

Full Length Research Paper

Effect of temperature on shelf life, chemical and microbial properties of cream cheese

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Cream cheese samples were analyzed to find out the effect of recommended storage temperature (4±1 °C) and ambient room temperature (21±1 °C) on pH, titratable acidity (% lactic acid), moisture content and microbial growth. Percent reduction in moisture content and increase in titratable acidity of cheeses were found to be directly proportional to the increase in storage period. There was a decrease in pH with progress in storage duration. Reduction was significantly higher ($P \leq 0.05$) in samples stored at 21±1 °C as compared to those refrigerated (4±1 °C). Sample 4 showed 41.03% reduction in moisture content and 39.73% increase in titratable acidity whereas, pH value was 3.39 at 21±1 °C on 28th day. All these values were significant as compared to the control ($P \leq 0.05$). Increase in CFU/g of LAB was more at 21±1 °C as compared to 4±1 °C. Comparison of data of titratable acidity and CFU log₁₀ of lactic acid bacteria (LAB) shows direct relationship between microbial growth and titratable acidity. Species of *Penicillium*, *Aspergillus*, *Cladosporium* and unknown actinomycetes were isolated from the samples. Distribution and frequency occurrence of fungi were higher at ambient room temperature than at refrigerated temperature.

Key words: Cream cheese, storage period, temperature, titratable acidity, microbial growth.

INTRODUCTION

Cheese is a dairy product with best nutritional value and health care function, and it is widely popular in many countries in the world with good taste and diverse flavor. Cheese has a long history in the human diet (Walther et al., 2008). In countries with developed dairy industry and some developing countries, cheese occupies an important position in the resident's dietary structure. Dairy products are extremely popular across the Gulf and wider Arab world and people are increasingly integrating more dairy products in their daily diets due to its durability in hot weather compared with milk as well as for its

convenience and nutritional value (Anonymous, 2006). Cheese are typically categorized according to their (1) moisture content as a) soft $\geq 50\%$, b) semi soft $> 35\% - < 50\%$ and c) hard $\leq 39\%$; (2) fat content in the initial mix as a single and double cream. Cream cheese is a soft, mild, rich, unripened cheese and is a creamy white, slightly acidic tasting product with a diacetyl flavor. It is usually manufactured by the coagulation of cream or mixture of milk and cream by acidification with starter culture and is ready for consumption after the manufacturing process is complete (Guinee et al., 1993). According to USDA (1994), cream cheese and related products should have a uniform white to light cream color with a slightly lactic acid and cultured diacetyl flavor and aroma; off-flavors such as bitter, sulfide, yeasty and unnatural flavor should not be present. The texture of the products should be smooth without lumps or grittiness, and the products should not show any indication of

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Abbreviation: LAB, Lactic acid bacteria.

cracking, or wheying off. Food spoilage is an enormous economic problem worldwide. Through microbial activity alone, approximately one-fourth of the world's food supply is lost (Laszlo, 2007). Milk is a highly nutritious food that serves as an excellent growth medium for a wide range of microorganisms. Undesirable microbes that can cause spoilage of dairy products are bacteria, yeasts, and molds. For this reason, increased emphases are placed on the microbiological examination of milk and dairy foods. Microbiological analyses are critical for the assessment of quality and safety, conformation with standards and specifications, and regulatory compliance (Tatini and Kauppi, 2003). Some cheeses are more susceptible than others to microbial contamination and spread. The causes include both intrinsic factors (nutrients, water activity, pH, inhibiting factors produced by starter cultures and non-starter microorganisms, competitive microflora, etc.) and extrinsic factors (microbial quality of raw milk, production phases, ripening and packaging conditions, etc) (Prencipe et al., 2010; Hosny et al., 2011; Giammanco et al., 2011).

