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Biochemical and microbiological changes during ripening of cheddar cheese from buffalo milk supplemented with goat milk and microencapsulated enzymes

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Effect of addition of goat milk (GM) and microencapsulated enzymes (ME) on biochemical and microbiological changes in buffalo milk cheddar cheese (BMCC) during ripening period of 8 months was investigated. Addition of GM had definite improvement in glycolysis (1.37-1.39% LA), proteolysis (8.9-11.2% soluble protein) and lipolysis (16.0-43.2 µ.eq FFA/g) of cheddar cheese as compared to the respective values of 1.20% LA, 8.3% soluble protein, and 12.2 µ.eq FFA/g of control cheese, while ME had positive impact on proteolysis (10.8% soluble protein) and lipolysis (36.8 u.eq FFA/g). The proteolysis could be advanced by 2 months through addition of GM or ME while lipolysis could be advanced by 2-4 months. Addition of both GM and ME had synergistic effect on the proteolysis and lipolysis during ripening. GM had definite stimulatory effect on the growth of starter culture (9.0 log cfu/g) and lactobacilli (6.6-6.8 log cfu/g) in BMCC. The samples containing ME showed higher total viable count (8.9 log cfu/g) and lactobacilli count (6.9 log cfu/g) throughout the ripening period. Yeast and mold count and spore count were unaffected by addition of GM or ME or both and did not follow any distinct growth pattern during ripening. Coliform count, on the other hand, decreased with the progress of storage in all the samples. Depending on the availability, addition of GM or ME or both is recommended to accelerate the biochemical and microbiological changes during ripening of BMCC.

Keywords: Cheddar cheese, Buffalo milk, Goat milk, Microencapsulated enzymes, Quality

Cheese ripening is a slow process, involving a concerted series of microbiological, biochemical and chemical reactions. Cheddar cheese requires prolonged ripening period at controlled temperature and humidity involving glycolysis, proteolysis and lipolysis for attaining its characteristic flavour (Fox et al 1996, Singh et al 2003). Therefore, ripening process represents a significant proportion of cost in cheese production. Particularly when cheddar cheese is made from buffalo milk, this problem is further aggravated because of its low moisture retention, resulting into inherent resistance to biochemical changes and microbial growth necessitating the prolonged ripening periods. As a result of extensive research work, the technology of cheddar cheese manufacture from buffalo milk has been partially modified resulting in fairly satisfactory product (Jha and Singh 1993, Kanawjia and Singh 1993, Singh et al 1993, Upadhyay 1996). The glycolysis and proteolysis have been improved considerably, but still the sharpness of typical cheddar cheese flavour is missing, probably due to inadequate lipolysis. Accelerating the biochemical and microbiological processes, therefore, may reduce the cost of cheese ripening.

Goat milk (GM) or GM fat fractions improved the lipolysis in BMCC (Arora and Rai 2000, Rao and Singh 2001), while additions of exogenous enzymes improved all the biochemical changes in BMCC (Kanawjia et al 1992, Rao and Singh 2001, Kheadr et al 2002, Azarnia et al 2006). The microbial ecology undergoes changes with progress of ripening which in turn brings about biochemical changes. Literature on the status of microbial changes during ripening of buffalo milk supplemented with GM and/or enzymes is scanty. Therefore, an attempt was made to study the effect of synergistic action of GM and ME on the acceleration of biochemical and microbial changes during ripening of BMCC.

Materials and methods

Buffalo milk (BM) was obtained from the herd of 'Murrah' buffaloes and GM from a flock of 'Beetal' and 'Alpine' crosses maintained at NDRI, Karnal. BM was supplemented with GM at 25% level.

LF-40 *(Lactococcus lactis* sub sp. *cremoris, L. lactis* sub sp. *lactis, L. lactis* sub sp. *diacetylactis, Leuconostoc mesenteroides* sub sp. *cremoris)* and *Lb. casei*-300 *(Lactobacillus casei)* were procured from Dairy Bacteriology Division of the Institute and added @ 0.5% along with LF-40 to cheese milk for cheddar cheese making. Meito rennet produced from *Mucor pusillus* var 'Lindt' was supplied by Meito Sangyo and Co Ltd, Japan, in granular form. Protease (Type II) produced from *Aspergillus oryzae* and lipase (Type VIII) produced from *Candida cylindracae* procured from M/s Sigma Chemical Co., USA, were used at the rate of 0.01 and 0.001%, respectively on curd basis as suggested by Kanawjia and Singh (1990). Enzymes to be added were encapsulated with milk fat by the method described by Magee and Olson (1981) and added to cheese milk before addition of coagulating enzyme.

