmen. Yogurt from hyperfiltrated, ultrafiltrated and evaporated milk and from milk with added milk powder. Milchwissenschaft. 35: 399-402, 1980.

[3] Afoakwa, E. O., F. M. K. Kpodo, B. B. Amoa, A. S. Budu, and F. K. Saalia. Effect of Ingredient Variation on Microbial Acidification, Susceptibility to Syneresis, Water Holding Capacity and Viscosity of Soy-Peanut-Cow Milk Yogurt. J. Nutr. Health Food Eng. 1: 00012, 2014.

[4] Ainaz, A. and M. R. Ehsani. Probiotic survival in yogurt made from ultrafiltered skim milk during refrigeration storage. Res. J. Biol. Sci., 3**:** 1163-1165, 2008.

[5] Al-Ashmawy, M. A. M. and J. I. Ibrahim. Influence of potassium sorbate on the growth of yeasts and moulds in yogurt. Int. J. Dairy Tech., 62: 224-227, 2009.

[6] Amatayakul, T., F. Sherkat, and N. P. Shah. Syneresis in Set Yogurt as Affected by EPS Starter Cultures and Levels of Solids. Int. J. of Dairy Tech. 59**:** 216-221, 2006.

[7] Becker, T. and Z. Puhan. Effect of different processes to increase the milk solids non-fat content on the rheological properties of yogurt. Milchwissenschaft. 44: 626–629, 1989.

[8] Biliaderis, C. G., M. M. Khan and G. Blank. Rheological and sensory properties of yogurt from skim milk and ultrafiltered retentate. Int. Dairy J. 2: 311-323, 1992.

[9] Bills, D. D., C. S. Yang, M. E. Morgan and F. W. Bodyfelt. Effect of Sucrose on the Production of Acetaldehyde and Acids by Yogurt Culture Bacteria. J. Dairy Sci. 55**:** 1570-1573, 1972.

[10] BIS. Rapid examination of milk. In: Handbook of Food Analysis (SP: 18 - Part XI), Dairy Products. Bureau of Indian Standards. Manak Bhavan, New Delhi. 5-20, 1981a.

[11] BIS. Chemical examination of milk. In: Handbook of Food Analysis (SP: 18 - Part XI): Dairy Products. Bureau of Indian Standards. Manak Bhavan, New Delhi. 21-44, 1981b.

[12] BIS Condenced milk. In: Handbook of Food Analysis (SP: 18 - Part XI): Dairy Products. Bureau of Indian Standards. Manak Bhavan, New Delhi. 120-126, 1981c.

[13] BIS. Methods for detection and estimation of coliform bacteria in food stuffs. IS: 5401 (1969). Bureau of Indian Standards. Manak Bhavan, New Delhi, 1969.

[14] BIS. Determination of fat by ther Gerber method - Milk products. IS: 1224 (part II) - 1977. Bureau of Indian Standards. Manak Bhavan, New Delhi, 1977.

[15] BIS. Methods of yeast and mould count of food stuffs and animal feeds. IS: 5403 (1999). Bureau of Indian Standards. Manak Bhavan, New Delhi, 1999.

[16] Celik, S. and I. Bakirci. Some properties of yogurt produced by adding mulberry perkmez (concentrated juice). Int. J. Dairy Tech. 56: 26-29, 2003.

[17] Dave, R. I. and N. P. Shah. The influence of ingredient supplementation on the textural characteristics of yogurt. Aust. J. Dairy Technol. 53: 180-184, 1988.

[18] FSSA. Food Safety and Standards Act. Food Safety and Standards Authority of India, New Delhi, 2006.

[19] Glover, F. A. Ultrafiltration and reverse osmosis for the dairy industry. In: Technical bulletin (Ed. F. A. Glover), National Institute for Research in Dairying, England, 5: 1–207, 1985.

[20] Hamdan, I. Y., J. E. Kunsman and D. D. Deane. Acetaldehyde production by combined yogurt cultures. J Dairy Sci. 54:1080–2, 1971.

