Effect Of Different Starter Culture Combination and Ripening Period on Free Fatty Acid Profile and Physicochemical Characteristics of Buffalo Cream

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Abstract— Cream ripening is a common technique used to enhance the flavour of dairy products like ghee and butter. The generation of flavour is influenced by a number of variables, including the kind and quantity of strains employed, the substrate found in the media, and the incubation temperature and duration. In this current work, Lactococcus lactis subsp lactis, Lactococcus lactis subsp diacetyllactis and Leuconostoc mesenteroides subsp dextranicum were used in five different combinations and incubated at the same temperature for 24 hours and 48 hours, respectively. The titratable acidity increased during ripening, and the increase was higher in samples added with citrate. The cream sample with Lactococcus lactis subsp lactis and Leuconostoc mesenteroides subsp dextranicum with and without citrate addition obtained the highest score for flavour during sensory evaluation. The incubation period had no significant impact on the flavour score. Diacetyl production by starters was observed at 6 hours of incubation, and maximums were observed at 18 hours of incubation. The free fatty acid profile of treatment E (Lactococcus. lactis subsp. lactis and Leuconostoc mesenteroides subsp.dextranicum (1:1) with citrate (1%)) by GC MS/MS showed an increase in free fatty acid content with increase in incubation period.

***Index Terms*—Cream, Ripening, Diacetyl, Fatty acid profile.**

1. Introduction

Cream, a fat rich product is a base ingredient in many sweets and cuisines as well as an intermediary product for products like butter and ghee. Ripened cream is formed by fermentation of cream by added lactic acid bacteria.it is mainly used as an intermediated product for ghee and butter. Many studies suggest that ripening has positive effect on butter and ghee flavour (Yadav and Srinivasan ,1984), (Pandya and Sharma ,2002). According to Ayad et.al., (2002) and Van Kranenburg et al. (2002). Lactic acid bacteria affect product's sensory quality, primarily through the production of flavor compounds during the fermentation process. The major ways that lactic acid bacteria contribute to the flavour of cultured milks are through the conversion of lactose to lactic acid and the creation of diacetyl and acetaldehyde (Seitz (1990), Smit (2004), Urbach, (1995)). Most bacteria produce diacetyl and acetoin from carbohydrate via pyruvate, but lactic acid bacteria do not produce them from carbohydrate unless an additional source of pyruvate is present. This is due to the necessity of converting all of the pyruvate generated from the carbohydrate to lactate in homofermentative organisms. The production of "surplus" pyruvate can be accomplished by citrate metabolism (Marshall, V. M. 1987). According to Hugenholtz (1993) diacetyl and acetaldehyde have highly distinct fragrance qualities and considerably improve the flavour of fermented products. Flavour production depends on several factors like strains used ,substrate present in media and environmental conditions such as temperature of incubation and time of incubation. In the present study different starter culture combinations were selected for the ripening of cream. Sensory evaluation methods were employed to select the starter culture combinations with the highest flavour scores at different incubation periods. The titratable acidity and total lactic bacteria count of cream samples were analysed before sensory evaluation to ensure that starter cultures were active. Cream samples with high sensory scores were further studied for pH, diacetyl production, and free fatty acid profile at different incubation periods.

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2. Materials and Methods

Freshly separated buffalo cream was purchased from Dairy Plant, Kerala Veterinary Animal Sciences University, Mannuthy, Thrissur. The quality of the cream was assessed by measuring the titratable acidity using the standard procedures outlined in the BIS (1966). Fat content of cream was standardized to 70 per cent fat using skim milk. The standardized 70 per cent cream was subjected to house hold pasteurisation at 80o C for 15 second (Tetra Pack Handbook) and cooled to room temperature.

A. Ripening of cream samples

As the first step of ripening lyophilised culture of Lactococcus lactis subsp lactis, Lactococcus lactis subsp diacetylactis and Leuconostoc mesenteroides subsp dextranicum procured from NCDC, Karnal. Throughout the entire study, this lyophilized culture that had been activated and propagated in skim milk was employed.

Pasteurized cream was divided into six lots of which one was kept as control. Five culture combinations which were added at a rate of two percentages are mentioned below. Citrate was also added at 0.1 percent level to two lots.

Treatment A: Lactococcus lactis subsp. diacetylactis

Treatment B: Lactococcus lactis subsp. lactis + Lactococcus lactis subsp. diacetylactis

Treatment C: Lactococcus lactis subsp. lactis + Lactococcus lactis subsp. diacetylactis + Citrate @ 0.1 per cent

Treatment D: Lactococcus. lactis subsp. lactis + Leuconostoc mesenteroides subsp .dextranicum

Treatment.E:Lactococcus. lactis subsp. lactis + Leuconostoc mesenteroides subsp.dextranicum+ Citrate @ 0.1per cent

Treatment F: Cream without culture (Control)

Each treatment was further divided into two based on the ripening period given. Six lots were kept for ripening at 25oC for 24 hours while the duplicates of six samples were kept at same temperature for 48 hours

B. Analysis of ripened cream

Ripened cream were analysed for acidity and microbial load to ensure the quality.Titratable acidity of ripened cream was determined according to BIS (1966). After the preparation of suitable decimal dilutions, the total lactic acid bacterial count (log cfu/ml) was enumerated by plating on M17 agar (AOAC, 1980).

