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Short communication

MICROBIOLOGICAL ASPECTS OF ERGO (*ITITU*) FERMENTATION

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ABSTRACT: *Ergo* is a traditionally fermented dairy product which has some resemblance to yogurt in its curd formation and acidity. Raw milk is allowed to ferment in to *Ergo* for about 24 h at ambient temperature before it is consumed. Time course studies on growth of the microorganisms involved in the fermentation of *Ergo* were made. Its fermentation is carried out by lactic acid bacteria belonging to the genera *Lactococcus*, *Streptococcus*, *Leuconostoc* and *Lactobacillus*. However, micrococci, sporeformers and coliforms were present in fairly high numbers during the first 14–16 h of fermentation. The Lactococci were the most dominant group throughout the fermentation ($1x10^5$ at time 0 h and $4x10^9$ colony forming unit cfu(ml)$^{-1}$ at end of fermentation (24 h), followed by Streptococci. The yeast population which was about $1x10^1$ cfu(ml)$^{-1}$ just after milking increased to about $1x10^5$ cfu(ml)$^{-1}$ at the end of 24 h of fermentation. The total aerobic mesophilic count (TAM) increased from about $1x10^5$ cfu(ml)$^{-1}$ to $4x10^9$ cfu(ml)$^{-1}$ and at the same time titratable acidity increased from 0.16 to 0.75% with concomitant drop in the pH.

Key words/phrases: *Ergo*, fermentation, lactic acid bacteria, milk

INTRODUCTION

In Ethiopia, when and where milk is available in large quantities or considered as one of the main diets as in some of the highland or arid regions, it may be

allowed to undergo natural fermentation in various types of containers for several hours to days (FAO, 1990; Mogessie Ashenafi, 1990; O'connor, 1992; Kumisa, 1993). The containers may be large gourds or earthen pot. However, in metropolitan areas and towns, the milk is allowed to ferment in glass or plastic containers. These are cleaned thoroughly before a second batch of milk is to be fermented. The traditional containers which have been in use continuously develop smooth slimy inner surfaces. They are frequently smoked with splinters of certain types of woods and then rinsed with water. The milk as well as the containers traditionally utilized in the rural areas may thus contribute their own organisms to the fermentative actions (FAO, 1990; O'Connor, 1992). The fermented product is known as *Ergo* (Amharic) or *Ititu* (Oromifa) by the people living in the highland regions of the country. The taste and odour of *Ergo* obtained using the traditional containers is different from that produced in towns using glasses or plastic cans. As a result of the natural fermentation, the milk coagulates and forms a soft curd. *Ergo* is usually spiced with various additives such as *Kochkcha* (mashed green pepper with onion and salt) or hot pepper when served to guests at any time of the day. A small portion of the curdled milk may be left in the containers to initiate the fermentation of freshly introduced raw milk. This process invariably ensures proper development of flavor and aroma of the fermented milk (*Ergo*).

Previous studies have characterized the microorganisms isolated from raw cow's milk and milk products (Mehari Tadesse and Berhanu Abegaz Gashe, 1990; Mogessie Ashenafi and Fekadu Beyene, 1994; Mogessie Ashenafi, 1994). The present report however, deals with the microbiological changes in *Ergo* and physico-chemical changes they bring about as the result of their activities during fermentation. Information of the type of the fermentative organisms could lead to the development and production of starter cultures and standardization of the product. In addition it may also lead to the commercial production of this important dairy product.

MATERIALS AND METHODS

Twenty five samples of raw milk (one liter or more of each) were purchased from private homes who keep milking cows in their back yards around Addis

Ababa. Fermentation was initiated by incubating 800–1000 ml of fresh raw milk in one liter-capacity sterilized glass containers at ambient temperature which varied 18–22° C. Each of these samples were analyzed separately. Aliquots (25 ml) were removed every 4–6 h for bacteriological analysis, pH and titratable acidity determinations. Titratable acidity was determined as percent lactic acid.

Total mesophilic aerobic count (TAM) and psychrophilic count represented those organisms which grew on plate count agar at 30° C within 24–48 h and at 7–10° C within 10 days, respectively (Marth, 1978). For counting spore-forming organisms and thermodurics, samples were heat-treated for 10 min at 80° C and for 30 min at 65° C, respectively (Mehari Tadesse and Berhanu Abegaz Gashe, 1990). After heat treatment, appropriately diluted samples were plated on plate count agar (Difco) and incubated for 24–48 h at 30° C. Thermophiles were estimated and isolated by incubating seeded plate count agar at 55° C for 24 h. MacConkey Agar, Mannitol Salt Agar, Rogosa Agar, Azide Blood Agar base and Slanetz and Barteley medium and Potato Dextrose Agar were used for enumerating and isolating coliforms, staphylococci, lactobacilli, streptococci and fungi, respectively.