Among the dairy products, cheese is the only product really susceptible to fungal growth and also production of mycotoxins. Commonly isolated fungi from cheese include *Penicillium*, *Aspergillus*, *Cladosporium*, *Geotrichum*, *Mucor* and *Trichoderma*. Incidence of moulds in cheese and fermented dairy products indicates that those most commonly found (predominant flora) belong to the genus *Penicillium*. Growth of these genera in or on cheese leads to undesirable changes affecting the quality of products (Berthiller et al., 2010). In most cases, spoilage is due to a mixed flora, where several different species contribute to spoilage. Microbial growth is dependent on a number of factors including water activity, temperature, substrate, strain of mould, gas composition, the presence of chemical preservatives and microbial interactions (Bullerman et al., 1984; Varga et al., 2005). Refrigeration slows the metabolism of microorganism and therefore the rate of multiplication and thus can delay spoilage. However, lower temperature does not slow the growth of different species of microorganism to the same degree.

Therefore, analyses of four different type of cream cheese were carried out to find out the role of temperature on various factors such as pH, titratable acidity (% lactic acid), percent reduction in moisture content and microbial growth, which determines spoilage of cheese.

MATERIALS AND METHODS

Cheese samples

Four different types of cream cheese were purchased from a super market at Riyadh. Two products were commercially packed whereas two were available as open cream cheese (locally prepared) in market. The details of cream cheese regarding type of cream cheese, expiry date and ingredients listed on the label are

provided in Table 1. Temperatures selected for the study were $4\pm 1^\circ\text{C}$ (recommended for the storage of cheese) and $21\pm 1^\circ\text{C}$ (ambient room temperature). Each sample was divided into two sets and throughout the study, one set was stored in a refrigerator and the other set in an incubator at 4 ± 1 and $21\pm 1^\circ\text{C}$, respectively. At sampling time (0, 7, 14, 21 and 28 days storage period), 100 g of each sample was taken out aseptically and was used to determine the moisture content, titratable acidity, pH and microbial growth.

Moisture content

Moisture content in cream cheese was determined by Method No. 926.08 of AOAC (1990). To determine the moisture content, porcelain dishes containing sand at base were dried in hot air oven (Haraeus, VT 5042EK, Germany) at 103°C for 1 h and after cooling, weight (W1) was measured, and later in each dish, 2 g sample (W2) was mixed with the help of glass rod. Prepared samples were placed in hot air oven at $103 \pm 5^\circ\text{C}$ till the constant weight of samples were obtained. Weight (W3) of the dishes was recorded after samples were cooled in the desiccators. Moisture was calculated by using the following expression:

$$\text{Percent moisture} = (W2 - W3 \times 100) / (W2 - W1)$$

Titratable acidity

Acidity in cream cheese was estimated by titration Method No. 920.124 of AOAC (1990). 1 g of each cream cheese sample was mixed with warm water and volume was made up to 10 ml in 100 ml conical flask; each sample was shaken vigorously and filtered. The filtrate was titrated with 0.1 N NaOH using phenolphthalein as indicator. Percent acidity was calculated by using the following expression:

$$\text{Titratable acidity \%} = 0.0090 \times \text{volume of NaOH used} \times 100 / \text{weight of the sample}$$

Estimation of pH

Pre weigh of each sample (20 g) of cream cheese was blended with 12 ml water to prepare the cream cheese slurry and pH was measured by a pH meter (Mettler Toledo, MP220 Switzerland) after calibrating it with fresh standard buffer solutions of pH 4.0 and 7.0 (Ong et al., 2007).

Microbiological analysis

Each cream cheese sample was prepared for microbiological examination according to ICMSF (1988). The standard dilution plate method was used for the isolation of bacteria and fungi on de Man, Rogosa and Sharpe (MRS) agar (oxid) and PDA media (Scharlau Chemie, Spain), respectively. Samples were examined for different types of fungi present in the sample. For identification, fungi were individually transferred to PDA plates to get the pure culture of each fungus. Isolated fungi were identified according to Nelson et al. (1983) whereas, viable cells of lactic acid bacteria (LAB) in cream cheese were determined on MRS agar (Dave and Shah, 1996).

