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"Manshanu" Production: Microbiological and Biochemical

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ABSTRACT

The microbiological and biochemical changes during the ripening of the milk for Manshanu (local fermented butter) production were assessed. The biochemical changes were evaluated in terms of titratable acidity, pH and diacetyl content. The flavor changes in the butter in terms of free fatty acids during the storage were also studied. The use of pure starter culture was found to improve the keeping quality of the butter compared to the butter produced by chance fermentation. The free fatty acids contents of the butter from the pure culture remained at its initial level of 0.168%, oleic acid for 16 days of frozen storage. There was an increase from 0.168% to 0.273% oleic acid after 16 days of storage under refrigeration. With the traditionally prepared butter the free fatty acids increased from 0.168% to 0.643% during 16 days of storage at refrigeration temperature. The microorganisms involved in the ripening of milk for manshanu production were isolated, identified, purified on nutrient agar slants and used as pure starter culture for the butter production. Streptococci lactis, Leuconostoccremoris and Lactobacillus lactis were isolated and identified. Lactobacillus lactiswas isolated only twice in the total of ten trials. A mixed starter culture of the dorminant organisms S. lactis and L. cremeris, when used as pure culture resulted in the butter production with better flavor and keeping quality.

Keywords: *manshanu, starter culture, ripening, diacetyl.*

INTRODUCTION

Fermented foods are foods of which their production is based on the activities of micro-organisms, foods are fermented to make for variety, increase in desired flavor and quality, increase in nutrient level and to prolong shelf life of products. Examples of such foods include cheese, butter and yoghurt; as well as 'manshanu.' Manshanu is a traditionally fermented milk product (butter) consumed mostly in the Northern part of Nigeria. The microbiological activities during the ripening of the milk for manshanu production result into 'manshanu' with different flavor and quality characteristics.

MATERIALS AND METHODS

Isolation of Pure Culture

This was done following the poured plate technique of Harrigan and Mc Cance (1976); nutrient Agar and blood agar as culture media, and incubated at 35^{O}C for 24 hours. After counting, representative colonies were picked, streaked and incubated at 35^{O}C to obtain pure colonies. The morphologies of the cells were observed under the microscope. The various cultures were inoculated on nutrient agar slopes and incubated at 35^{O}C for 24hours.

Preparation of Starter Culture

Pasteurized milk (10ml) was inoculated with isolated microorganisms from the pure culture. This was incubated at 45^{O}C for 4hours. The 10ml culture was used to seed 200 ml pasteurized milk in a conical flask and incubated at 45^{0}C for 4hours. The 200ml culture was used as starter in the laboratory preparation of manshanu.

Preparation for Butter (Manshanu)

Fresh milk from farm was pasteurized at 70^{0}C for 10 minutes cooled to 45^{0}C and inoculated with the starter culture. The entire mixture was then incubated at 45^{0}C for 8hours. The fermented milk was cooled and churned (45 minutes) to separate the butterfat from the butter milk. The separated butter fat was washed with distilled water to remove excess butter milk. The fat was worked to remove excess water and to homogenize the constituents. The 'manshanu' was stored in the freezer apartment of the refrigerator for further analysis.

Determination of Biochemical Roles of the Isolated Micro Organisms

Three batches of 500ml of pasteurized milk were inoculated with pure culture isolates individually and with a mixed culture of the isolates respectively. These samples were then incubated under refrigeration and at 45^{O}C for 24 hours. At time intervals samples were withdrawn asceptically and tested for pH, titratable acidity and diacetyl. pH determination was done using the pH meter (Kent Eil 7020), Titratable acidity was determined by Pearson (1978) method using 1% phenolphalein as indicator, acidity was expressed as % oleic acid. Diacetyl was estimated by Spectrophotometric method (Walsh and Cogan, 1974).

Results and Discussion

Table1. *Titratable Acidity (% lactic acid) in butter samples*

Samples	% Lactic Acid
A	0.030 ± 0.02
B	0.458 ±0.054
C	0.499±0.016
D	0.320 ±0.0

Table2. *The lactic bacteria associated with manshanu production*

Organism	Isolation medium	Number of Isolations
Streptococcus lactis	Nutrient agar/blood agar	+ (10)
Leuconostoc Cremoris	Nutrient agar/blood agar	+ (10)
Lactobacillus Lactis	Nutrient agar/blood agar	+ (2)

+ = Isolated; (- -) = Figures in brackets indicate number of isolations made out of 10 streaking.

