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Potential Significance of Adjunct Cultures and Raw Milk on Physicochemical Analysis of Cheddar Cheese

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Abstract: Present exploration was an attempt to investigate the physicochemical and sensory evaluation of the cheddar cheese using different concentrations of starter's cultures at different storage intervals. For this purpose, cheddar cheese was prepared through *Streptococcus thermophillus* and *Lactobacillus bulgaricus* in combination with starter cultures. In ripening, three types of starter cultures are used including *Lc. Cremoris* and *Lc. lactis* (95:5) 95% + *Str.thermophilus* and *Lb.bulgaricus* 5% (T_1), *Lc. Cremoris* and *Lc. lactis* (95:5) 90% + *Str. Thermophilus* and *Lb. bulgaricus*10%, *Lc. Cremoris* and *Lc. lactis* (95:5) 85% + *Str. Thermophilus* and *Lb. bulgaricus* 15% (T_3) and along with T0**:** *Lc. Cremoris* and *Lc. lactis* (95:5)100% @0.0025% were prepared for comparison purpose. During study, storage imparts substantial effect on pH and acidity. In pH, storage caused a significant reduction in pH from 5.20±0.001 to 5.09±0.005% at 0 and 90th day, correspondingly. Likewise, the recorded acidity values of cheddar cheese *i.e.*T0, T_1, T_2 and T_3 were 0.86±0.04, 0.84±0.06, 0.91± and 1.05±0.05, respectively. However during storage, values for ash contents ranged from 3.92±0.03 to 4.01±0.04 at the initiation till the termination of trial. Similarly, protein contents are decreased from 28.31±0.83 to 26.41±0.72% at 0 and 90th day of storage, correspondingly. Moreover, non protein nitrogen (NPN) values of cheddar cheese for treatments T0, T1, T2 and T3 were 1.51±0.07, 1.60±0.06, 1.81±0.05 and 1.92±0.06, respectively whilst during storage, NPN significantly increased from 1.03±0.54 to 2.28±0.43. Conclusively, storage and treatments imparted the substantial effect on the cheddar cheese.

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1. Introduction

Milk is a promising source of energy, proteins, calcium, minerals, vitamins and bioactive constituents. It is used to prepare the yoghurt, butter, ice-cream, dry milk powder and cheese. There are more than four thousand varieties of cheeses exist in world. The world production of cheese is approximately 15million tones/year (Contarini and Povolo, 2013).

Cheddar cheese is more popular in the world and also in Pakistan due to its distinguishing flavor and texture. It is fairly hard, pale yellow to orange, sharp tasting cheese produced by coagulation, whey drainage and ripening (Azarnia et al., 2006). Cheddar cheese is a biochemically dynamic product that undergoes significant changes during ripening. Cheddar cheese is used as an ingredient to improve the color, taste, flavor, texture and nutritional qualities in many food based products. It is also considered to be a good addition to special products like low fat foods (Chevanan and Muthukumarappan, 2008).

Hedonic response is a key indicator used to determine the acceptability and preference of the product through trained taste panel. Sensory evaluation is a documented method to characterize the foods on the basis of natural senses including smell, taste, sight etc. carried out by trained taste panel (Kuti, 2004). The sensory assessment of a product is correlated with consumer approach, believe and awareness (Aaron et al., 1994). The products containing phyto-chemicals need careful assessment not only to evaluate consumer suitability but also find their effect on specific segment of population (Quílez et al., 2006). Considering the importance, scientists working on the development of functional foods are not only emphasizing on their physiological

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functionality but also paid attention towards hedonic aspects.

The ripening of cheddar cheese involves a series of physico-chemical, microbiological, biochemical changes that are collectively responsible for the development of its characteristics structure, flavor and aroma (Mahony et al., 2005). During ripening, cheese texture become soften due to the hydrolysis of casein micelle by proteolysis and changes to water binding ability of curd and changes in pH. The protein network structure inside the cheddar cheese changes continuously during ripening and affects the texture and rheological properties (McSweeney, 2004). The pH affects curd demineralization which is greater when acid is produced prior to whey drainage. During cheese manufacture, as the pH decrease, Ca is lost to whey more rapidly than PO_4 that decreased the mineral contents (Hill et al., 1985; Lucey and Fox, 1993). Keeping in view the importance of cheddar cheese and to reduce the repining duration the project was under take to achieve the following objectives.