Preparation of cheddar cheese: The cheddar cheese was prepared from mixture of BM and GM using the method described by Burde and Srinivasan (1967) with modifications suggested by Rao and Singh (2001). The modifications included i) standardization of milk to higher casein to fat ratio, 0.75; ii) addition of less starter culture, 1.5%; iii) lower setting time, 30-35 min and iv) higher cooking time, 50-60 min. Cheddar cheese was ripened at 8±1°C and analyzed for biochemical and microbiological changes during ripening kept for 8 months.

Biochemical analysis: Titratable acidity (AOAC 1990), soluble protein content of cheddar cheese (Kosikowski 1982) and lipolytic changes in terms of free fatty

acids (FFA) content by extraction titration method (Deeth and Fitz-Gerald 1976) were determined.

Microbiological analysis: Different microbiological ready-made media obtained from Hi-Media, Bombay were used for microbiological analysis. Plate count agar was used as nutrient medium to determine the total viable count (TVC) and spore count (SC) in cheddar cheese. Lactobacilli agar, Potato dextrose agar and Violet red bile agar were used for the enumeration of Lactobacilli, yeast and mold (YMC) and coliform (CC) counts, respectively, in cheese. The cheese samples were prepared for microbiological examination and enumerated according to ICMSF (1996).

Statistical analysis: Experimental data from 3 replications on biochemical and microbiological changes during ripening of cheddar cheese ripened for 8 months were analyzed statistically by ANOVA method (Snedecor and Cochran 1984).

Results and discussion

Results are presented in Table 1.

Glycolytic changes: GM supplemented cheese had higher acidity than the control throughout the ripening ($p<0.01$). This is due to the increased moisture retention and the stimulatory effect of GM. Higher titratable acidity was observed in fermented products obtained from GM as compared to those made from cow milk or BM (Abrahamsen et al 1978, Manjunath et al 1983). Addition of ME could not improve the acidity of BMCC significantly during ripening. However, addition of both GM and ME had positive and significant impact on the development of acidity in cheese ($p<0.05$).

Regardless of addition of GM or ME or both, the titratable acidity increased at a faster rate towards the end of 2 months followed by a consistent increase until 6 months and then leveled off. Addition of GM is the most simple and effective approach to improve glycolysis in BMCC. Cheese made with an excessive acidity or faster rate of acid production reported to exhibit faster breakdown of its constituents because depletion of minerals from curd allows easier access to the caseins for the proteolytic enzymes. It also helps to regulate biological agents responsible for the flavour development in cheddar cheese (Fox et al 1990). Lactic acid, the product of glycolysis serves as a background for more distinctive flavour compounds typical for the cheddar cheese (Singh et al 2003).

Table 1. Effect of addition of goat milk (GM) and microencapsulated enzymes (ME) on the biochemical and microbiological quality of buffalo milk Cheddar cheese during ripening at 8 ± 1^0C