Table3. *Total variable bacterial count during fermentation*

Time (hrs)	Number of Organism/ml
0	8 x10^{4}
2	2.1x10^{5}
4	1.38x10^{6}
6	3.3x10^{7}
8	3.3x10^{7}
12	6.2x10^{7}
24	5.2x10^{8}

Growth medium- Nutrient agar/Blood agar

Inoculum- Mixed culture of S. lactis and L. cremeris

Table4. *Diacetyl (ppm) production by strains of lactic acid bacteria during manshanu production*

Isolated organism	Hours							
	0	4	8	12	16	18	20	24
Streptococcus lactis	1.8	2.4	4.8	4.8	ND	ND	ND	4.6
Leuconostoccremoris	1.8	5.9	18.6	20.2	ND	ND	ND	16.8
Mixed culture of Strep lactis and Leuconostoccremoris	1.8	6.2	20.4	24.2	ND	ND	ND	16.2

Table5. *Effect of pH on the diacetyl content during ripening of milk with mixed culture of S. lactis and L.cremoris*

Time (hr)	pH	Diacetyl (ppm)
0	6.8	1.8
4	6.2	5.5
8	4.5	20.4
12	4.2	24.2
16	4.0	18.4
20	-	-
24	4.0	16.2

Table6. *Levels of Diacetyl inmanshanu samples*

Samples	Diacetyl content (ppm)
A	8.2±2.4
B	6.5±1.8
C	3.1±1.2
D	12.0±2.6

A= sample from Konduga; B= sample from Railway market; C= sample from Monday market; D= Sample prepared in the lab using traditional process