Statistical analysis

All experiments were carried out in triplicate and statistical analysis

Table 1. Properties and ingredients of cheese samples as mentioned on labels.

Sample code	Sample type	Form	Ingredients information on labels	Manufacturing and expiry date
1	Double cream cheese	Spreadable creamy cheese cubes (commercially packed in foil)	Cheese (microbial rennet), cream (30%) milk proteins, 68% fat in dry matter and emulsifiers.	28/4/2010, 1 year from date of manufacture
2	Double cream cheese	Spreadable creamy cheese spread (commercially packed in bottle)	Cheese, milk fat, butter, emulsifier, milk protein, mineral salt, sugar, milk solid non fat, salt, food acids, preservatives, rennet, 60% fat in dry matter.	1/3/2010, 1 year from date of manufacture
3	Double cream cheese	Non spreadable creamy cheese block (openly available)	Not mentioned	15/4/2010, 1 year from date of manufacture
4	Double cream cheese	Non spreadable creamy cheese block (openly available)	Not mentioned	30/4/2010, 1 year from date of manufacture

(ANOVA) was performed using XLSTAT 2011 Software. Standard deviation of mean was used to describe data; Duncan multiple range test was used to evaluate the significant differences between control and cream cheese samples. P value was considered significant at 95%.

RESULTS

The sensory evaluation of cheese samples showed that all samples stored at $21\pm 1^\circ\text{C}$ were in unacceptable state on the 28th day of storage and thus no further analyses were conducted (data not presented).

Moisture content

Changes in moisture content at 4 ± 1 and $21\pm 1^\circ\text{C}$ during storage period of 0, 7, 14, 21, 28 days (Figure 1) shows that moisture content of cream cheese in all samples studied was significantly ($P\leq 0.05$) decreased at both temperature with the progress in storage days. The decrease in

moisture content of samples stored at room temperature was significantly ($P\leq 0.05$) more compared to samples stored at refrigerated temperature. Maximum decrease in moisture content (41.03%) was observed in Sample 4 at $21\pm 1^\circ\text{C}$ and stored for 28 days.

Titratable acidity

The changes in titratable acidity (% lactic acid) of cream cheese during storage (0 to 28 days) at room temperature and at refrigerated temperature are shown in Table 2.

The titratable acidity of all cream cheeses increased significantly ($P\leq 0.05$) at both temperatures with the increase in storage time as compared to the control (value at zero days). Sample 2 showed non-significant changes in the titratable acidity with the progress of storage time. The increase in acidity of samples stored at room temperature was significantly ($P\leq 0.05$) more compared to samples stored at the refrigerated

temperature.

This trend was same for all cream cheese samples. Sample 4 had maximum titratable acidity at 28 days of storage and the increase was 39.73% as compared to titratable acidity at the beginning of the study and it was at par with the value of Sample 3 at same storage time and temperature.

Estimation of pH

The pH of cream cheese in Samples 1, 2 and 3 was found to increased up to 14 days and after the 14th day, it showed decreasing trend till the 28th day (Table 3).

In sample 2, pH decreased to 5.88 and 5.65 at 4 ± 1 and $21\pm 1^\circ\text{C}$, respectively on 28th day which was 6.02 at the beginning of the study whereas, pH of Sample 4 was found to increase on the 7th day (4.75); after that, it showed a gradual decrease and on the 28th day, it dropped to the lowest value (3.39). The decrease in pH of samples stored at room temperature was