1. To evaluate the significance of thermophillic lactic culture in cheddar cheese
2. To determine the physicochemical analysis of the cheddar cheese at different storage intervals

2. Materials and Methods

The current research work was conducted in the Dairy Technology and Food Analysis Laboratories, National Institute of Food Science and Technology (NIFSAT), University of Agriculture, Faisalabad. The detail of the materials used and analytical methods employed during research work is given below.

2.1. Raw Milk

Buffalo milk for cheese preparation was procured from Farm House, Institute of Animal Nutrition and Feed Technology, University of Agriculture, Faisalabad. Starter cultures, mesophilic (*Lactococcus lactis* ssp. c*remoris* and *Lactococcus lactis* ssp. *lactis)* CH-R704 and thermophilic (*Streptococcus salivarius subsp. thermophilus and Lactobacillus delbrueckii subsp. Bulgaricus)* CHOOZIT™ TM 81 LYO for the preparation of cheese was taken from Dairy Technology Laboratory, National Institute of Food Science and Technology (NIFSAT), University of Agriculture Faisalabad. For the curd formation rennet powder was procured from the scientific store. Sodium and calcium chloride were taken from Dairy Laboratory that was pure, refined and free of any anti-caking agent.

2.2. Raw milk analysis:

The pH of milk was evaluated by electronic digital pH meter (WTW series 720) according to the guidelines of Ong et al. (2007). Similarly, acidity and fat were determined through AOAC (2000) whilst, fat was evaluated by using Gerber method as mentioned by Marshall (1993). Lactose and SNF in milk samples were determined through Lactoscope (COMP-1.0). Protein contents were determined through Kjeldahl method as discussed by Fox and McSweeney (1998).

Plan for Different Concentrations of Cultures

T0: *Lc. Cremoris* and *Lc. lactis* (95:5)100% @0.0025%

⇩

T_1:*Lc. cremoris*and*Lc. lactis* (95:5) 95% + *Str.thermophilus* and *Lb.bulgaricus*5%

⇩

T_2: *Lc. cremoris* and *Lc. lactis* (95:5) 90% + *Str. thermophilus* and *Lb. bulgaricus*10%

⇩

T_3: *Lc. Cremoris* and *Lc. lactis* (95:5) 85% + *Str. thermophilus* and *Lb. bulgaricus*15%

2.3. Cheese manufacturing:

Cheddar cheese was prepared from buffalo milk (pasteurized milk 90% and raw milk 10%) in cheese VAT FT20-MKII following the standard protocol as described by Rehman et al. (2000).

2.4. Quality evaluation of cheese

Cheese samples were analyzed for physiochemical, textural and sensory characteristics as described below. All tests were performed after 0, 30, 60 and 90 days.

2.4.1. Physico-chemical analysis

pH was measured by electronic digital pH meter WTW series 720 available in the laboratory of Dairy technology. Buffer solution of pH 4 and 7 were used for the calibration of the pH meter. Cheese sample was taken in a butter paper. Electrode of pH meter was immersed in the sample and reading was recorded (Ong et al., 2007).

Acidity in cheese was determined by method given in AOAC (2000). 10 g prepared cheese sample was taken in beaker at temperature of 40^0C volume was made up to 105mL, shaken vigorously and filtered. 25mL portion (representing 2.5 g sample) of filtrate was titrated against standard 0.1N NaOH using phenolphthalein indicator. Results were expressed in terms of lactic acid.

$$\% \text{ Acidity} = \frac{0.009 \times \text{Volume of NaOH used}}{Wt.of\ sample} \times 100 \qquad [1]$$

For estmation of fat contents Gerber test as described by Pearson (1999) was used. Cream butyrometer with special rubber cork stoppers was used for analysis. Firstly, 10mL of sulpheuric acid was poured into butyrometer and then 3g of prepared cheese sample was added followed by 5 mL warm water and 2 mL of isoamyl alcohol. Butyrometer was bunged with rubber stopper; its contents were thoroughly mixed and immediately centrifuged at 1100 rpm by centrifugal machine (Model: 800, China) for 5 minutes. Then fat was noted from the column.