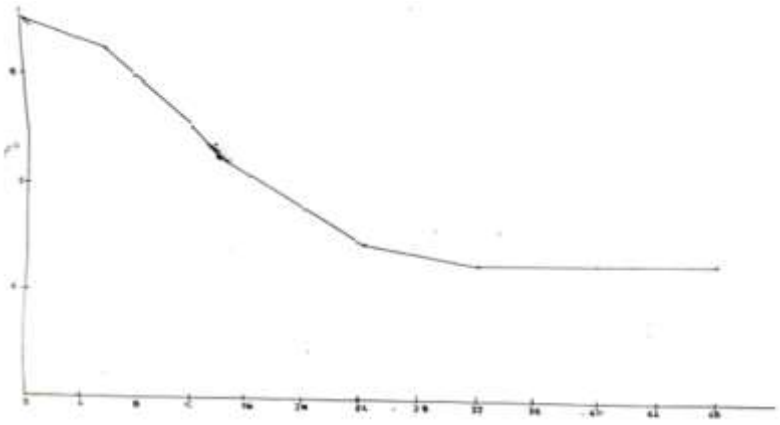

Figure1. *pH changes during traditional processing of butter*

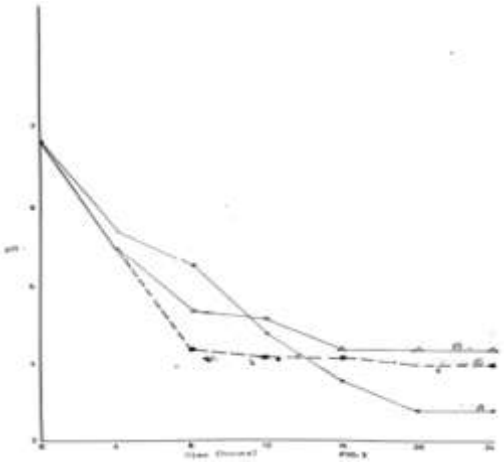

Figure2. *P^H changes during concentration of milk at 45^0C*

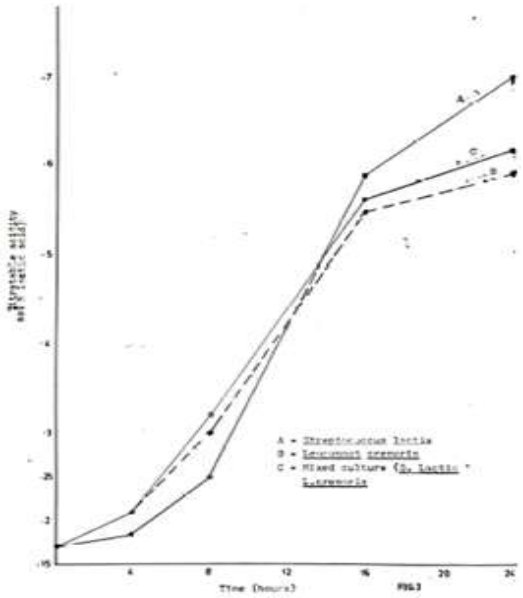

Figure3. *Changes in Titratable Acidity (% Lactic acid) during fermentation of milk at 45^oC*

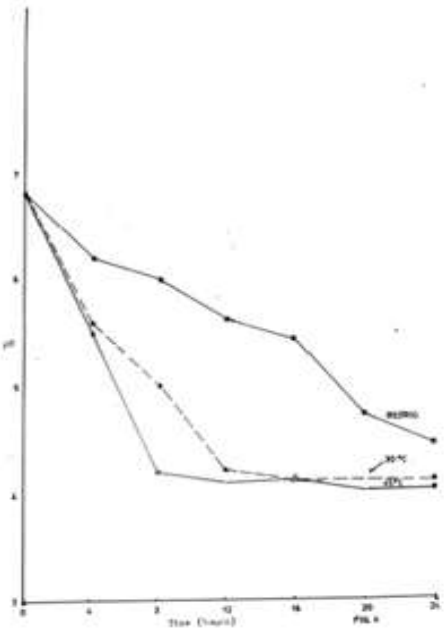

Figure4.

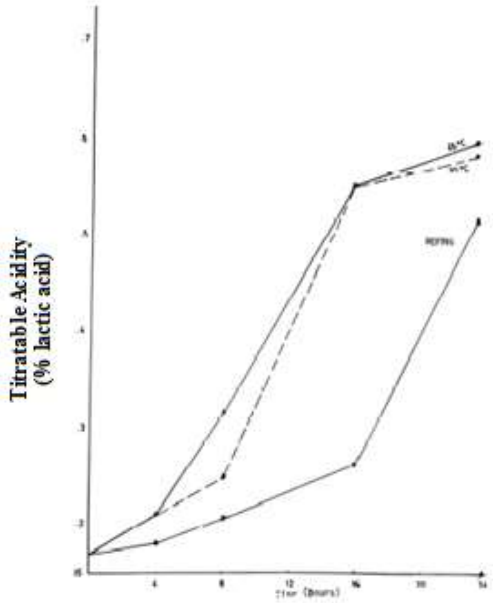

Figure5. *Effect of temperature of incubation on titratable acidity of the fermenting mash*

Microbiological And Biochemical Changes During Butter Production

During the traditional processing of "manshanu", the pH decreased to 4.2 after 32 hours of incubation, thereafter becoming stable (Fig.1) under the laboratory condition, but simulating the traditional technique and incubating at 45^OC, the pH value of 4.2 was attained after eight hours. (Fig.2)

The microorganisms associated with the ripening of milk during "manshanu" preparation were isolated and identified as *Streptococcus lactis* and *Leuconostoccremoris*. Ten isolations were obtained for each of these organisms. The third lactic acid bacteria, *Lactobacillus lactis* was isolated two times out of a total of ten experiments (Table 2). It was therefore concluded that naturally occurring *Streptococcus lactis* and *Leuconostoccremoris* dominated the fermentation process in "manshanu" production. The result of their growth was pleasant and flavorful butter. Various Lactic acid bacteria have been reported to be associated with the production of fermented milk products such as yoghurt, cheese and cultured butter. These include species of the genera streptococcus, for example; *S. lactis, S. cremoris, S. diacetyl lactis, S. thermophilus;* those of the genera *lactobactillus*: examples; *S. bulgaricus, L. casei, and Leuconostoccitrovorum, Leuconostoccremoris,* (Rose, 1981).

The isolated organisms were used separately and their biochemical roles with respect to pH, tritratable acidity and diacetyl production during the fermentation process were established.