Treatment	Ripening period, months				
	0	2	4	6	8
	Glycolytic changes (titratable acidity, %LA)				
Control	0.60	1.07	1.12	1.18	1.20
GM	0.65	1.12	1.26	1.35	1.37
ME	0.61	1.09	1.15	1.20	1.21
GM + ME	0.65	1.16	1.27	1.38	1.39
	Proteolytic changes (soluble protein), %				
Control	1.3	3.9	6.7	7.3	8.3
GM	1.4	4.2	7.1	8.4	8.9
ME	1.4	4.5	7.5	9.3	10.8
GM+ ME	1.5	4.8	8.4	10.0	11.2
	Lipolytic changes (FFA), µ.eq/g				
Control	1.1	5.4	8.6	10.8	12.2
GM	1.2	7.0	10.8	14.0	16.0
ME	1.5	12.6	22.5	30.0	36.8
GM + ME	1.6	14.1	24.6	36.0	43.2
	Total viable count, log cfu/g				
Control	7.6	8.5	8.7	7.0	6.8
GM	7.8	9.0	8.1	6.9	6.8
ME	7.8	8.9	8.4	7.0	6.9
GM + ME	7.8	9.0	8.0	6.9	6.8
	***Lactobacillus* count, log cfu/g**				
Control	4.9	5.3	6.5	6.6	6.6
GM	4.6	5.8	6.6	6.5	6.6
ME	4.8	5.6	6.9	6.9	6.9
GM + ME	5.0	5.7	6.7	6.8	6.7
	Yeast and mold count, log cfu/g				
Control	2.3	2.5	2.5	2.4	2.5
GM	2.5	2.6	2.4	2.5	2.6
ME	2.2	2.5	2.4	2.3	2.3
GM+ ME	2.3	2.4	2.5	2.4	2.6
	Spore count, log cfu/g				
Control	2.0	1.9	2.1	1.8	2.0
GM	2.1	2.0	2.0	2.0	2.1
ME	1.9	2.1	2.0	1.9	2.0
GM + ME	1.9	1.8	1.9	1.9	1.8
	Coliform count, log cfu/g				
Control	0.84	0.48	0.48	NT	NT
GM	0.84	NT	NT	NT	NT
ME	0.78	0.30	0.30	NT	NT
GM + ME	0.70	NT	0.60	NT	NT

n =3, NT=not traceable

Proteolytic changes: The soluble protein content of fresh cheeses ranged between 1.3 and 1.5%, the higher values being noted in cheese supplemented with GM and ME. In all the samples, the extent of proteolysis increased with the increasing ripening period. The rate and extent of proteolysis, in general, was considerably higher in cheese supplemented with GM and ME than in cheeses containing either GM or ME ($p<0.01$). The same trend continued throughout the ripening period. The proteolysis increased sharply upto 4 months and then tended to rise gradually reaching the maximum values of 8.9, 10.8 and 11.2% in cheeses with GM, ME, GM and ME, respectively.

Addition of GM or ME advanced the proteolysis by 2 months, while addition of both GM and ME by 4 months in BMCC. Kheadr et al (2000) reported that encap-

sulated bacterial proteinases could be successfully used to accelerate proteolysis for attaining acceptable flavour within 4 months in cheddar cheese.

Lipolytic changes: Initial FFA values ranged between 1.1 and 1.6 µ.eq/g, the higher value being in cheese supplemented with GM and ME. In all the cheeses, FFA content increased with progress of ripening period. The rate and extent of lipolysis were higher in cheese containing both GM amd ME than those of cheeses incorporated either only GM or ME. The trend continued throughout the ripening period. The maximum FFA content in control cheese was 12.2 µ.eq/g while the cheese containing GM, ME, GM and ME showed 16, 36.8 and 43.2 µ.eq/g, respectively at the end of ripening. The higher moisture retention in cheese supplemented with GM could be one of the factors responsible for the enhanced lipolytic activity. Smaller sized fat globules in GM could be another factor contributing to the increased surface area of fat. GM was also reported to be sensitive to the spontaneous lipolysis because of different lipase distribution (Juarez and Ramos 1986). Our observations of enhanced lipolysis in cheese supplemented with ME are similar to the reports of Kheadr et al (2002). Addition of GM or ME advanced the lipolysis by 2 months and 4 months, respectively, while addition of both GM and ME by 6 months in BMCC.

Changes in total viable count (*TVC*): The initial TVC of fresh cheese was 7.6-7.8 log cfu/g, which increased to the maximum (8.5-9.0 log cfu/g) in all the cheeses towards the end of 2 months of storage followed by a consistent decrease till the end of ripening (6.8-6.9 log cfu/g). It is due to the fact that the starter culture, which largely contributes to the TVC, multiplies at faster rate during the early stages of ripening. However, as the ripening progresses, starter flora number declines autolyzing themselves in the changing environment in cheese (Mansson 1987). Lactic acid bacterial counts increased during the 1st month of ripening and then decreased gradually towards the end of ripening in Emmental cheese from BM, cow milk and their mixtures (Ladkani and Srinivasan 1990). The TVC of GM cheeses gradually decreased during aging in refrigerated storage (Park et al 2004).