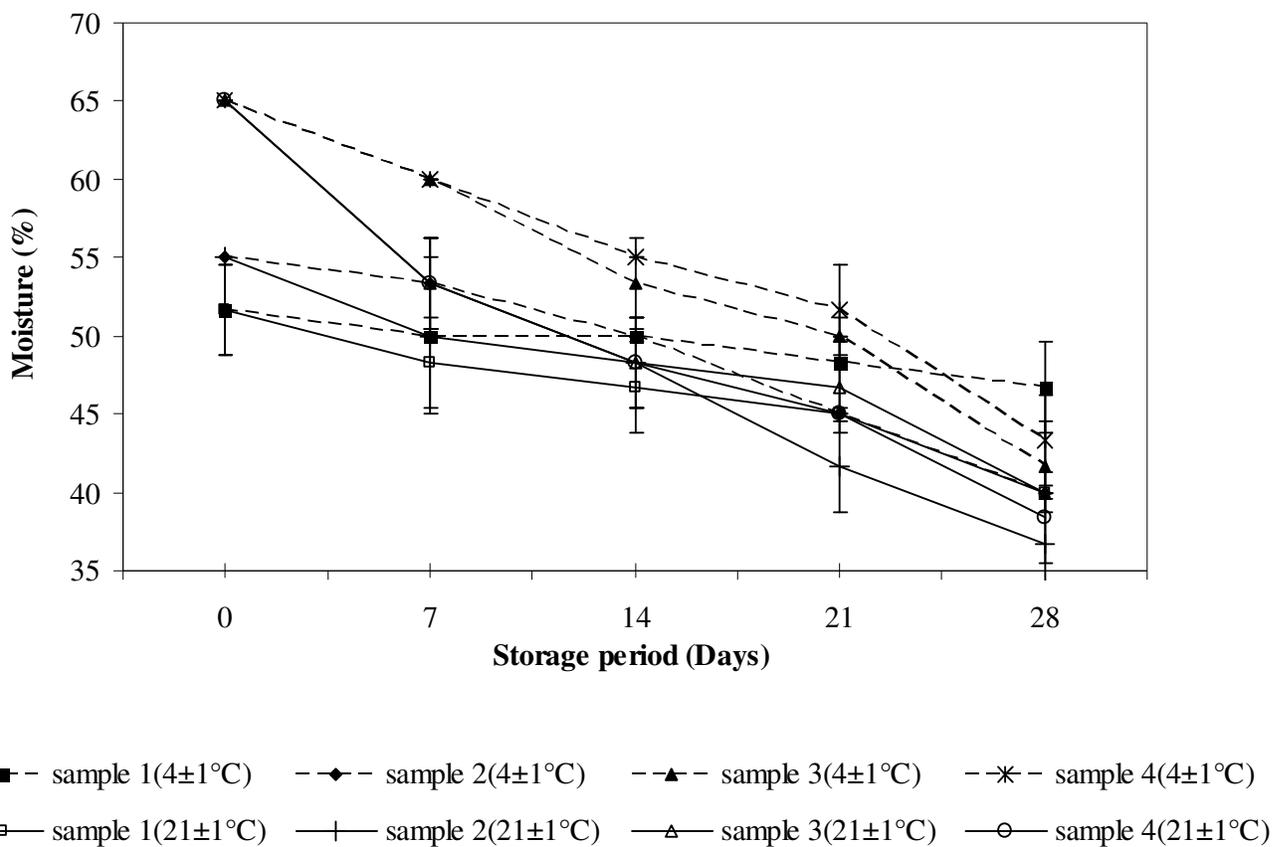


Figure 1. Changes in moisture content of cream cheeses stored at 4±1 and 21±1 °C for 28 days. Error bars for mean value (n=3) ±SD.

significantly more ($P \leq 0.05$) as compared to samples stored at refrigerated temperature.