Ash content was determined by igniting the 3g cheese sample first on flame and then in a furnace at 550^0C until the white ash obtained (AOAC 935.42, 2000). The crucible containing ash was placed in desiccators and cooled. Quick weighing of crucible was done to prevent moisture absorption. Then ash percentage was calculated by using formula.

$$\text{Ash}\% = \frac{Wt.of\ ash}{Wt.of\ ash\ in\ sample} \times 100 \qquad [2]$$

Moisture was determined by following method number 926.08 (AOAC, 2000). For moisture determination 2-3 g cheese sample was taken in pre-dried moisture dish and placed in oven at 100 ± 5^0C till constant weight was obtained. The samples was removed from oven, cooled, and weighed. Moisture percentage was calculated from following formula.

$$\text{Moisture}\% = \frac{Wt\ before\ \ drying\ -Oven\ dried\ \ Wt.}{Wt.of\ \ sample} \times 100 \qquad [3]$$

The percentage of crude protein was estimated through Kjeltech Apparatus (Model: D-40599, Behr Labor Technik, Gmbh-Germany) by adopting the protocol of AOAC (2000). Initially, sample was digested with conc. H_2SO_4 and digestion mixture for 6 hr till light greenish color. Afterwards, 250 mL dilution of digested sample was made. The diluted sample was distilled by taking 10 mL of sample and 10 mL of 40% NaOH solution in the distillation assembly. The liberated ammonia was trapped in 2% boric acid solution. Lastly, distillate was titrated against 0.1 N H_2SO_4 till golden brown end point.

Accurately weighed cheese sample of 1 gram was taken in a conical flask. To this 20 mL of 12% TCA solution was added. All constituents were shaken well at 90^0C for 15 minutes. After this, filtration was done through filter paper by using funnel. Residue on filter paper was washed twice with 5 mL distilled water in filter paper. Washing started when all the liquid was drained out. Then filtrate was poured in digestion flask, 5g digestion mixture and 25 mL of sulpheuric acid was added. Again the whole procedure for proximate analysis of protein was used to get results.

Salts contents in cheese samples was determined by Volhard method (AOAC 975.20, 2000). 2 g of grated and shredded cheese sample was weighed into a 50 mL beaker. 20 mL warm water was added and stirred to break the particles and form the slurry. The slurry was transferred to 250 mL Erlenmeyer flask. Te beaker was rinsed with 10 mL warn water and rinse was added to the flask. 25 mL of 0.1N silver nitrate and immediately 10 mL of nitric acid and 50 mL of distilled water was added. Then it was placed on a hot plate and solution was boiled in a hood. When solution started boiling, potassium permanganate (4%) was added in 5 mL portions until the solution turned brown and remained brown for at least 5 minutes on gentle boiling. Heating was continued until the brown color disappeared and resulted in a straw colored clear solution.

There was white curd like particles in the solution indicating the silver chloride aggregates. Then the hard solution was filtered into a clean 250 mL Erlenmeyer flask. Filter paper was washed thoroughly with 10 mL hot water. Solution was cooled to room temperature and 2 mL of ferric ammonium sulfate indicator (saturated) was added. Excessive silver nitrate was titrated with 0.1N potassium thiocyanate to first pale reddish brown color (lasted for 30 sec.).

From the final titration obtained, the blank value obtained using 2 mL water in place of 2gram of cheese sample, was subtracted. The salt (sodium chloride) content was calculated using the following formula:

$$\text{NaCl}\% = \frac{[(\text{mL x 0.1N AgNO3}) - (\text{mL x 0.1N KSCN})] \text{ x } 0.0585}{Wt.of\ sample} \times 100 \qquad [4]$$

2.5.Texture analysis

Texture profile analysis (TPA) was performed using a TA-XT2i Texture Analyzer (Stable Micro System Ltd., Godalming, Surrey,U.K.). Four cubic samples (1.3 x 1.3 x 1.3cm) were obtained from each cheese block at different depth's to minimize the effects of surface drying (Jack et al., 1993). The samples were held at room temperature (21-24°C) for 1 hour before testing. Each sample was compressed axially to 50% of their original height into single compression by 30mm diameter flat crosshead, crosshead speed 1.0mm per second and contact speed 5 gm (IDF, 1991).

2.6. Sensory evaluation

The prepared cheddar cheese (T_0, T_1, $T_2$and T_3) were evaluated for sensory response using 9-points hedonic scale ranged from extremely liking to disliking (9 = like extremely; 1 = dislike extremely) as mentioned in Appendix-I, following the guidelines of Meilgaard et al. (2007).

