RELATIVE CHANGES IN pH AND ACIDITY DURING RIPENING OF CHEDDAR CHEESE, MANUFACTURED BY USING *LACTOBACILLUS RHAMNOSUS* AS AN ADJUNCT CULTURE

— research paper —

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Abstract: Cheddar cheese is produced by acidification and concentration of milk following gel formation with rennet, starter cultures promote acid development and in combination with adjunct cultures they confer distinct flavor and texture properties to cheese. *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis* are commonly used starter cultures for Cheddar cheese. *Lactobacillus rhamnosus* used as adjunct culture improves the flavour, imparts therapeutic properties, decreases bitterness and accelerates proteolysis of Cheddar cheese. For the present project, Cheddar cheese was manufactured from buffalo milk by using *Lactobacillus rhamnosus* in combination with starter cultures. Raw milk was tested for fat, total proteins, lactose, acidity, total solids, SNF and pH. After manufacturing, cheese was ripened at 6°C for a period of 90 days. During ripening cheese was evaluated for pH and acidity at the intervals of 0, 30, 60 and 90 days. Results obtained were statistically analyzed to assess the influence of the *Lactobacillus rhamnosus* on Cheddar cheese quality. On the basis of statistical evaluation, it was found that there is gradual decrease in pH and gradual increase in acidity during 90 day's ripening, while treatment T_2 was evaluated to be the best during storage by its texture and sensory characteristics, with *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. l*actis* (95:5) @ 1.5% + *Lb. Rhamnosus* @ 0.5% combination.

Keywords : Cheddar cheese, Physico-chemical analysis, , Storage study, Sensory Evaluation.

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INTRODUCTION

Cheese is a concentrated and coagulated form of milk solids, it is the fresh or matured solid or semisolid product obtained by coagulating milk, skimmed milk, partly skimmed milk, cream or any combination of these materials, through the action of rennet or other suitable coagulating agents, and by partially draining the whey resulting from such coagulation". Comparative nutrition value of cheese is same as milk considering proteins, vitamins and minerals. In addition, digestibility of proteins in ripened cheese increases due to the proteolytic activity during cheese ripening. Cheese is also a suitable nutrient for patients who have diabetes or lactose malabsorption, because of the low lactose ratio it contains (Demirci, 1990).

Cheddar is a hard ripened cheese, and is popular throughout the world due to its distinct flavour, taste and aroma. It was firstly manufactured in a town, Cheddar George (England) but now a days it is being manufactured in many parts of the world. Cheddar cheese is produced by acidification and concentration of milk following gel formation with rennet (Banks, 2002). It is a complex mixture, consisting of protein, fat, carbohydrates, vitamins and minerals. The chnges during ripening depend on the biochemical conditions of curd i.e., water activity, pH, oxidation reduction potential, mineral contents, ripening temperature, level and method of salt addition and nature of secondary microbiota (Law, 1999).

Cheddar cheese ripening is a very complex microbiological and biochemical process which involves the enzymatic digestion of the curd components (Spreer, 1998) (Choisy et al., 2000). The process of Cheddar cheese ripening involves the fermentation of lactose and the degradation of proteins and fats (Laleye et al., 1987) resulting in the decline of pH in cheese (Azarnia, 2006).

The lactic acid bacteria known as starters added in milk for cheese production initiate the acidification. The acidity prevents the growth of spoilage organisms, affects the activity of coagulant during manufacturing and ripening, solubilises the colloidal calcium phosphate, promotes synersis and influences the activity of enzymes during ripening. Thus, it affects the cheese texture and flavour quality (Amarita et al., 2001) (McSweeney and Fox, 2004).

Lactococcus lactis subsp. lactis and Lactococcus lactis subsp. cremoris are the main cultures used for Cheddar cheese in combination of 95-98% respectively (Teuber et al., 1991). Various strains of lactobacilli are used as adjuncts to improve flavour development and accelerate cheese ripening. These adjuncts influence proteolysis, resulting in the formation of high

concentration of free amino acid (FAA) and improved flavour as compared to the control cheese (Broome et al., 1990).
pH is a critical factor in several aspects of the manufacture and ripening of cheese curd, and affects cheese physiology (McSweeney and Sousa, 2000).
In this project *Lactobacillus rhamnosus* was used as an adjunct culture in Cheddar cheese and its impact on pH and acidity of Cheddar cheese is studied.

MATERIALS AND METHODS

All the research work was conducted in the Dairy and Food Analysis Laboratories, National Institute of Food Science and Technology, University of Agriculture, Faisalabad. The details of the materials used and analytical methods employed during the study are given below.

Raw materials

Buffalo milk of a specific breed was obtained from farm house, Department of Live Stock Management, University of Agriculture, Faisalabad. Commercially available Cheddar cheese cultures and rennet was purchased from scientific stores in Faisalabad.