C. Sensory Evaluation

Sensory evaluation of the ripened cream samples was done by a trained panel of 5 judges with the help of an ISI score card, in which flavour was given the highest score. The sample with the highest score was analysed for pH, titratable acidity, and diacetyl production at different incubation periods.

pH, Titratable Acidity and Qualitative analysis of Diacetyl production.

The pH of optimised samples was analysed using a calibrated pH metre. Titratable acidity was determined using standard procedures outlined in the BIS (1966). Diacetyl, one of the main flavour compounds in fermented milk products, was analysed by the creatine test. In this, 2 ml of the cream sample and 2 ml of the 40% w/v NaOH solution were combined in a clean tube. A pinch of powdered creatine monohydrate has been mixed with it. For 10 minutes, the tube was kept in the dark. The appearance of a pink colour band indicated the formation of diacetyl flavour (Ivanova et al., 2012).

D. Fatty Acid profile

To analyse the variation in free fatty acid profile of cream samples at 24- and 48-hours incubation were analysed using GC-MS/MS.

3. Results and Discussion

A. Development of acidity on ripening of cream

Fresh buffalo cream had an average acidity of 0.042 ± 0.02% LA. As ripening periods prolonged, acidity increased in all samples, with less acidity in the control sample (Table 1). Citrate-added samples C and E show higher titratable acidity than corresponding samples B and D, respectively, without citrate. This result was correlated with Garcia-Quintans (2008) and Goupry (2000); according to them, co-fermentation of citrate and lactose in milk by Lactococcus bacteria results in the accumulation of lactic acid as the main end product and several minor products that are important for the flavour and textural qualities of the milk. There is roughly equal utilisation of glucose and citrate for the buildup of lactic acid, diacetyl, and acetoin. The level of increase in titratable acidity in cream samples C and E was more or less similar after 48 hours of incubation. This may be due to the hindering effect of developed acidity.

B. Lactic acid bacteria count

Lactococcus lactis subsp. diacetyl lactis is a slow grower and the result was reflected in Sample A, which contains only Lactococcus lactis subsp. diacetyl lactis. Sample A showed the lowest count compared to other ripened cream samples except the control sample (Table 2). Samples D and E both contains Lactococcus. lactis subsp. lactis and Leuconostoc mesenteroides subsp. dextranicumbut different only in citrate content in sample E have almost the same bacterial count, indicating that citrate has no effect on bacterial growth. Samples C and E, which contain added citrate, have a reduced bacterial load after 48 hours of incubation; this may be due to the hindering effect of developed.

C. Sensory Evaluation

The Lactococcus lactis subsp. lactis and Leuconostoc mesenteroides subsp. dextranicum was selected as the best starter combinations for flavour production. Samples D and E secured the highest flavour score in both 24 hours and 48 hours of incubation (Tables 3 and Table 4). Incubation period had no impact on flavour score in this incubation range. No significant

difference was observed in the flavour score of samples D and E inoculated with Lactococcus lactis subsp. lactis and Leuconostoc mesenteroides subsp. dextranicum with and without citrate. Citrate at 0.1 percent is not sufficient to make a noticeable change, or the diacetyl produced may be converted into the flavourless compound ,3-dutanediol. Control cream samples showed a lower flavour score compared to other cream samples. It indicates that ripening increases the flavour score of cream.

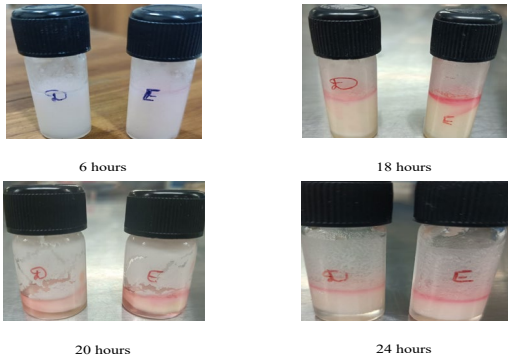

Figure :1 Qualitative analysis of Diacetyl *production*

D. Diacetyl Production

Both Sample D and E showed a positive test for diacetyl production (Figure: 1). Diacetyl production starts in sample E at 6 hours of incubation when pH reduced to 4.65 and titrable acidity reaches 0.068 percent LA (Table 5). Maximum diacetyl production was observed in samples D and E at 18 hours of incubation when pH dropped to 4.6 for sample D and 4.5 for sample E and acidity reached 2.02 and 2.81 percent LA, respectively. The intensity of the pink band decreased later on.