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Table 1. Effect of different concentrations of cultures on physicochemical parameters of Cheddar cheese

Treatments	pH	Acidity	Fat	Ash
T_0	5.09±0.087c	0.86±0.19c	31.93±0.34a	3.94±0.03c
T_1	5.14±0.072b	0.84±0.16c	31.66±0.37ab	3.92±0.03d
T_2	5.17±0.070ab	0.91±0.06b	31.23±0.42bc	3.97±0.02b
T_3	5.18±0.015a	1.05±0.07a	30.98±0.48c	3.98±0.04a

Where T0 is *Lc. Cremoris* and *Lc. lactis* (95:5)100% @0.0025% ; T_1 is *Lc. Cremoris* and *Lc. lactis* (95:5) 95% + *Str.thermophilus* and *Lb.bulgaricus* 5% ; T_2 is *Lc. Cremoris and Lc. lactis* (95:5) 90% + *Str. Thermophilus and Lb. bulgaricus*10%; T_3 is *Lc. Cremoris* and *Lc. lactis* (95:5) 85% + *Str. Thermophilus and Lb. bulgaricus* 15%.

The sensory behavior of cheddar cheese drinks for various characteristics like flavor, taste, aroma, texture, and overall acceptability was assessed on monthly basis during storage. The sensory profiling was performed in the Sensory Evaluation Laboratory at National Institute of Food Science and Technology (NIFSAT), University of Agriculture Faisalabad. Each panelist was provided with written instructions to award scores for the prepared cheese. During hedonic evaluation, panelists were seated in separate booths equipped with white fluorescent light and cheese was presented in polystyrene cups labeled with random codes at room temperature. For enhancing the accuracy, evaluators were provided water and unsalted crackers to neutralize their mouth feel between samples testing. Samples were presented to the judges randomly to avoid any biasness and asked to rate their acceptance by assigning score for selected parameters. Moreover, panelists names were concealed to maintain secrecy.

2.7. Statistical analysis

The collected data obtained for each parameter was subjected to two factor factorial analysis. Furthermore, analysis of variance was applied to determine the level of significance (Steel et al., 1997).

3. Results and Discussion

3.1 Physicochemical Analysis

Milk used for cheese making was analyzed for total protein, acidity, pH, fat, lactose, and milk solids-not-fat (MSNF) contents. The milk contained, 5.5% fat, 4.98% lactose, 9.18% SNF contents, 14.68% TSS, pH 6.62, acidity 0.139% and total protein 3.45%. The pH in cheddar cheese including T0, T1, T2 and T3 was observed as 5.09±0.087, 5.14±0.072, 5.17±0.070 and 5.18±0.015%, respectively. However, storage caused a significant reduction in pH from 5.20±0.05 to 5.09±0.03% at 0 and 90th day, correspondingly. Likewise, the recorded acidity values of cheddar cheese i.e.T0, T1, T2 and T3 was 0.86±0.19, 0.84±0.16, 0.91±0.06 and 1.05±0.07, respectively. During storage, acidity significantly increased from 0.83±0.05 to 1.07±0.06. The fat of the cheddar cheese T0, T1, T2 and T3 were 31.93±0.34, 31.66±0.37, 31.23±0.42 and 30.98±0.48, respectively. Likewise, storage also revealed non-significant enhancement in fat and observed values at 0 and 90th days were 31.73±0.39 and 31.06±0.56, respectively.

Ash contents varied non-significantly among the different cheese T0, T1, T2 and T3 as 3.94±0.03, 3.92±0.03, 3.97±0.02 and 3.98±0.04, in respective drinks. However during storage, scores for ash increased significantly from 3.92±0.03 to 4.01±0.04 at the initiation till the termination of trial (Table 1 and 2).

The observed values for moisture contents in cheddar cheese T0, T1, T2 and T3 were 37.98±0.35, 37.48±0.37, 38.19±0.50 and 37.92±0.50, respectively. Nevertheless, 90 days storage resulted a significant reduction in moisture contents from 38.34±0.43 to 37.45±0.22. The protein contents in cheddar cheese including T0, T1, T2 and T3 was observed as 27.06±0.48, 27.05±0.86, 27.48±0.93 and 27.34±1.06%, respectively. However, storage caused a significant reduction in protein contents of cheddar cheese from 28.31±0.83 to 26.41±0.72% at 0 and 90th day, correspondingly.