Milk analysis

The raw milk after collecting from farm house was analyzed for its fat content (Marshall, 1992), total protein, pH value, lactose content, total solids, solid not fat and titratable acidity, according to the standard methods of AOAC (2000).

Cheese manufacturing

Cheese was manufactured by following the protocol as described by (Scott, 1981).

Standardization

For Cheddar cheese milk was passed through the cream separator then standardized at 3.5% fat by mixing the cream and skim milk then mixing it again according to the standard.

Homogenization and pasteurization

After standardization of milk, it was homogenized with a homogenizer and pasteurized at controlled temperature of 65^0C for 30 minutes in water bath. Milk was shifted into processing vat and cooled at to 31^0C.

Addition of additives

The milk was divided into five portions. To each portion, additives were added as given in the Table 1.

Table 1. Experimental design describing the addition of microbial cultures and rennet

Treatments	Cultures	Rennet
T_o	*Lactococcus lactis ssp. cremoris* and *Lactococcus lactis ssp. lactis* (95:5) @ 2.0%	0.006%
T_1	*Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis* (95:5) @ 1.75% + *Lactobacillus rhamnosus* @ 0.25%	0.006%
T_2	*Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. l*actis* (95:5) @ 1.5% + *Lactobacillus rhamnosus* @ 0.5%	0.006%
T_3	*Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. l*actis* (95:5) @ 1.25% + *Lactobacillus rhamnosus* @ 0.75%	0.006%
T_4	*Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. l*actis* (95:5) @ 1% + *Lactobacillus rhamnosus* @ 1%	0.006%

Curd formation

The milk was placed undisturbed for curd formation for 30-45 minutes at 31^0C. The coagulum formation was checked after every 15 min. for this purpose a stem of thermometer was plunged below the surface layer and lifted the coagulum causing it to break in a cleavage line. A clear cleavage with green whey separation at the base of cleft indicated that the curd was ready to be cut.

Cutting

The coagulum was cut into 1/4 inch cubes by stainless steel knife. A smaller cube size will yield the cheese with lower moisture whereas a large cube size will result in a high moisture cheese. The cubes were allowed to set again for 10-15 minutes.

Scalding and stirring

After cutting the coagulum was scalded (cooked) at 39-40^0C for first 15 minutes, then the curd was stirred for 45-50 minutes. The scalding promotes

synersis and whey expulsion from the curd, while stirring aids the uniform heat distribution throughout the curd particles.

Whey drainage
After scalding, whey was drained off from the curd. Firm curd body was washed with hot water at 80^0C and second cooking was carried out at 32^0C for 10-15 minutes to settle down the firm curd and the whey separated during heating was removed continuously from the curd.

Texturing/ Cheddaring
Having removed the whey, the curd was divided into blocks and pilled up every 15-20 min. Cheddaring a procedure which involves two basic steps, stretching and in attaining curd particles coalesce, drainage of whey continues and finally the curd attain the characteristic texture. Traditional Cheddaring process involves manually pilling and turning the curd.

Milling
The curd was milled into finger sized pieces, cooled to 25^0C to enclose fat particles before salting.

Salting
Salting was done @ 1% by sprinkling the salt on the curd pieces and mixed well. After salting curd pieces were placed for 5 minutes.

Moulding and pressing
After salting firm curd was scooped out and placed in cheese mould and then during pressing, a pressure of 25 Psi was applied for 2-3 hours (the pressure was increased gradually) to give curd the final shape, firm surface and correct final moisture content.

Storage/ripening
Following moulding and pressing, cheese obtained was coated with food grade wax, wrapped in clean aluminum foil and stored for ripening at temperatures 6 ± 2 ^{0}C for a period of 3-months.

RESULTS AND DISCUSSIONS

pH of cheese

The effect of storage on pH of Cheddar cheese is given in Table 2 and the analysis of variance for pH in Cheddar cheese in Table 3.

Table 2. Effect of ripening on pH in Cheddar cheese

Ripening days/ Treatments	1	30	60	90	Means
T_0	5.58	5.54	5.53	5.5	5.5375e
T_1	5.6	5.57	5.55	5.54	5.565b
T_2	5.63	5.6	5.59	5.57	5.5975a
T_3	5.6	5.57	5.54	5.52	5.5575c
T_4	5.57	5.55	5.54	5.51	5.5425d
Means	5.596a	5.566b	5.55c	5.528d	

Table 3. Analysis of variance for pH in Cheddar cheese

SOV	df	SS	MS	F-value
Ripening days (S)	3	0.0357	0.0119	356.98NS
Treatments (T)	4	0.03204	0.00801	240.32NS
S X T	12	0.00161	0.0001342	4.03NS
Error	40	0.00133	0.0000333	
Total	59	0.0708		

**: Highly significant; *: Significant; ^{NS}: Non-Significant

The pH values of T_0, T_1, T_2, T_3 and T_4 were 5.58, 5.6, 5.63, 5.6 and 5.57 respectively in one day old cheese. There was a steady decline in pH of Cheddar cheese during the 90 days of ripening. Lactic acid is primarily responsible for decrease in pH. The highest decrease (0.08) in pH was recorded in T_0 and T_3 while the lowest (0.06) was in sample T_1, T_2 and T_4 during 90 days of aging.

