

Incidence of Enterococci In Buffalo Milk Cheddar Cheese

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ABSTRACT

The incidence of enterococci in buffalo milk cheddar cheese was studied. The counts of enterococci varied from 1×10^4 to 88×10^4 /g of cheese. The number of enterococci increased upto second month of ripening and then constantly decreased upto the end of seventh month. In all 44 isolates were examined and among them twenty-nine were *Str. faecium*, *Str. liquefaciens*, *Str. faecalis* and *Str. durans* were two each, while *Str. bovis* and *Str. equinus* comprised eight and one respectively. *Str. faecium* was the most dominant species. The proteolytic activities of enterococci varied widely both in cow and buffalo skim milks at 14°C. The proteolysis continued to increase till 90 days with only exception of strain CH-253.

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INTRODUCTION

In the present study an attempt has been made to examine the number and types of enterococci present in buffalo milk cheese and to test their proteolytic activities in cow and buffalo milk. These characteristics may serve as an index in selecting more proteolytic strains as starter additives to hasten the ripening process in the buffalo milk cheese with probability of enhancement in flavour score.

MATERIALS AND METHODS

Collection, Handling and Treatment of Cheese samples :

Fourteen buffalo milk cheddar cheese samples were collected from two batches of cheese, manufactured at the Amul Dairy, Anand. The samples were im-

mediately analysed using the procedure described in Standard methods for the examination of dairy products (1960).

Isolation and Identification of Enterococci :

The counting was done using the method of IDF (1967).

A few colonies from each plate were randomly picked up and transferred into chalk-litmus milk incubated at 37°C for 48 hr. and then stored at -8°C till further study for identification.

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

Proteolytic Ability of the Selected Isolates:

From among the above isolates two strains of each species were examined for proteolytic abilities in cow and buffalo skim milk at 14°C for a period of three

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months. 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 Spectronic-20 Spectrophotometer at 650 nm.

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

RESULTS AND DISCUSSION

TABLE 1

Enterococcal count of buffalo milk cheese at different stages of ripening (incubated at 44°C for 48 hr.).

Age of cheese (Months)	pH of cheese		Enterococcal count ($\times 10^4$)/g	
	Batch I	Batch II	Batch I	Batch II
1	5.2	5.2	12	8.6
2	5.4	5.4	88	53
3	5.4	5.5	7	38.3
4	5.5	5.5	7	9.7
5	5.5	5.5	4	7
6	5.5	5.5	3	6.1
7	5.6	5.6	1	3.8

The data presented in 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 counts of enterococci showed an initial increase upto second month of ripening but a decline was noticed till the end of maturation period. The maximum counts recorded were 88×10^4 and 53×10^4 per gram in batch I and II respectively at the second month of maturation. The high incidence of enterococci in young cheddar cheese made from cow milk, has also been recorded by Mattick and Shattock (1943), Hanay and Newland (1950) and Franklin and Sharpe (1962). The decrease in the

number of enterococci after two months of ripening could be ascribed to (a) depletion in the quantity of fermentable energy source, (b) competition by other bacteria growing in the cheese and (c) over all change in the physico-chemical conditions in the product.

Table-2 contains the results on identification of enterococcal isolates.

The isolates were identified to species level using the scheme of Sharpe *et al* (1968). The results observed were quite consistent with the said scheme, except that isolate No. 5 did not ferment lactose.

The results documented in Table 2 indicated that *Str. faecium* was most dominant species encountered. They comprised 29 in a total of 44 isolates. The

rest were *Str. liquefaciens*, *Str. faecalis*, *Str. durans*, *Str. bovis* and *Str. equinus*; numbering 2, 2, 2, 8 and 1 respectively.

