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Application of Osmometry in Monitoring Fermentation of Milk

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Abstract: Fermentation of milk yields many other products apart from lactic acid. This study was carried out to monitor the progress of fermentation by measuring the total solute concentration i.e. osmolality in fermenting milk. Five samples each of raw milk, pasteurised milk and UHT (ultra-high temperature treated) milk were incubated aerobically without starter culture at 37°C for 3 days. Osmolality was measured by freezing point depression at the beginning of incubation and at exactly the same time for the next 3 days. Osmolality of raw milk (mean±SD) increased from 290.2±8.0 to 405.6±31.06 mOsmoles kg^{-1} in one day and stabilized around this value thereafter. For pasteurised milk, osmolality rose from 294.5±7.3 to a constant value of 403.4±18.2 mOsmoles kg^{-1} in two days. Osmolality of UHT milk increased almost linearly from 289.3±3.3 to 472.7±10.1 mOsmoles kg^{-1} over 3 days. The results showed that osmometry is a viable tool in monitoring the progress of milk fermentation. Using this method, maximum rate of spontaneous fermentation was obtained with raw milk, whereas maximum fermentation potential resided in UHT milk.

Key words: Osmolality · Raw Milk · Pasteurised Milk · UHT Milk · Fermentation · Monitoring

INTRODUCTION

Sour milk is produced by bacterial fermentation of lactose in fresh milk to lactic acid by a class of anaerobic, Gram-positive bacteria known as lactic acid bacteria (LAB). There are twelve genera of LAB, including *Lactobacillus spp*, *Lactococcus spp*, *Streptococcus spp* and *Leuconostoc spp*. Previous studies [1] indicated that the most important lactic acid-producing bacteria in milk belong to the genus *Lactobacillus*. Spontaneous fermentation of raw milk is brought about by its native microbial population. During commercial production of sour milk, the fermentation process is aided by addition of lactic acid-producing bacteria to milk to ferment it since the native microbial population would have been destroyed by heat treatment [2]. The attributes of fermented milk are steadily unfolding. As a result of vitamin synthesis by symbiotic bacteria, fermented milk contains higher levels of B-vitamins than fresh milk, particularly folic acid, niacin, biotin, pantothenic acid, pyridoxine and cyanocobalamin. Apart from producing fermented dairy milk, a number of LAB are also being used as probiotics in fermented dairy products [3] including, *Lactobacillus plantarium*, *Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus delbrueckii and Lactobacillus rhamnosus* Furthermore, some lactic acid bacteria produce bacteriocins during fermentation of milk, as has been demonstrated with *Lactobacillus plantarium* from naturally fermented milk [4]. These developments make the need for adequate monitoring of the progress of fermentation essential. Early studies on ethanol production from sucrose fermentation showed that the process of fermentation is associated with an increase in osmolality i.e. total concentration of solutes [5]. The objective of the present study was to assess the validity of osmometry as a tool for monitoring the progress of fermentation of milk.

MATERIALS AND METHODS

Osmometry is the measurement of the total concentration of solutes (osmolality) in a solution. Principles and practices of osmometry have been previously reviewed [6]. Basically, the freezing point of

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a solution decreases as its osmolality increases. A cryoscopic osmometer measures the osmolality of a test solution by measuring the freezing point. Pure water freezes at 0°C. One mole of a solute dissolved in one kilogram of water will result in a freezing point depression of 1.86°C. The freezing point of raw milk is between -0.525 and -0.565°C. This translates to an osmolality of 282-304 mOsmoles kg^{-1} (7). Fermentation of milk yields soluble products resulting in a net increase in osmolality, which is then detected by the osmometer.