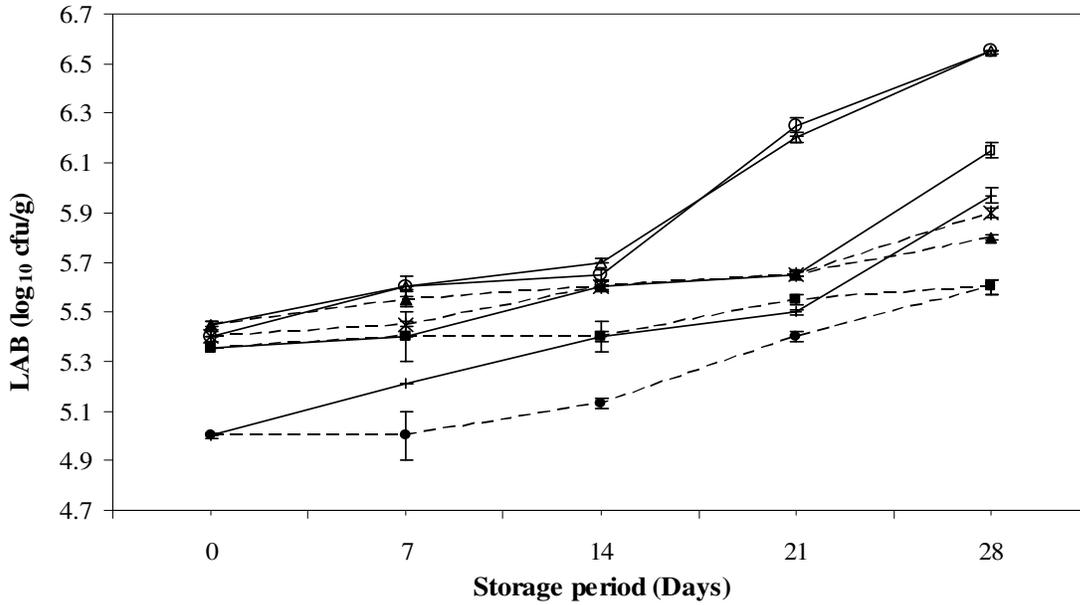
Microbiological analysis

The data presented in Figure 2 show that the total viable count of LAB was over 10^4 CFU/g ($5.00 \log_{10}$ CFU/g) in all samples and it increased with progress in storage time. The increase in CFU/g of LAB was more at 21±1 °C as compared to 4±1 °C. The comparison of data of titratable acidity and CFU \log_{10} of LAB shows direct relationship between microbial growth and acidity. Examination of isolated pure colonies of fungi revealed the presence of species of *Penicillium*, *Aspergillus*, *Cladosporium* and unknown actinomycetes. Sample 1, 2 and 3 showed the presence of moulds whereas Sample 4 showed only the presence of actinomycetes (Table 4). Further, it was found that *Penicillium* species dominated in all samples and frequency occurrence of *Penicillium* species was higher (62.5%) at the refrigerated temperature as compared to cream cheese samples stored at room temperature (40%). However, the isolation of different

species of fungi was higher at 21±1 °C as compared to 4±1 °C (Figure 3). *Aspergillus niger* was isolated only from Sample 1 stored at room temperature whereas at same temperature *Cladosporium* sp. was isolated from Samples 1, 2 and 3.

DISCUSSION

Temperature-dependent storage of most foods has three major roles to allow for curing/ripening of foods that contain added active starter cultures and enzymes, to prevent quality defects, and to control microbial growth (Bishop and Smukowski, 2006). Reduction in moisture content of cheeses with increase in storage period were observed in all the samples (Figure 1); the reduction was higher in samples which were stored at room temperature as compared to those refrigerated. Acidity (specifically reduced pH) causes the protein matrix in the curd to contract and squeeze out moisture. That process of contraction is called syneresis. Cream cheese has very poor water-holding capacity (WHC) and is highly susceptible to syneresis (Fox et al., 2000). Samples 3 and 4 showed higher loss of moisture which may be due



--■-- sample 1(4±1°C) --●-- sample 2(4±1°C) --▲-- sample 3(4±1°C) --*-- sample 4(4±1°C)
 —□— sample 1(21±1°C) —+— sample 2(21±1°C) —△— sample 3(21±1°C) —○— sample 4(21±1°C)

Figure 2. Changes in total viable count of lactic acid bacteria (LAB) of cream cheeses stored at 4±1 and 21±1°C for 28 days. Error bars for mean value (n=3) ±SD.