Figures 2 and 3 shows changes in pH and tritratable acidity due to the various strains of the lactic acid bacteria. *Streptococcus lactis* produced the highest amount of acid during the 24 hours of incubation which depressed the pH to 3.4 at the end of fermentation. Tritratable acidity expressed as 1% Lactic acid) increased from 0.18% to 0.7%. Acid production by *Leuconostoccremoris* was at a faster rate during the first eight hours but by the end of the 24 hours of incubation, the pH had dropped to 4.6 which was higher than that produced by the *Streptococcuslactis.* Tritratable acidity increased from an initial value of 0.18% to 0.58%. Acid production during "manshanu" production was attributed to the growth of *Streptococcus lactis.* Acid production was also found to be optimum at 45ºC as against 30ºC and at refrigeration temperature (Figures 4 & 5).

Chemical compounds formed in milk by lactis acid culture increased the tritratable acidity and decreased the pH. During the first few hours of fermentation the amount of lactis acid formed was rather small because the microorganisms added were not in an active phase of growth and the number present was small. Increase in bacteria population causes increase in rate of acid development. The acid present in the latter part of fermentation, restricted the growth of lactis acid producing bacteria and acid was produced more slowly. Thus, more lactose was present then can be completely fermented, there was a significant increase ($p<0.05$) in total microbial count during the first 6 hours of fermentation (Table 3). The count increased from 10^4 to $10^{7.}$

The flavour of "manshanu" which was determined by the amount of diacetyl produced during fermentation was attributed to the activity of the *Leuconostoc* species since it produced the highest amount, 20.2ppmdiacetyl within 12 hours of incubation as against 4.4ppm produced by *Streptococcus lactis* (Table 4). Fresh milk contain about 0.15-0.20% citric acid as citrates which is fermented by the lactis acid culture to diacetyl, volatile acids and carbon dioxide. These by-products affect flavour and odor of milk. The citric acid content of milk is fermented completely during the fermentation period. Acetoin, diacetyl and volatile acids accumulate in large amounts after pH decrease to 5.0 or less. Thus citric acid is the limiting factor in the formation of flavour compounds. The performance of the mixed (with respect to pH, tritratable acidity and diacetyl) production (figures 2 to 5 and table 4) suggests an associative growth of the acid producers and the flavour producer.

Information gathered in the step to step investigations on the activities of the organisms (just reported) was taken into consideration.

These include:

- Optimum production of acid and diacetyl at 45ºC
- Optimum size of inoculums being 5% v/v
- The desirable pH of between 4.2-5.0 was obtained at 8 hours of incubation at 45ºc.
- Very low pH values (4 and below) results in a decrease in diacetyl content.

The significant increase in free fatty acids in the traditional butter, under refrigeration, may be as a result of activities of contaminating microorganisms.

Size of inoculum used as starter is most critical in industrial fermentations. When the amount of starter is much lower than the substrate, fermentations are unduly prolonged and the substrate is not properly utilized. The use of too large inoculum is also considered to be a waste (Prescott and Dunn, 1974). For these reasons, the best level of starter for "manshanu" production was established. The results are shown in figure 6. Two (2% v/v) percent starter was found to be unsuitable. While there was no significant difference between the levels of acid produced using 10% v/v and 5% v/v. For economic reasons therefore, 5% v/v starter was adjudged the best.

Flavour Development in "Manshanu"

During the studies on diacetyl production - the main compound responsible for the butter flavor (ICMSF, 1978), it was observed that increase in acidity affected the level of diacetyl produced by the starter organisms. Table 5 gives the effect of the pH of ripened milk on the diacetyl content. There was a gradual decline in the diacetyl content when pH goes down below 4.5. This observation is in agreement with that of Walsh and Cogan (1974). During their study of the separation and estimation of diacetyl and Acetoin in fermenting milk, it was noted that low pH values activates an enzyme, diacetylreductase which reduces this compound to odorless products – 2, 3 –butanediol. The diacetyl content () of the fermented butter produced from the laboratory was higher than those of the butter samples purchased from the local markets (12.0ppm), Table 6.

Conclusion

Manshanu at present is mostly produced on small house-hold scale under highly variable

conditions which result in butter of unpredictable quality. In the transformation of the production from small scale to a large-scale factory situation where the flavour and other characteristics can be controlled, it is suggested that information gathered in this present study be used. This is the first clear departure from small scale production and it should be used on the results of laboratory experiments. Finally, the industrial plant stage can then be launched.

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