Representatives of each of the dominant colonies were picked based on cultural characteristic of the colonies and purified. The pure cultures were then subjected to various morphological (Gram's, spore, capsule, polybetahydroxy-butyrate stains, cell arrangement, size) and biochemical (utilization of various sugars, polyols, sugar acids, amino acids, mono-and dicarboxylic acids, growth characteristics in liquid media) test (Harrigan and Margaret, 1966; Collinos and Lyne, 1976). Naming of the isolates were carried out following the scheme described in Bergey's Manual (Sneath *et al.*, 1984).

RESULTS AND DISCUSSION

The microbial counts of raw milk before and after fermentation are shown in Table 1. Initially (0 h), the total aerobic mesophilic count (TAM) was about $1x10^5$ colony forming units $cfu(ml)^{-1}$. The majority of the bacteria isolated (>90%) were cocci (Table 1). The rest were either thermodurics and/or thermophiles. Fungi (mostly yeasts) and coliforms were also present in low

numbers. The changes in the pH value and titratable acidity, expressed as percent lactic acid, of the fermenting milk are given in Table 1. The milk soon after collection had a pH of about 6.6 and titratable acidity of 0.16%. The fall in pH during the first 8 h of fermentation was about 0.4 units. However, during this period the population of the microorganisms increased by several fold (Table 1). Curd formation and wheying-off occurred 14–16 h after the initiation of fermentation. At the time when curd formation appeared, the total mesophilic count, titratable acidity, and pH was about $2x10^8$ cfu(ml)$^{-1}$, 0.5% and 5.4, respectively. Microbial population and titratable acidity values continued to increase for another 8–10 h. When the product was ready for consumption (24 h), the microbial population, titratable acidity and pH were about $4x10^9$ cfu(ml)$^{-1}$, 0.75% and 4.5, respectively. *Ergo* at 24 h of fermentation had low pH values ($\leq$4.75) with insignificant variation within samples ($p>0.1$). Variation within samples, in all counts and at each time interval of fermentation were also markedly low ($p>0.5$) again.

The most abundant species which were isolated belonged to the genera *Lactococcus*, *Streptococcus*, *Leuconostoc*, *Lactobacillus* and yeast (Table 2). However, *Micrococcus* sp., coliforms and spore-formers were also present in fairly high numbers during the first 12–14 h of fermentation. Their population decreased substantially thereafter which implies an antimicrobial activity besides low pH in the fermented milk. Similarly, antimicrobial effects have been found in other lactic fermentations and the inhibitors have been suggested to be antibiotic substances (Fernandes *et al.*, 1987; Mbugua and Njenga, 1992). Species belonging to genus *Lactococcus* were the most predominant lactic acid bacteria (LAB) throughout the fermentation period. Their population increased from about $1x10^5$ cfu(ml)$^{-1}$ at time of collection to about $4x10^9$ cfu(ml)$^{-1}$ at time of consumption (24 h). The second most abundant bacteria present were species of *Streptococcus*, followed by lactobacilli and *Leuconostoc* sp. (Table 2). The yeast population increased from about $1x10^1$ cfu(ml)$^{-1}$ (at time 0) to $1x10^5$ cfu(ml)$^{-1}$ (at 24 h) consistent with the findings for raw milk (Mogessie Ashenafi, 1990). Their population was almost the same as those of *Leuconostoc* and *Lactobacillus* species in the fermented product.

Table 1. Changes in pH, titratable acidity (%) and population of the various groups of microorganisms cfu(ml)$^{-1}$ during fermentation of raw milk to prepare *Ergo*.

Fermen. Time, h	pH	Titratable acidity	TAM	Thermo-durics	Therm-ophiles	Psychr-ophiles	Gram +cocci	Gram + rods	Gram - rods	Yeasts
0	6.60	0.16	$4.0x10^5$	$2.0x10^2$	$1.1x10^2$	$2.1x10^5$	$5.0x10^5$	$2.0x10^2$	$7.0x10^3$	$4.0x10^2$
4	6.52	0.18	$2.0x10^7$	$4.0x10^2$	$5.1x10^3$	$2.0x10^7$	$1.6x10^7$	$9.0x10^4$	$5.0x10^5$	$2.0x10^3$
8	5.35	0.26	$1.4x10^8$	$5.1x10^2$	$4.0x10^5$	$4.1x10^7$	$1.1x10^8$	$6.1x10^5$	$4.0x10^6$	$7.1x10^3$
12	5.34	0.50	$4.1x10^8$	$7.1x10^2$	$7.1x10^5$	$8.1x10^7$	$2.1x10^8$	$2.1x10^6$	$2.1x10^5$	$4.0x10^4$
16	5.15	0.53	$4.2x10^9$	$8.0x10^2$	$8.0x10^5$	$9.0x10^8$	$5.6x10^9$	$5.2x10^7$	$1.1x10^3$	$8.0x10^4$
20	5.00	0.57	$1.2x10^{10}$	$9.0x10^2$	$9.1x10^5$	$5.1x10^9$	$8.0x10^9$	$7.0x10^7$	——	$3.1x10^5$
24	4.75	0.62	$9.8x10^{10}$	$9.8x10^3$	$1.0x10^6$	$7.9x10^9$	$9.0x10^9$	$9.0x10^7$	——	$9.0x10^5$

TAM, Total aerobic mesophlic count.