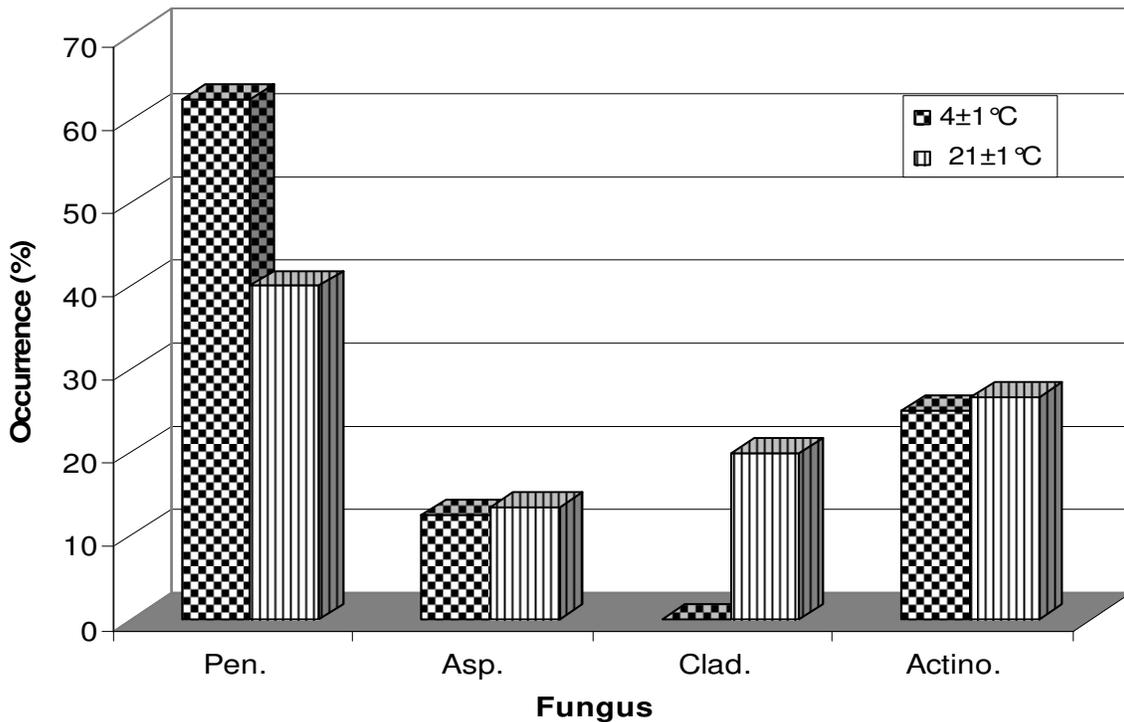


Figure 3. Percent occurrence of fungi and actinomycetes in cream cheeses stored at 4±1 and 21±1°C for 28 days. Pen= *Penicillium* sp., Asp= *Aspergillus* sp., Clad= *Cladosporium* sp., Actino= Actinomycetes.

Table 2. Changes in titratable acidity (% lactic acid) of cream cheeses stored at 4±1 and 21±1 °C for 28 days.