Two strains from each species were selected to study their proteolytic activity in cow and buffalo milk at 14°C for a period of 90 days. Strain CH₂₅₃ was included to get comparative information. The results in respect of proteolytic activity are recorded in Table 3. It may be observed that proteolytic activity of different enterococcal isolates varied appreciably with the species. *Str. liquefaciens*-8 exhibited the highest proteolysis in both cow and buffalo milk, while the lowest proteolysis was shown by CH₂₅₃. Proteolytic ability also varied widely among different strains of the

same species and with the type of milk used as substrate. The data also revealed that the increase in solubilized tyrosine continued till the end of incubation period except in case of starter (CH₂₅₃) where it decreased slightly at one stage.

These findings indicate the need for further studies to select a right strain as a starter component for cheese preparation.

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TABLE 2

Identification of enterococcal isolates from buffalo milk cheese.

Number of isolates out of 44	Growth at			Fermentation of Sugars				Beta type	Ammonia from Arginine	Gelatin liquefaction	Tetra zolium reduction	Species
	10°C	45°C	50°C	pH 9.6	Lac-tose	Arabi-nose	Meli-biose					
29	+	+	+	+	+	+	+	-	+	-	-	<i>Streptococcus facium</i>
2	+	+	-	+	+	-	-	-	+	-	-	<i>Streptococcus liquefaciens</i>
2	+	+	-	+	+	-	-	-	+	-	+	<i>Streptococcus faecalis</i>
2	+	+	-	+	+	-	-	-	+	-	-	<i>Streptococcus durans</i>
8	-	+	-	-	+	+	+	-	-	-	+	<i>Streptococcus bovis</i>
1	-	+	-	-	-	-	-	-	-	-	-	<i>Streptococcus equinus</i>

*1 isolate did not ferment lactose.

TABLE 3

Proteolytic activity of selected enterococcal isolates at 14°C.

Organism	Type of Milk	Period of incubation (days)					
		15	30	45	60	75	90
		(mg of tyrosine/5 ml TCA filtrate)					
<i>Str. faecium-4*</i>	cow	0.15	0.31	0.52	0.63	1.20	1.20
	buffalo	0.018	0.02	0.04	0.12	0.30	0.50
<i>Str. faecium-28</i>	cow	0.16	0.23	0.45	0.6	0.65	0.8
	buffalo	0.15	0.2	0.47	0.6	0.72	0.85
<i>Str. durans-25</i>	cow	0.29	0.6	0.75	0.9	0.96	1.3
	buffalo	0.15	0.3	0.5	0.55	0.7	0.9
<i>Str. durans-26</i>	cow	0.33	0.59	0.8	0.9	1.2	1.5
	buffalo	0.1	0.25	0.46	0.7	0.8	0.85
<i>Str. faecalis-13</i>	cow	0.1	0.3	0.6	0.7	0.9	1.2
	buffalo	0.06	0.08	0.14	0.44	0.6	0.8
<i>Str. faecalis-15</i>	cow	0.03	0.06	0.09	0.12	0.3	0.6
	buffalo	0.05	0.07	0.1	0.2	0.5	0.6
<i>Str. liquefaciens-8</i>	cow	0.72	1.02	1.21	1.6	2.0	2.8
	buffalo	0.84	1.2	1.6	2.1	2.8	3.2
<i>Str. liquefaciens-11</i>	cow	0.19	0.36	0.62	0.8	1.1	1.4
	buffalo	0.03	0.05	0.09	0.23	0.62	0.8
<i>Str. bovis-32</i>	cow	0.3	0.5	0.8	1.2	1.4	1.6
	buffalo	0.06	0.08	0.2	0.6	0.7	0.8
<i>Str. bovis-34</i>	cow	0.04	0.06	0.08	0.14	0.4	0.6
	buffalo	0.05	0.08	0.12	0.32	0.5	0.7
CH ₂₅₃	cow	0.035	0.06	0.05	0.07	0.07	0.1
	buffalo	0.05	0.08	0.06	0.08	0.09	0.14

Number suffixed to the strain of bacteria represents isolate number.

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