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Incidence of Lactobacilli in Buffalo Milk Cheddar Cheese

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ABSTRACT

The incidence of Lactobacilli in buffalo milk cheddar cheese was studied. The counts of Lactobacilli varied from 3×10^6 to 50×10^6 /g of cheese. The number of Lactobacilli was highest during the first and the fourth months of ripening in batch I and II respectively; subsequently started to decline. In a total of 105 isolates *L. casei*, *L. plantarum*, *L. leichmanii*, *L. buchneri*, *L. fermenti*, *L. brevis* and *L. cellobiosus* were 58, 37, 1, 5, 1, 1 and 2 respectively. Proteolytic abilities varied significantly with regard to strains, types of milk and the period of incubation. *L. buchneri–8* exhibited highest proteolysis in cow milk and *L. casei–3* in buffalo milk. The pH of the cheeses varied between 5.2 to 5.6.

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INTRODUCTION

Lactobacilli, the most dominant group of bacteria in mature cheddar cheese, when occurring naturally in cheese would be supposed to be able to better survive cheese as an environment and selected strains might grow profusely in cheese if used as an additive. Such strains, if possess desirable charcteristics, can be successfully employed to hasten the ripening process in cheese, Johns and Cole (1959), Feagan and Dawson (1959), Lane and Hammer (1935), Sherwood (1939) and Jagannath *et al.* (1973).

The present study was planned to examine the buffalo milk Cheddar cheese samples for the number and types of Lactobacilli present in it and examine their proteolytic ability in cow and buffalo milk, which would serve as the basis for selecting more proteolytic strains as starter additives to hasten the rate of ripening in the cheddar cheese made from buffalo milk probably with some enhancement in organoleptic score.

Published data on the types of Lactobacilli occurring in buffalo milk cheddar cheese and their characteristics are not available.

MATERIALS AND METHODS

Collection, handling and treatment of cheese samples:

Fourteen buffalo milk cheddar cheese samples of varying age were collected from two batches of cheese manufactured at the Amul Dairy, Anand. The samples were immediately analysed using the procedure described in the Standard Methods for the Examination of Dairy Products (1960).

Counting, isolation and identification of lactobacilli:

The counting was done using the medium of Mann, Rogosa and Sharpe (MRS) (1960).

A few colonies from each plate were randomly picked up and replated on the

MRS Agar for purification and then transferred into MRS Agar stabs incubated at 30° C for 5 days and then stored at –8° C till further use for identification.

All the isolates were identified to species level using the scheme of Sharpe *et al.* (1968).

Judging proteolytic ability of the selected isolates:

From among the isolates two strains of each species were examined for proteolytic abilities in cow and buffalo skim milk at 14° C for a period of seventyfive days. Sixteen hour old milk cultures of the isolates were inoculated into autoclaved skim milk at the rate of 1% (v/v). Proteolysis was judged at 15 days interval using the method of Hull (1947). Colour intensity was measured on Bausch and Lomb Spectronic-20 Spectrophotometer at 650 nm.

The pH of the cheese was measured by the method of Franklin and Sharpe (1963).

Statistical analysis:

The statistical analysis was done by the methods of Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The data presented in the above table indicate that the pH of cheese in both the batches ranged between 5.2 to 5.6 during the ripening period of seven months, as normally observed in cow milk cheese.

The count of Lactobacilli were quite high in numbers, almost comparable to the other two counts viz. of total viable bacteria and the Enterococci which ranged between 7.3×10^6 to 1.2×10^8/g and 1×10^4 to $8.8. \times 10^5$/g respectively. In the cheese from batch I, they were highest in numbers i.e. 50×10^6/g at one month and then gradually declined till seventh month. The high number present could probably be ascribed to the higher rate of contamination with these bacteria during the manufacturing process of cheese, especially from vats, handlers and other sources on the plant. Batch II had little lower count in the beginning, then increasing upto fourth month and subsequently showing a decline probably due to both, accumulation of toxic metabolites and depletion of nutrients.

TABLE 1

Lactobacillus count of Buffalo Milk Cheese at *Different Stages of Ripening.* (incubated at 30° C for 5 days).

Age of cheese	*pH of cheese*		*Lactobacillus Count/g (x 10^6)*	
Months	*Batch I*	*Batch II*	*Batch I*	*Batch II*
1	5.2	5.2	50	5
2	5.4	5.4	35	28
3	5.4	5.5	18	27
4	5.5	5.5	23	29
5	5.5	5.5	3	12
6	5.5	5.5	3	6
7	5.6	5.6	3	4

Table 2 contains the data on identification of the Lactobacilli. The isolates were identified to species level using the scheme of Sharpe *et al.* (1968). The results observed indicated that ability to produce NH_3 from arginine varied with the strains. Homo—as well as Heterofermentative strains showed this ability. Sharpe (1962) accepted some exceptions in using this test as a substitute for the type of lactic fermentation. About 50% of the L. plantarum strains failed to ferment arabinose.

TABLE 2

Identification of lactobacillus isolates from buffalo milk cheddar cheese.