Statistical analysis showed non significant effect of storage, treatments and their interaction on pH of cheese during ripening.

During Cheddar cheese manufacture, starter bacteria ferment lactose to lactic acid. In the case of Cheddar-type cheeses, most of the lactic acid is produced in the vat before salting and molding. Even after losing about 98% of the total milk lactose in the whey as lactose or lactate, the cheese curd still

contains 0.8 to 1.5% lactose at the end of manufacture. The pH decreases after salting, presumably due to the action of starter (Fox et al., 1990) (Singh et al., 2003).
Higher decline in pH of T_4 indicates that the adjuncts metabolite lactose and produce lactic acid. A similar result was obtained by (Johnson et al., 1994).

Acidity of cheese

Cheese is a fermented dairy product and hence the controlled production of lactic acid from lactose by lactic acid bacteria is an essential step during the manufacturing and ripening. Results pertaining to acidity of Cheddar cheese are presented in Table 4 and the analysis of variance for acidity in Cheddar cheese in Table 5.

Table 4. Effect of ripening on acidity (%) in Cheddar cheese

Ripening days/ Treatments	1	30	60	90	Means
T_0	0.84	0.86	0.88	0.885	0.86625b
T_1	0.855	0.87	0.89	0.91	0.88125b
T_2	0.825	0.85	0.88	0.91	0.86625b
T_3	0.848	0.87	0.9	0.92	0.8845b
T_4	0.87	0.9	0.915	0.92	0.90125a
Means	0.8476d	0.87c	0.893b	0.909a	

Table 5. Analysis of variance for acidity in Cheddar cheese

SOV	df	SS	MS	F-value
Ripening days (S)	3	0.03418	0.01139	27.80NS
Treatments (T)	4	0.008735	0.002184	5.33NS
S X T	12	0.00407	0.000339	0.83**
Error	40	0.0164	0.000410	
Total	59	0.06337		

** Highly significant; * Significant; NS Non-Significant

The mean values of acidity for T_0, T_1, T_2, T_3 and T_4 are 0.866%, 0.881%, 0.866%, 0.884% and 0.901% respectively. During 90 days of ripening, there was a gradual increase in acidity of all Cheddar cheese sample. The highest acidity (0.901%) was observed in Cheddar cheese sample T_4 at 90 days of ripening and least acidity (0.86%) was noted in Cheddar cheese sample T_0

prepared at one days of ripening. After 30, 60 and 90 days of ripening a gradual increase was observed in acidity contents of Cheddar cheese.
Statistical analysis (Table 4.16) showed non significant effect of storage and treatments on the acidity of Cheddar cheese, but their interaction indicates highly significant effect on acidity.
The value of acidity found in the experiment is in accordance with (Vernam and Sutherland, 1994), who reported the mean acidity of Cheddar cheese as 0.9-1.0%. 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) (Amarita et al., 2006). A critical factor in the control of Cheddar cheese quality is consistency in the rate and extent of acid production by the starter cultures (Banks, 2002).
During ripening, microbiological and biochemical changes like lipolysis, proteolysis and conversion of residual lactose to lactate and citrate occur. Residual lactose is metabolized rapidly to lactate during the early stages of ripening. Lactate is an important precursor for a series of reactions including racemization, oxidation or microbial metabolism that lead to the production of acidity and result in flavouring compounds (McSweeney, 2004) (Ong et al., 2007).

CONCLUSIONS

The mean acidity of cheddar cheese is 0.9-1.0%. The evaluation of Cheddar cheese revealed that interaction of storage and treatments has highly significant effect on acidity, As the highest acidity (0.901%) was observed in Cheddar cheese sample T_4 at 90 days of ripening and least acidity (0.86%) was noted in Cheddar cheese sample T_0 prepared at one days of ripening, indicates the gradual increase in the conversion of lactose into lactic acid as a result of increasing the concentration of adjunct *Lb. Rhamnosus*, which controls the spoilage microorganism. There was a steady decline in pH of Cheddar cheese during the 90 days of ripening due to incresed Lactic acid concentration that is responsible for decrease in pH.
The highest decrease (0.08) in pH was recorded in T_0 and T_3 while the lowest (0.06) was in sample T_1, T_2 and T_4 during 90 days of aging. There was a steady and constant pH decrease, while acidity increase in T_2. On the basis of overall texture sensory evaluation, it was found that treatment T_2 , *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. l*actis* (95:5) @ 1.5% + *Lb. Rhamnosus* @ 0.5% combination seems to be the best and can be

suggested as an ideal combination for Cheddar cheese production having best texture and maximum control over spoilage microbes growth.

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