Likewise, the recorded NPN values of cheddar cheese i.e.T0, T1, T2 and T3 were 1.51±0.07, 1.60±0.06, 1.81±0.05 and 1.92±0.06, respectively. During storage, non-protein nitrogen (NPN) significantly increased from 1.03±0.54 to 2.28±0.43. The observed values for salts contents in cheddar cheese T0, T1, T2 and T3 were 1.48±0.042, 1.49±0.050, 1.48±0.056 and 1.50±0.047, respectively.

Table 2. Effect of storage days on different parameters of Cheddar cheese

Days	pH	Acidity	Fat	Ash
0	5.20±0.05a	0.83±0.05d	31.73±0.39	3.92±0.03d
30	5.18±0.03a	0.88±0.04c	31.41±0.28	3.94±0.02c
60	5.12±0.02b	0.95±0.05b	31.59±0.74	3.96±0.03b
90	5.09±0.03b	1.07±0.06a	31.06±0.56	4.01±0.04a

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Table 3. Effect of different concentrations of cultures on physicochemical parameters of Cheddar cheese

Treatments	Moisture contents%	Protein %	NPN%	Salt%
T_0	37.98±0.35b	27.06±0.48a	1.51±0.07b	1.48±0.042ab
T_1	37.48±0.37d	27.56±0.86a	1.60±0.06ab	1.49±0.050b
T_2	38.19±0.50a	27.48±0.93a	1.81±0.05ab	1.48±0.056ab
T_3	37.92±0.50c	27.34±1.06a	1.92±0.06a	1.50±0.047a

Where T0 is *Lc. Cremoris* and *Lc. lactis* (95:5)100% @0.0025% ; T_1 is *Lc. Cremoris* and *Lc. lactis* (95:5) 95% + *Str.thermophilus* and *Lb.bulgaricus* 5% ; T_2 is *Lc. Cremoris and Lc. lactis* (95:5) 90% + *Str. Thermophilus and Lb. bulgaricus*10%; T_3 is *Lc. Cremoris* and *Lc. lactis* (95:5) 85% + *Str. Thermophilus and Lb. bulgaricus* 15%.

Nevertheless, 90 days storage resulted a significant enhancement in moisture contents from 1.44±0.04 to 1.56±0.02 (Table 3 and 4).

The pH of cheddar cheese decreased during pressing and the first several months of ripening due to lactic acid bacteria (Shakeel-Ur-Rehman et al., 2004). During cheddar cheese manufacture starter bacteria ferment lactose to lactic acid which causes reduction in pH (Fox et al., 1996a; Singh et al., 2003). The process of cheddar cheese ripening involves the fermentation of lactose and the degradation of proteins and fats (Laleye et al., 1990) resulting in the decline in cheese pH (Azarnia et al., 2006). The pH of cheese is influenced by the growth of both starter and non-starter lactic acid bacteria in raw and pasteurized milk cheeses.

The results of acidity found are in accordance with the results of Salwa et al. (2002) who reported that during ripening acidity increase in all type of cheese. This may be attributed to lactic acid bacteria which are mainly responsible for acid production (Marth and Steele, 2001). The higher acidity shows the activity of starters, because the primary function of starters is the conversion of lactose and other sugars in milk to lactic and other acids (Hill and Ross, 1998). Similarly, Durmos et al. (2007) reported an increase and decrease of fat content during 90 days ripening. Fenelon and Guinee (1999) reported that there is a slight difference in fat content of cheddar cheese during storage. Difference in fat could be due to lipolysis during ripening (Farkye, 2004; McSweeney, 2004; Ong et al., 2007). Raw milk contains non-starter bacteria and indigenous milk enzymes that are inactivated during pasteurization, which cannot take part in lipolysis to produce full aroma (Grappin and Beuvier, 1997).

Moreover, Hughes and Willenberg (1993) who stated that ash nearly remain constant during storage.

Moisture is the major component of cheese which acts as a plasticizer in the protein matrix, thereby making it less elastic and more susceptible to fracture upon compression. It provides the media for biochemical reactions occurring within cheese (Fox et al., 2000).