Five samples each of raw milk, pasteurised milk and UHT (ultra high temperature-treated) milk were used in this investigation. Raw milk samples were collected from the bulk tank at the Dairy Unit of the University of Zimbabwe Farm whereas pasteurised milk and UHT milk were sourced from leading retail supermarkets. During transportation, the milk samples were maintained below 4°C with the aid of ice water. All milk samples were kept in a refrigerator at 4°C prior to experimentation (maximum 1 day). From each sample, 20 mL of milk were measured into sterile 100 mL glass bottles, which were then loosely closed to allow communication with the outside environment. The bottled milk was then incubated without starter culture in a water bath (Gallenkamp Thermo stirrer 85, Germany) at 37°C for 3 days [8, 9]. At the beginning of incubation (day 0) and at exactly the same time on subsequent days, 50 µL were withdrawn from the milk with a micropipette and placed into an Eppendorf tube for measurement of osmolality using a cryoscopic osmometer (Osmomat 030, Germany).

RESULTS AND DISCUSSION

Statistical analysis of results was performed using Sigma Plot version 1.02 (Jandel Scientific). Fermentation was invariably accompanied with an increase in osmolality of milk ($p<0.05$). However, the rate and magnitude of the increase differed for the three milk products.

Raw milk had the fastest change, with osmolality (mean±SD) rising from 290.2±8.0 to 405.6±31.06mOsmoles kg^{-1} in one day. Thereafter, the osmolality was maintained at nearly constant value (Figure 1).

The osmolality of pasteurised milk rose from 294.5±7.3 to a maximum of 403.4±18.2mOsmoles kg^{-1} in 2 days and remained steady thereafter (Figure 2).

In contrast to raw and pasteurised milk, the osmolality of UHT milk increased linearly without attaining a stable maximum value, from 289.3±3.3 on day 1 to 472.7±10.1mOsmoles kg^{-1} on day 4 (Figure 3).

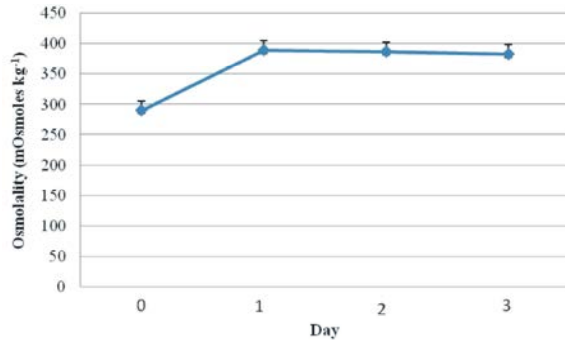

Fig. 1: Osmotic changes during fermentation of raw milk

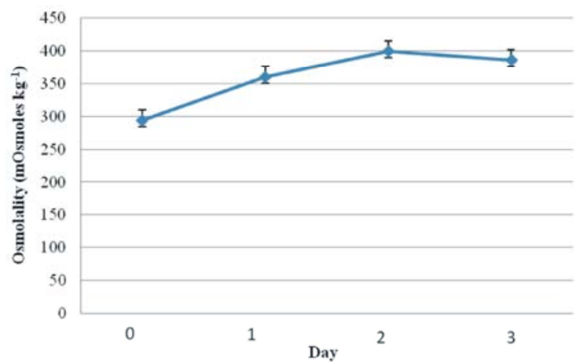

Fig. 2: Osmolality of pasteurised milk during fermentation

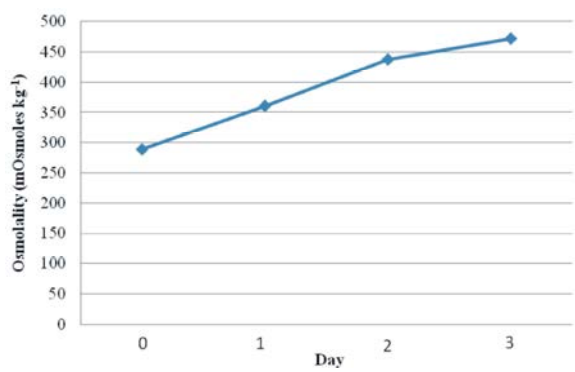

Fig. 3: Osmotic changes during fermentation of UHT milk

The rate of increase in osmolality was in the order raw milk>UHT milk>pasteurised milk. There was no difference in maximum osmolality between raw and pasteurised milk, but the maximum osmolality of UHT milk was significantly greater than that of the two products ($p<0.05$).