[21] Hassan, A. and I. Amjad. Nutritional evaluation of yogurt prepared by different starter cultures and their physicochemical analysis during storage. Afr. J. of Biotechnol. 9**:** 2913-2917, 2010.

[22] ISO. Yogurt-Determination of titratable acidity-Potentiometric method. ISO 11869:1997(E). International Organization for Standardization, Geneva, Switzerland, 1997.

[23] Kagan, J. Yogurt-a rising star in the dairy industry. Cultured Dairy Products Journal. 20- 24., 1985.

[24] Kristo, E., C. G. Biliaderis and N. Tzanetakis. Modelling of the acidification process and rheological properties of milk fermented with a yogurt starter culture using response surface methodology. Food Chem. 83: 437–446, 2003.

[25] Kücükcetin, A., M. Demir, A. Asci and E. M. Comak. Graininess and roughness of stirred yogurt made with goat's, cow's or a mixture of goat's and cow's milk. Short communication. Small Ruminant Res. 96: 173–177, 2011.

[26] Kumar, P. and H. N. Mishra. Effect of mango pulp and soy milk fortification on the texture profile of set yogurt made from buffalo milk. J. Text. Stud. 34: 249-269, 2003.

[27] Lucey, J. A. and H.Singh. Formation and physical properties of acid milk gels: A review. Food Res. Int. 30: 529-542, 1998.

[28] MacBean, R. D. Packaging and the Shelf Life of Yogurt. In: Food packaging and shelf life-A practical guide. (Ed. G. L. Robertson). CRC Press, Taylor and Francis Group, Boca Raton, FL. 143-156, 2010.

[29] Menefee, S. G. and O. R. Overman. Semi micro kjeldhal method for determination of total nitrogen in milk. J. Dairy Sci. 23: 1177 – 1185, 1940.

[30] Narayana, N. M. N. K. and V. K. Gupta. Effect of total milk solid content adjusted by adding ultrafiltered milk retentate on quality of set mango yogurt. Int. J of Dairy Tech. 66: 570-575, 2013.

[31] Parnell-Clunies, E. M., Y. Kakuda, K. Mullen, D. R.. Arnot, J. M. DeMan. Physical properties of yogurt: A comparison of vat versus continuous heating systems of milk, J. Dairy Sci. 69: 2593-2603, 1986.

[32] Premaratne, A. J. and M. A. Cousin. Microbiological analysis and starter culture growth in retentates. J. Dairy Sci. 74: 3284-3292, 1991.

[33] Ranganadham, M. and S. K. Gupta. Sensory evaluation of dahi and yogurt. Indian Dairyman. 39: 493-497, 1987.

[34] Routray, W. and H. N. Mishra. Scientific and Technical Aspects of Yogurt Aroma and taste: A Review. Compr. Rev. Food Sci. Food Saf. 10: 208-220, 2011.

[35] Saint-Eve, A., C. Levy, M. Le Moigne, V. Ducruet and I. Souchon. Quality changes in yogurt during storage in different packaging materials. Food Chem., 110: 285-293, 2008.

[36] Salvador, A. and S. M. Fiszman. 2004. Textural and sensory characteristics of whole and skimmed flavored set type yogurt during long storage. J. Dairy Sci., 87: 4033-4041.

[37] Sichani, M. S., S. A. Y. Ardakani, and M. Danesh. 2014. The effect of Cress seed gum & Locust bean gum on textural properties of low fat set yogurt. Indian J. Sci. Res 5: 439-445.

[38] Singh, G. and K. Muthukumarappan. 2008. Influence of calcium fortification on sensory, physical and rheological characteristics of fruit yogurt. LWT. 41**:** 1145-1152.

[39] Supavititpatana, P., T. I. Wirjantoro and P. Raviyan. 2009. Effect of sodium caseinate and whey protein isolate fortification on the physical properties and microstructure of corn milk yogurt. CMU. J. Nat. Sci., 8: 247-263.

[40] Tamime, A. Y. and H. C. Deeth 1980. Yogurt: technology and biochemistry. J. Food Prot., 43: 939-977.