Number of isolates out of 105	*Growth at*			*Fermentation of*						*NH_3 from*	*Asculin Hydro-*	*Lactic*	*Species*
	0.4%	*15°C*	*45°C*	*Treha-lose*	*Sucrose*	*Xylose*	*Meli-biose*	*Lactose*	*Arabi-nose*	*Argi-nine*	*lysis*		
58	—	+	—(36)	—(47)	—(19)	—(57)	—	—(36)	—(56)	—(54)	—(1)	Hm.	*Lactobacillus casei.*
			+ (22)	+(11)	+(39)	+(1)		+(22)	+(2)	+(4)	+(57)		
37	—(21)	+	—(20)	—(17)	+	+	+	—(2)	—(18)	—(26)	+	Hm.	*Lactobacillus plantarum*
	+ (16)		+ (17)	+(20)				+(35)	+(19)	+(11)	+		
5	—(1)	+	+	—	—(2)	—	+	—(1)	+	+	+	Ht.	*Lactobacillus buchnari*
	+ (5)				+(3)	—	+	+(4)					
1	—	—	+	+	+	+	—	+	—	—	+	Hm.	*Lactobacillus leichmanii*
1	+	—	+	—	+	—	—	+	—	+	—	Ht.	*Lactobacillus fermanti*
1	+	+	—	—	+	+	—	+	+	+	+	Ht.	*Lactobacillus brevis.*
2	+	+	—	+	+	+	+	+	—	+	+	Ht.	*Lactobacillus cellobiosus*

Figures in the parentheses show number of isolates.
Hm = Homofermentative. Ht = Heterofermentative.

The results exhibited in Table 2 indicated that *L. casei* and *L. plantarum* were the most dominant species encountered. Fiftyeight *L. casei* and thirtyseven *L. plantarum* made a total of 95 out of 105 total isolates. *L. leichmanii*, *L. buchneri*, *L. fermenti*, *L. brevis* and *L. cellobiosus* were 1, 5, 1, 1 and 2 respectively.

Table 3 covers the data on proteolytic abilities of selected strains from among the isolates. *L. buchneri*-8 exhibited highest proteolytic ability in cow milk and *L. casei*-3 in buffalo milk.

TABLE 3

Proteolytic activity of selected Lactobacillus Isolates at 14°C.

Organism	*Type of Milk*	*Time of incubation (days)*				
		15	*30*	*45*	*60*	*75*
		(mg of tyrosine/5 ml TCA filtrate)				
L.casei–1*	Cow	0.66	0.9	1.1	1.6	2.0
	buffalo	0.64	0.8	1.0	1.8	1.8
L.casei–3	cow	0.23	0.5	0.7	1.1	1.5
	buffalo	0.18	0.4	0.8	2.1	2.4
L.plantarum–4	cow	0.24	0.61	0.72	1.2	1.9
	buffalo	0.2	0.3	0.6	0.8	1.2
L.plantarum–5	cow	0.15	0.25	0.42	0.82	1.3
	buffalo	0.16	0.35	0.63	0.9	1.5
L.buchneri–8	cow	0.32	0.72	0.93	1.4	2.4
	buffalo	0.26	0.50	0.7	0.9	1.6
L.buchneri–29	cow	0.45	0.89	1.2	1.6	1.8
	buffalo	0.51	0.9	1.3	1.8	2.1
L. fermenti–57	cow	0.7	1.1	1.5	1.8	2.6
	buffalo	0.5	0.7	0.9	1.3	1.6
L.leichmanii–46	cow	0.02	0.04	0.04	0.08	0.1
	buffalo	0.01	0.02	0.06	0.06	0.08
L.cellobiosus–96	cow	0.31	0.41	0.62	0.87	1.2
	buffalo	0.4	0.6	0.82	0.93	1.5
L.brevis–81	cow	0.2	0.4	0.42	0.5	0.8
	buffalo	0.3	0.3	0.5	0.58	0.9

*Number suffixed to the strain of bacteria represents isolate number.

There was a vide variation in the proteolytic abilities of different species and of different strains within a species. The release of tyrosine increased progressively with the time of incubation in all cases. The type of milk i.e. cow or buffao also had effect on this activity.

The results presented in Table-4 showed that the strains of Lactobacilli significantly (5% level) differed in their average proteolytic activity. *L.casei*-1, *L. buchneri*-29 and *L.fermenti*-57 topped the list for their proteolytic activity. However, in view of the heterofermen.

Table 4

Effect of types of milk on average proteolytic activity of different strains of Lactobacilli.

Strains	*mg of tyrosine/5ml TCA filtrate*		*Mean*
	Cow milk	*Buffalo milk*	
L. casei–1	1.25	1.20	1.23
L. casei–3	0.80	1.17	0.98
L. plantarum–4	0.93	0.62	0.78
L. plantarum–5	0.58	0.70	0.64
L. buchneri–8	1.15	0.79	0.97
L. buchneri–29	1.18	1.32	1.25
L. fermenti–57	1.54	1.00	1.27
L. leichmanii–46	0.05	0.04	0.04
L. cellobiosus–96	0.68	0.85	0.77
L. brevis–81	0.46	0.51	0.48
Mean	00.862	0.820	0.841

Note: The milk samples were incubated at 14° C for 75 days.

S.E. Strain–0.0559 C.D. 0.1604

S.E. Milk x Strains–0.0790 C.D. 0.227

C.V.% = 20.91%

tative nature of the latter two strains it is suggested that *L.casei*-1 has the potentiality to be used as a proteolytic starter additive in cheddar cheese-making. Further, the said strain behaved almost uniformly in both the types of milk used.

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