The results of moisture contents regarding cheddar cheese are close to the previous finding of Salwa et al. (2002) who reported decrease in moisture content during 90 days ripening period. The reduction in moisture content may be attributed due to loss of moisture from surface. Use of different combination of culture to cheese milk had non-significant effect on the protein contents of the manufactured cheese (Fenelon et al., 2002). The decrease in protein contents during ripening of cheddar cheese supported by studies of Murtaza et al. (2008) and Murtaza et al. 2014). Decrease in protein contents occur due to proteolysis during ripening. Proteolysis is caused by enzymes contained in milk (plasmin), rennet (pepsin and chymosin) and microbial enzymes released by starter cultures.

The activities of these enzymes hydrolyze the caseins (α_{s1}-, α_{s2}-, β- and k-casein), which leads to theformation of large and intermediate size peptides (Cliffe et al., 1993; Lynch et al.,1999; Ochi et al., 2013). The proteolytic enzymes produced by certain adjuncts were also found to degrade bitter peptides (Koka and Weimer 2000; Broadbent et al., 2003). There is a combined effect of fat, moisture, salt content and the storage on the proteolysis in cheddar cheese that expressed through the levels of non-protein nitrogen (Bertola et al., 1996).

Table 4. Effect of storage days on physicohemical parameters of Cheddar cheese

Days	Moisture %	Protein %	NPN%	Salt%
0	38.34±0.43a	28.31±0.83a	1.03±0.54c	1.44±0.04b
30	38.08±0.27b	27.70±0.62ab	1.54±0.34b	1.47±0.04ab
60	37.72±0.28c	27.08±0.40bc	1.99±0.28a	1.50±0.03ab
90	37.45±0.22d	26.41±0.72c	2.28±0.43a	1.56±0.02a

Where T0 is *Lc. Cremoris* and *Lc. lactis* (95:5)100% @0.0025% ; T_1 is *Lc. Cremoris* and *Lc. lactis* (95:5) 95% + *Str.thermophilus* and *Lb.bulgaricus* 5% ; T_2 is *Lc. Cremoris and Lc. lactis* (95:5) 90% + *Str. Thermophilus and Lb. bulgaricus*10%; T_3 is *Lc. Cremoris* and *Lc. lactis* (95:5) 85% + *Str. Thermophilus and Lb. bulgaricus* 15%.

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Table 5. Effect of treatments on hardness and fracture of cheddar cheese

Treatments	Hardness(mm/min)	Fracture
T_0	2466.1±729.40	69.29±2.35
T_1	2865.4±358.15	71.20±2.61
T_2	5021.8±992.14	71.16±2.28
T_3	3720.1±1122.16	71.26±2.23

Where T0 is *Lc. Cremoris* and *Lc. lactis* (95:5)100% @0.0025% ; T_1 is *Lc. Cremoris* and *Lc. lactis* (95:5) 95% + *Str.thermophilus* and *Lb.bulgaricus* 5% T_2 is *Lc. Cremoris and Lc. lactis* (95:5) 90% + *Str. Thermophilus and Lb. bulgaricus*10%; T_3 is *Lc. Cremoris Lc. lactis* (95:5) 85% + *Str. Thermophilus and Lb. bulgaricus* 15%.

During ripening of cheddar cheese, proteolytic activity of microorganisms *St. thermophillus* and *Lb. bulgaricus* caused a significant increase in non-casein nitrogen (NCN) and NPN. The lower molecular weight peptides and amino acids from the cheese are soluble in the 12% TCA solution. Salt is a major determinant of water activity and thereby exerts control over microbial growth, enzymes activity, biochemical changes during cheese ripening and the simultaneous development of desired flavor and aroma (Guinee, 2004). The concentration of volatile compounds enhanced due to the reduction in salt contents but it negatively effect sensory attributes. (Murtaza et al., 2014).

3.2. Texture analysis

The mean values of hardness for T_0, T_1, T_2 and T_3 are 2466.1±729.40, 2865.4±358.15, 5021.8±992.14 and 3720.1±1122.16 whereas for storage intervals values were 4359.2±722.07, 3097.1±523.01, 3668.1±68.30 and 2948.9±78.48 at 0 to 90 days. During 30, 60, and 90 days a progressive decrease in hardiness was observed in all cheese samples. The fracture ability differed non-significantly in treatment T0, T1, T2 and T3 as 69.29±2.35, 71.20±2.61, 71.16±2.28 and 71.26±2.23, respectively. Likewise, means for storage effect 72.44±1.54, 71.55±1.35, 69.98±0.78 and 68.96±1.05 (Table 5 and 6). In current results it was observed a decrease in the hardness and fracture ability of raw milk cheese during the end of ripening. This could be mainly due to the higher proteolysis in raw milk cheese than in pasteurized milk cheese.