The pathway of fermentation involves breakdown of the milk sugar lactose to its constituent monosaccharides glucose and galactose [10]. Since they lack enzymes of the Krebs cycle, LAB utilise substrate level phosphorylation to further metabolise glucose. Homofermentors (*Lactobacillus, Lactococcus and Streptococcus* species) yield two molecules of lactic acid from breakdown of each glucose molecule. Heterofermentors (*Leuconostoc* and some *Lactobacillus* species) produce one molecule of lactic acid as well as acetic acid, ethanol and carbon dioxide. On average, the lactose content of raw milk decreases from about 5 to 3.5-4% during fermentation [11]. The addition of glucose, galactose as well as lactic acid and other products of fermentation to the aqueous phase of milk during fermentation exceeds removal of lactose, resulting in an increase in total solute concentration *i.e.* osmolality of milk. Furthermore, lactic acid causes inorganic components of milk that are insoluble to go into solution. In particular, calcium is released into solution because lactic acid causes transformation of calcium caseinate to calcium lactate and casein. The free casein is subsequently precipitated, resulting in curdling of the milk. Thus an increase in osmolality of milk during fermentation occurs not only as a result of breakdown of lactose, but also with liberation of calcium and phosphate ions from the colloidal phase into the aqueous phase. Accordingly, measurement of changes in osmolality of milk during fermentation is a reliable indicator of the progress and extent of fermentation.

In the present study, the rate of fermentation was fastest with raw milk. Raw milk left at ambient temperature will undergo natural souring as a result of lactic acid produced from lactose by native LAB as well as yeasts [12, 13]. When osmotic changes occurring in raw milk were compared with UHT milk, it became evident that fermentation of raw milk does not reach its full potential. Apparently, competition from contaminating organisms in milk is one of the limiting factors in fermentation of raw milk. During the process of pasteurisation, all pathogenic organisms in milk are destroyed. Most of the native LAB and contaminating micro-organisms in milk are also destroyed. However, a few thermoduric LAB species and spoilage organisms remain. These organisms are ultimately responsible for spoilage of pasteurised milk if kept beyond the expiry date. The surviving LAB will also multiply and cause milk to undergo fermentation if provided with an ideal temperature, as has been observed in this investigation. Since the starting LAB population is small, the lag phase of growth is relatively prolonged giving rise to a slower rate of fermentation with pasteurised milk when compared to raw milk. The extent of fermentation is nevertheless similar for the two milk types. The UHT process resulted in milk sterilization i.e. total destruction of all micro-organisms present in raw milk. As such, spontaneous fermentation of UHT milk is entirely by LAB present in the environment. This necessitates adequate colonization of the milk by environmental LAB prior to growth and activity of the fermentative microbial population. Therefore the rate of fermentation is slower with UHT milk in comparison to the raw milk. However, due to absence of competition from contaminating or thermoduric bacteria, fermentation attained its greatest potential in UHT milk compared to raw milk or pasteurised milk.

To date, the progress of fermentation has been monitored by measurement of changes in pH [9, 14]. Lactic acid production causes the pH to drop from an average of 6.5 to between 4.5 and 4.0 during fermentation of cow's milk [11, 15]. However, fermented milk contains a number of organic compounds that do not influence pH, including acetyldehyde, acetone, 2-methy propanal, 2-methyl-1-propanol, 3-methyl-1-butanol, 3-methyl-1-butanal [16], ethanol, succinate [17], diacetyl and acetoin [18]. In addition, partial hydrolysis of protein during fermentation yields free amino acids as well as peptides [19]. Since osmolality measures the total concentration of all soluble products of fermentation, osmometry would be a useful complement to measurement of pH in monitoring progress of fermentation.

The rate and extent of fermentation was successfully monitored by measuring changes in osmolality of milk occurring during the fermentation process. Traditional methods of preparing fermented milk favor natural fermentation of raw milk. However, fermentation does not reach its full potential with raw milk. Pasteurisation makes milk safer for consumption, but does not improve its fermentation potential. In contrast, sterilization of milk results in a greater fermentation potential as compared to raw milk, albeit at a slower pace.

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