Table 2. Changes in lactic acid bacteria population [cfu(ml)$^{-1}$] during fermentation of raw milk to prepare *Ergo*.

Fermen. Time, h	*Lactococcus cremoris*	*Lactococcus lactis*	*Leuconostoc cremoris*	*Lactobacillus mesentroides*	*Streptococcus thermophillus*	*Micrococcus* sp.	*Lactobacillus deluburki*	*Lactobacillus homi*
0	$1.9x10^3$	$3.9x10^5$	$5.4x10^5$	$8.8x10^5$	$4.4x10^5$	$6.6x10^3$	$2.0x10^3$	$8.0x10^3$
4	$5.4x10^5$	$3.8x10^6$	$6.2x10^6$	$7.1x10^5$	$3.0x10^7$	$1.8x10^4$	$2.5x10^4$	$5.0x10^3$
8	$4.0x10^6$	$1.2x10^8$	$7.1x10^7$	$3.5x10^7$	$1.5x10^8$	$2.1x10^5$	$1.1x10^5$	$4.0x10^4$
12	$6.2x10^6$	$7.0x10^8$	$1.1x10^8$	$5.3x10^7$	$2.0x10^8$	$5.1x10^5$	$2.6x10^5$	$1.2x10^5$
16	$5.7x10^7$	$2.4x10^8$	$8.4x10^8$	$3.9x10^8$	$1.9x10^9$	$5.8x10^5$	$5.7x10^5$	$2.3x10^5$
20	$5.0x10^7$	$8.0x10^8$	$9.0x10^8$	$1.2x10^9$	$4.0x10^8$	$8.0x10^5$	$7.5x10^5$	$3.0x10^5$
24	$6.1x10^7$	$1.8x10^9$	$2.8x10^9$	$1.2x10^9$	$6.2x10^9$	$2.2x10^6$	$4.4x10^6$	$5.0x10^5$

Mixtures of lactococci, streptococci and lactobacilli are responsible for fermentation of milk under natural conditions (Jay, 1994; Robhinson, 1990; Steinkraus, 1983). Since *Ergo* is fermented naturally, the microorganisms responsible for the fermentation were heterogenous; but the most predominant ones were the lactic acid bacteria (LAB). The LAB and yeasts were present throughout the fermentation; therefore, they must be responsible for the fermentation of the product in agreement with results obtained for sour milk (Rohm *et al.*, 1992). At times, *Ergo*, which is obtained from the market or from some households, imparts odor and taste which is not desirable to most consumers. This may be due to the presence of large number of coliforms during the fermentation. Presence of coliforms in large number and their subsequent activity in foods imparts undesirable flavor and aroma (Jay, 1994; Motarjemi and Nout, 1995). In this study, the coliform population was about $1x10^3$ cfu(ml)$^{-1}$ initially, but increased to $5x10^5$ cfu(ml)$^{-1}$ during the first 8 h of fermentation. They were the second most abundant species between 4–12 h of fermentation. Since coliforms are capable of fermenting lactose, they must contribute to the decreased pH during the initial stages of the fermentation. We believe that the initial role played by coliform and spore-formers in acidifying the milk and paving the way for growth of LAB can not be undermined. The population of the coliforms decreased to a non-detectable level after 14 h of fermentation. Similar results were observed for *Ergo* (Mogessie Ashenafi, 1994) and lactic fermented cereals (Svanberg *et al.*, 1992).

Streptococcus lactis (now classified as *Lactococcus lactis*) is mainly responsible for acid production with some assistance from coliforms, enterococci, lactobacilli and micrococci when raw milk is maintained at 10–37° C, (Robhinson, 1990). A strain which resembles *Lactococcus lactis* was the most dominant group present in the fermented product in our study. This compliments previous findings reported on naturally fermented dairy products. Strains of lactic acid bacteria belonging to *Lactococcus lactis* species were similarly isolated from naturally fermented milk products in Ethiopia (Fekadu Beyene, 1994). The container we used was sterile, hence, it did not contribute any microorganisms to the milk.

All the microorganisms which were encountered during this study were present in the raw milk used for the study. These microorganisms must have gained

access into the milk as a result of the invasion of the teat canals or contamination of the milk during milking from the external sources. We are now attempting to determine the contribution of traditional containers to the microflora of the fermenting product. Selection of appropriate cultures for future is also being attempted.

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