Storage period	Sample 1		Sample 2		Sample 3		Sample 4	
	4±1 °C	21±1 °C	4±1 °C	21±1 °C	4±1 °C	21±1 °C	4±1 °C	21±1 °C
0 day	0.086 ^b ± 0.001	-	0.062 ^b ± 0.001	-	0.099 ^d ± 0.016	-	0.093 ^c ± 0.010	-
7 days	0.089 ^b ± 0.003	0.099 ^c ± 0.016	0.063 ^c ± 0.00	0.083 ^c ± 0.003	0.102 ^e ± 0.010	0.109 ^e ± 0.007	0.096 ^d ± 0.005	0.108 ^d ± 0.00
14 days	0.092 ^c ± 0.009	0.102 ^c ± 0.010	0.072 ^c ± 0.00	0.090 ^c ± 0.009	0.108 ^{be} ± 0.008	0.118 ^{ce} ± 0.014	0.109 ^{ad} ± 0.002	0.119 ^{ad} ± 0.004
21 days	0.108 ^c ± 0.00	0.113 ^c ± 0.011	0.084 ^c ± 0.005	0.099 ^c ± 0.016	0.115 ^{be} ± 0.004	0.136 ^c ± 0.002	0.114 ^{ad} ± 0.005	0.141 ^d ± 0.005
28 days	0.113 ^c ± 0.007	0.133 ^a ± 0.005	0.112 ^c ± 0.003	0.132 ^a ± 0.007	0.129 ^{ad} ± 0.010	0.153 ^a ± 0.009	0.132 ^a ± 0.014	0.154 ^e ± 0.017

Data are means ± S.D of three replicates. Values followed by same superscript are not significantly different ($P > 0.05$).

to loose packaging as cheese loses its moisture during storage if it is not properly wrapped and thus reduce its yield (Connor, 1994). Titratable acidity is calculated in terms of percent lactic acid. The result indicates (Table 2) that the titratable acidity got increased with storage period and this increase was more in Samples 3 and 4 as compared to Samples 1 and 2. Earlier studies reveals that the increase (about 80 to 90%) in acidity during storage of cheese at both temperatures (refrigerated and ambient) is mainly due to the lactic acid formed by a predominating lactic acid bacteria (True and Patel, 1973; Jelen, 1992; Mahran et al., 2000; El Owni and Hamid, 2008).

The higher acidity found in this study may be due to activity of starters, as the primary function of starters is the conversion of lactose and other sugars in milk to lactic and other acids (Hill and Ross, 1998; Amarita et al., 2001). Results are in synchronization with the results of Joshi and Sharma (2009) who reported that titratable acidity increased with the advancement of fermentation period up to 16 days. El Owni and Hamed (2009) added that the titratable acidity of cheese samples stored at room temperature was higher as compared to those stored at refrigerator

temperature. In all the cheese samples, pH decreased with increase in time (Table 3), although changes were irregular. These results are similar to the outcome of the study by Hough et al. (1999) which reported irregular changes in pH of ricotta cheese during storage at various temperatures. The reason for increase in pH is unclear, however the reduction of pH values observed during storage of cream cheese is a natural process caused by continuous production of lactic acid and other organic acids by the starter and the probiotic cultures LAB. This might be attributed to the fact that storage at room temperature tends to increase lactose fermentation which leads to decrease in pH values. Reduction of pH value during storage of cream cheese was also reported by Kuipers et al. (2000) and Shah (2007). LABs are considered to be important components of the microbiota playing a large variety of health-promoting functions. The present data indicates that CFU log₁₀ of LAB has increased with progress in storage time. The increase in CFU log₁₀ of LAB was more at 21±1 °C as compared to 4±1 °C. Results also reveal that storage temperature is the most important factor affecting the growth of LAB.

Higher multiplication enhances production of

acidity which results in very strong off flavor and unacceptability of cheese. Fungal profile of cream cheese reveals the presence of genera *Penicillium*, *Aspergillus* and *Cladosporium*; unknown actinomycetes were also found in some samples (Table 4). Several reports indicate that the major fungi isolated from cheese include *Penicillium*, *Aspergillus*, *Cladosporium*, *Geotrichum*, *Mucor* and *Trichoderma*. However, the most commonly found fungus (predominant flora) belongs to the genus *Penicillium* (Filtborg et al., 1996; De Santi et al., 2010). Occurrence of *Penicillium* was found much higher (62.5%) at 4±1 °C as compared to 21±1 °C (40%). For most part, *Penicillium* species liked the temperature on the cool side. Moreover, LAB has been highlighted as having the ability to repress mould growth through the production of several low molecular weight antifungal metabolites (Dalie et al., 2010; Tharmaraj and Shah, 2009). Shah (2007) reported that LAB produces some components which have a bacteriocidal and bacteriostatic effect, which lead to the delaying and/or disappearance of fungal growth in samples during storage period. The production of lactic acid was higher at 21±1 °C as shown by the analysis of titratable acidity (Table 2) which may

Table 3. Changes in pH of cream cheese stored at 4±1 and 21±1 °C for 28 days.