Table 6. Effect of storage days on hardness and fracture of cheddar cheese

Days	Hardness(mm/min)	Fracture
0	4359.2±722.07	72.44±1.54
30	3097.1±523.01	71.55±1.35
60	3668.1±68.30	69.98±0.78
90	2948.9±78.48	68.96±1.05

Hardness has been reported to have a good correlation with proteolysis. It is reported that adjunct cultures improve the texture of cheddar cheese (Rehman et al., 2000). The hardness of cheddar cheese decreased during the ripening of cheese but proteolysis increased. (Murtaza et al., 2014) It was attributed that *Lb. bulgaricus* had slight effect on the texture of cheese (Drake et al., 1997).

3.3. Sensory evaluation

The primary concern of a sensory panel is to assess and quantify sensory characteristics of food products. Evaluation was conducted for various sensory attributes like flavor, aroma, texture, taste, and overall acceptability using the 9 point hedonic scale. Means for flavor scores indicated non-substantial variations from 5.75±0.8 to 7.30±0.7 in T_0 and T_3, respectively. Likewise, storage showed momentous decline in flavor scores from 5.52±0.4 at 0 day to 7.15±0.6 at 90th day. Statistical interpretation elucidated that taste was affected non-significantly by treatments and storage. The maximum taste scores were recorded in $T_3$7.62±0.9 followed by T_2 6.35±0.6, T_1 5.74±0.8 and T_0 5.75±0.7. The assigned scores for taste at 0 and 90th days were 5.90±0.2 and 6.71±0.4, respectively.

The aroma differed non-significantly in treatment T_0, T_1, T_2 and T_3 as 5.92±0.8, 6.56±0.6, 6.15±0.7 and 7.50±0.9, respectively. A significant declining trend was noticed for aroma, at the initiation, recorded scores were 6.32±0.03 that increased to 6.81±0.4 at the termination of study. Likewise, texture varied non-substantially among the T_0, T_1, T_2 and T_3 treatments as 6.50±0.7, 6.51±0.9, 6.70±0.8 and 7.51±0.6, in respective product. However during storage, scores for texture increased significantly from 5.50±0.3 to 8.25±0.5 at the initiation till the termination of trial.

Table 7. Effect of different concentrations of cultures on sensorial evaluation of cheddar cheese

Treatments	Flavor	Taste	Aroma	Texture	Overall acceptability
T_0	5.75±0.8	5.75±0.7	5.92±0.8	6.50±0.7	6.70±0.8
T_1	6.15±0.9	5.74±0.8	6.56±0.6	6.51±0.9	6.60±0.9
T_2	6.37±0.6	6.35±0.6	6.15±0.7	6.70±0.8	6.67±0.8
T_3	7.30±0.7	7.62±0.9	7.50±0.9	7.51±0.6	8.12±0.7

Where T0 is *Lc. Cremoris* and *Lc. lactis* (95:5)100% @0.0025% ; T_1 is *Lc. Cremoris* and *Lc. lactis* (95:5) 95% + *Str.thermophilus* and *Lb.bulgaricus* 5% ; T_2 is *Lc. Cremoris and Lc. lactis* (95:5) 90% + *Str. Thermophilus and Lb. bulgaricus*10%; T_3 is *Lc. Cremoris* and *Lc. lactis* (95:5) 85% + *Str. Thermophilus and Lb. bulgaricus* 15%.