E. Free fatty acid profiling

The fatty acid profile indicates that Sample D is independent of the incubation period. Its concentration remains more or less similar after 48 hours of incubation. By comparing the fatty acid profile of sample D at 24 hours of incubation with that of sample E, the concentration was much higher in sample D

The dominant saturated fatty acids detected in ripened cream samples were palmatic acid, myristic acid, and stearic acid. Unsaturated fatty acids predominant in the ripened cream sample were oleic acid. In sample E, all the fatty acid concentrations increased as the incubation time increased. A major rise was observed in oleic acid (6 times), followed by palmitic (5 times), myristic (4.8 times), and stearic (4 times).

4. Conclusion

Lactococcus. Lactis subsp. lactis and Leuconostoc mesenteroides subsp. dextranicum were selected as the best starter combination. Samples D and E obtained the highest sensory score in both incubation periods, indicating that the incubation period did not make any noticeable changes in the flavour profile. The cream samples D and E, without and with citrate at the 0.1 per cent level, don't show a significant difference in flavour. Indicate citrate at this level doesn't make an impact on the flavour profile of cream samples. Maximum diacetyl production was observed qualitatively at 18 hours of incubation.

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Table 1
Titratable acidity of cream samples

Sample	**Acidity % LA**	
Fresh cream	0.042 ±0.02	
Ripened cream	**24 hours**	**48 hours**
A	0.25±0.03	0.41±0.09
B	0.27±0.04	0.31±0.06
C	0.32±0.03	0.35±0.04
D	0.26±0.04	0.32±0.05
E	0.31±0.02	0.35±0.05

Data represents mean ± standard error of four replicates

Table 2
Total Lactic acid bacteria Count

Samples	**Viable count Log cfu/ml**	
	24 h	**48 h**
A	5.31 ±0.02	6.9 ±0.03
B	7.62 ±0.01	7.9 ±0.01
C	7.61 ±0.02	7.1 ±0.02
D	9.5 ±0.02	9.8 ±0.01
E	9.6 ±0.01	9.6 ±0.02

Data represents mean ± standard error of four replicates.

Table: 3
Sensory score of cream sample after 24 hours of incubation

Sample	Flavour	Texture	Colour	Freedom From Impurities	Package
A	44.75±1.60	17.50±0.87	9.75±0.25	14.5±0.5	5±0
B	45.38±1.28	16.75±1.44	9.75±0.25	14.5±0.5	5±0
C	45.75±0.48	16.5±1.66	9.75±0.25	14.5±0.5	5±0
D	46.25±1.25	17.50±0.87	9.75±0.25	14.5±0.5	5±0
E	46.25±0.63	18.50±0.87	9.75±0.25	14.5±0.5	5±0
F	42.75±1.93	16.50±1.66	9.75±0.25	14.5±0.5	5±0
Chi square	2.98 ns	1.64 ns	0 ns	0 ns	0 ns

Data represents mean ± standard error of four replicates, ns –non significant(p>0.05)

Table 4
sensory score of cream sample after 48 hours of incubation

Sample	**Flavour**	**Texture**	**Colour**	**Freedom From Impurities**	**Package**
A	43.75± 1.65	19.50± 0.29	10±0	15±0	5±0
B	44.75± 0.95	19.25±0.48	10±0.	15±0	5±0
C	45.25 ±1.25	19.50 ±0.50	10±0	15±0	5±0
D	46.00±1.04	20.00±0.00	10±0.	15±0	5±0
E	45.75±1.14	19.5±0.5	10±0	15±0	5±0
F	41.75±3.19	19.5±0.5	10±0.	15±0	5±0
Chi square	2.70 ns	2.53 ns	0 ns	0 ns	0 ns

Data represents mean ± standard error of four replicates, ns –non significant(p>0.05)

Table 5
Free fatty acid analysis

Free fatty acid (ug/ml)	**Sample D**		**Sample E**	
	24 hours	**48 hours**	**24 hours**	**48 hours**
Caprylic_acid_	39.895	37.197	23.839	42.280
Capric_acid_	72.295	67.049	28.050	81.192
Undecylic_acid_	16.071	14.867	15.671	15.160
Lauric_acid_	144.629	142.297	77.075	172.911
Myristic_acid_	422.116	427.964	110.214	530.890
Myristoleic_acid_	44.193	80.772	22.622	100.519
Palmitic_acid_	1126.160	1108.518	266.852	1367.965
Margaric_acid_	30.371	27.881	14.560	32.776
Stearic_acid_	414.664	362.042	109.890	441.142
Oleic_acid_	864.127	825.361	162.491	1021.191
Linoleic_acid_	48.779	41.822	21.413	54.573
Gamma_Linolenic	NF	NF	NF	28.406
Linolenic_acid_	27.281	27.192	17.668	26.631
Arachidic_acid_	22.487	25.343	20.664	25.514
Behenic_acid_	23.533	23.943	22.502	27.686