Storage period	Sample 1		Sample 2		Sample 3		Sample 4	
	4±1 °C	21±1 °C	4±1 °C	21±1 °C	4±1 °C	21±1 °C	4±1 °C	21±1 °C
0 day	5.2 ^{be} ± 0.006	-	6.03 ^{ad} ± 0.021	-	4.67 ^{ce} ± 0.006	-	4.69 ^a ± 0.021	-
7 days	5.4 ^{ad} ± 0.010	5.24 ^{be} ± 0.021	6.07 ^c ± 0.006	6.04 ^c ± 0.006	4.94 ^{ad} ± 0.00	4.70 ^{ad} ± 0.006	4.98 ^a ± 0.010	4.75 ^a ± 0.006
14 days	6.25 ^a ± 0.023	6.01 ^c ± 0.006	6.10 ^a ± 0.011	6.07 ^c ± 0.006	5.42 ^a ± 0.010	5.23 ^c ± 0.020	4.33 ^{ad} ± 0.030	4.19 ^{ad} ± 0.005
21 days	5.41 ^{ad} ± 0.006	5.1 ^e ± 0.020	5.96 ^{be} ± 0.025	5.85 ^{de} ± 0.03	4.92 ^{ad} ± 0.011	4.72 ^{ce} ± 0.023	4.13 ^{ad} ± 0.026	3.69 ^d ± 0.006
28 days	4.99 ^e ± 0.025	4.60 ^d ± 0.011	5.88 ^e ± 0.011	5.65 ^f ± 0.006	4.23 ^e ± 0.011	3.99 ^d ± 0.011	3.62 ^c ± 0.020	3.39 ^c ± 0.001

Data are means ± S.D of three replicates. Values followed by same superscript are not significantly different ($P > 0.05$).

Table 4. Distribution of fungi and actinomycetes in cream cheese samples during storage at 4±1 and 21±1 °C.

Fungi	4 ±1 °C				21±1 °C			
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 1	Sample 2	Sample 3	Sample 4
<i>P. chrysogenum</i>	+	+	+	-	-	+	+	-
<i>Penicillium</i> sp.	+	-	+	-	+	-	-	-
<i>A. niger</i>	-	-	-	-	+	-	-	-
<i>A. flavus</i>	-	+	-	-	-	+	-	-
<i>Cladosporium</i> sp.	-	-	-	-	+	+	+	-
Actinomycetes	-	-	-	+	-	-	+	+

+ Present; - Absent. Data are means of three replicates.

be the reason for lower frequency of *Penicillium* spp. at this temperature, whereas the variation of fungal species may be due to the difference of optimum temperature. Presence of actinomycetes in Samples 3 and 4 may be due to atmospheric contamination since these two cheeses were openly available in the market. The cream cheeses stored at room temperature were in unacceptable condition after 28 days; however the spoilage rate was higher in cream cheese openly available in the supermarket. At the end of the study, all the samples of cream cheese stored at

temperature 4±1°C were found to be in an acceptable condition. Results of the present study show that the temperature is an important factor affecting the shelf life, the chemical and microbiological quality of cheese. The improper storage temperature may prolong survival of the microorganisms or even enhance their multiplication. Our results are in agreement with the results of Collombo et al. (1992) who reported that low temperature was more effective for prolonged cheese storage than high temperature. Therefore, hygienic production and prevention of any kind of

contamination is one of the most important principles of producing cheese. However, post production temperature plays the most important role for maintaining the quality, microbial growth and shelf life of cheese.

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