Addition of GM has significantly increased the TVC in BMCC throughout the ripening. Cheese containing GM had about 3 times higher bacterial count as against the control cheese (9.0 and 8.5 log cfu/g). It indicated that the GM has definite positive stimulatory effect on the growth of starter culture in cheese. It can be further substantiated by the results of titratable acidity in GM cheeses, which is higher than in control cheese during the ripening. Abrahamsen et al (1978) and Manjunath et al (1983) also reported high levels of biological activities in GM. They attributed it to the smaller peptides, phospholipids and higher non-protein nitrogen contents of GM. Supplementation with ME also improved the TVC in BMCC. Casein hydrolysis products might have stimulated the growth of culture in enzyme treated cheese.

Changes in Lactobacillus count: Lactobacillus count was generally higher in experimental cheeses as compared to control cheese during ripening. In particular, it was significantly higher during 2-4 months of ripening. *Lactobacillus* count remained at higher levels even towards the end of ripening.

It is interesting to note that the final counts of lactobacilli were almost 70% of TVC recorded in cheeses at the end of ripening. It shows that as soon as the growth of starter bacteria is impeded, lactobacilli have become prominent flora in cheese especially after 4 months in cheddar cheese. Gardiner et al (1998) reported that non-starter lactic acid bacteria principally composed of lactobacilli, which proliferate as cheese ripens and sustain high viability in cheddar cheese during ripening. *Lactobacillus* species seemed to be resistant to the varying conditions of pH, moisture and salt content in cheese during ripening. Our observations are similar to the results of earliar workers, who studied the microbial changes in cheddar cheese during ripening (Dave et al 1983, Upadhyay et al 1985, Drake et al 1996).

Changes in yeast and mold count: The initial count of yeasts and molds in cheese varied between 2.2 and 2.5 log cfu/g. The population of yeasts and molds, in general, increased slightly during ripening. However, counts did not show any definite growth pattern in cheeses during ripening. Degree of contamination during manufacture and storage must have affected the number of yeasts and molds in cheese rather than the supplementation of either GM or ME. Our observations are in contrast to the reports of Freitas et al (1995), who noted an increase in the counts of yeasts and molds during the initial stage of ripening, followed by significant decrease towards 130 days in GM cheeses. Park et al (2004) also noted a gradual decrease in their number during storage in GM cheeses.

Changes in spore count (SC): The initial SC in cheeses ranged between 1.9 and 2.1 log cfu/g. The SC unlike TVC and *Lactobacillus* counts did not follow any growth pattern during storage. GM supplementation, enzyme addition and storage periods did not have any bearing on the number of spores in cheese. The variations in the SC can be attributed to the initial number of spores and degree of contamination during manufacture. Our observation is in contrast with the finding of Salwa and Galal (2002), who reported a gradual increase in SC during storage in cheese from buffalo milk.

Changes in coliform count (CC): The initial number of coliforms in cheese ranged from 0.78 to 0.84 log cfu/g. During storage, the CC in cheeses varied between 0.30 and 0.60 log cfu/g. Addition of GM or ME showed no influence on the number of coliforms. All the samples were negative for coliforms, especially during 6-8 months of ripening. The results are in agreement with the observations on BM and GM cheeses by Salwa and Galal (2002) and Park et al (2004), respectively.

Conclusion

Addition of GM to BM had definite improvement in glycolysis, proteolysis and lipolysis of cheddar cheese. The proteolysis was advanced by 2 months through addition of GM or ME while lipolysis by 2-4 months. Addition of both GM and ME had synergistic effect on the glycolysis, proteolysis and lipolysis during ripening. GM had definite positive stimulatory effect on the growth of starter culture and lactobacilli in BMCC. The samples containing ME showed higher TVC and

lactobacilli count throughout the ripening period. The population of yeasts and molds increased slightly during ripening. The spore count did not follow any growth pattern during storage, while the number of coliforms decreased with the progress of storage in all the cheese samples. Addition of GM or ME or both is recommended to eliminate inherent resistance of BM to glycolysis, proteolysis and lipolysis in cheddar cheese. GM or/and ME showed definite positive stimulatory effect on accelerating the biochemical and microbiological processes in BMCC.

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