Table 8.Effect of storage intervals on sensorial evaluation of cheddar cheese

Treatments	Flavor	Taste	Aroma	Texture	Overall acceptability
0	5.52±0.4	5.90±0.2	6.32±0.3	5.50±0.3	6.30±0.2
30	6.23±0.5	6.24±0.3	6.42±0.2	6.25±0.2	6.87±0.3
60	6.72±0.3	6.55±0.2	6.60±0.2	7.25±0.4	7.25±0.4
90	7.15±0.6	6.71±0.4	6.81±0.4	8.25±0.5	7.67±0.3

Where T0 is *Lc. Cremoris* and *Lc. lactis* (95:5)100% @0.0025% ; T_1 is *Lc. Cremoris* and *Lc. lactis* (95:5) 95% + *Str.thermophilus* and *Lb.bulgaricus* 5% T_2 is *Lc. Cremoris and Lc. lactis* (95:5) 90% + *Str. Thermophilus and Lb. bulgaricus*10%; T_3 is *Lc. Cremoris Lc. lactis* (95:5) 85% + *Str. Thermophilus and Lb. bulgaricus* 15%.

The observed scores for overall acceptability in T0, T_1, T_2 and T_3 were 6.70±0.8, 6.60±0.9, 6.67±0.8 and 8.12±0.7, respectively. Nevertheless, 90 days storage resulted a significant enhancement in overall acceptability scores from 6.30±0.2 to 7.67±0.3 (Table 7 and 8).

The current results are in line with the findings of Fox and Wallace (1997) supported that flavor development in cheese is the result of complex series of microbiological, biochemical and chemical processes that occur during ripening. The findings of Fox et al. (1996b) and McSweeney and Sousa (2000) also strengthens the proteolysis plays a major role in the development of flavor and texture in most rennet curd cheese varieties. Small peptides, amino acids and especially products of amino acid catabolism, e.g., amines and thiols contribute directly to cheese flavor.

The higher numerical values for taste suggest a contribution of culture in taste improvement of cheddar cheese because *Lactobacilli* have a potent proteolytic system (Kenny et al., 2003). Moreover cheeses prepared from raw milk have a higher rate of proteolysis than cheeses prepared from pasteurized milk (Lucey and Fox, 1993; Joshi et al., 2004). However proteolysis is the most important biochemical reaction involved in cheese manufacturing and ripening (Farkye, 2004; McSweeney, 2004; Ong et al., 2007).

Fox et al. (2000) determined that food aroma appreciation is of the first evaluation signals (along with food appearance and texture) encountered by consumers during eating of food. Furthermore, it is well known that this food characteristic strongly influences consumer's acceptability. Similarly, Hannon et al. (2007) observed those lactic acid bacteria (LAB) such as *Lb. bulgaricus* and its lysis enhanced flavor attributes such as creamy, caramel and sweet odor. During cheese manufacture a number of factors including: milk composition, milk quality, temperature, the rate and extent of acidification by the starter bacteria, the pH history of cheese, the concentration of Ca salts, extent and type of proteolysis and other ripening reactions influenced the physical structure and color. There have been numerous studies concerning the impact of these factors on composition, texture and functional attributes of cheese (Pastorino et al., 2003; Sheehan and Guinee, 2004).

It is reported that adjunct cultures improve the texture of cheddar cheese (Rehman et al., 2000). Calcium also plays an important role in cheese texture as Ca promoted protein to protein interactions, possibly through Ca bridging and charge neutralization serum was expelled from protein matrix and cheese became firmer. Accordingly in Cheddar cheese for any given pH there was tendency for cheese to become firmer as Ca content of cheese increased (Metzger et al., 2001).

The content of Ca affects the extent and degree of protein aggregation determining the basic structure and texture of cheese. The cheeses with a high concentration of Ca have a high level of protein aggregation, bigger protein aggregates and tend to be firmer and less melt able in comparison with cheeses with lower Ca content. These differences in protein aggregation determine the contrasting structure and texture of cheese such as cheddar (Guinee et al., 2002; Joshi et al., 2004).

4. Conclusion

The dairy industries are producing processed cheddar and mozzarella cheese for pizza preparation under local conditions. These locally produced cheese varieties have low standards and low quality and yet are unable to meet country's requirements. In this context, cheddar cheese was produced by using *Streptococcus thermophillus, Lactobacillus bulgaricus* and raw milk (10%). Likewise, physico-chemical analysis, texture analysis and sensorial evaluation of cheddar cheese and possible effects of starter culture combinations to the ripening period at different storage intervals were examined. pH and moisture decreased during ripening period while the acidity increased during storage. The ripening had significant effects on acidity and pH but highly significantly effect on texture of cheese. Physicochemical and sensory evaluation indicated that treatment prepared from 15% *Streptococcus thermophillus, Lactobacillus bulgaricus* and raw milk (10%) is the best treatment.

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