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The physicochemical and sensory evaluation of commercial sour milk (*amasi*) products

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The physicochemical and sensory properties of five commercially available sour milk (*amasi*) products-AoA, AoB, AoC, AoD and AoE were analyzed in 3 batches. Samples were collected from retail shops in Thohoyandou. The products were examined for *Escherichia coli*, lactic acid bacteria (LAB), total plate count (TPC), pH, titratable acidity (TA), colour, viscosity and sensory properties. Microbial analysis results for LAB ranged between 1.25 × 10^5 and 1.97 × 10^6cfu with sample AoA and AoB being the highest by 1.96 × 10^6 and 1.97 × 10^6 cfu, respectively. *E. coli* count ranged from 1.22 × 10^4 to 1.78×10^5 cfu with sample AoE and AoC being the lowest and highest ($p < 0.05$), respectively. TPC had the least number of counts with the values between 4.2 × 10^3 and 9.1 × 10^4 cfu. The pH values of the products ranged between 4.22 and 4.34 and TA ranged between 0.80 and 0.84. Colour measurements gave L* values of the products ranging from 33.77 to 40.19, while a* and b* values were 3.08 to 6.43 and 13.17 to 18.77, respectively. Differences existed in viscosities of the products that were not significantly different ($p < 0.05$). Sensory score for the overall acceptability indicated that consumers did like the sour milk, as the values ranged from 3.8 to 4.4. Although there were significant differences ($p < 0.05$) in terms of sweetness, smoothness and astringency, they did not affect the consumer acceptability of the products. Presence of *E. coli* in the sour milk products can be of health concern to consumers.

Key words: Sour milk, *amasi*, physicochemical, microbial.

INTRODUCTION

Traditionally, sour milk known as *amasi* among the *Zulus* in South Africa is a fermented product produced in clay pots and gourds which are used repeatedly through spontaneous fermentation of raw milk that occurs naturally at ambient temperature of ± 5°C (Bryant, 1949; Keller and Jordan 1990; Gadaga et al., 2001). Fermentation is one of the oldest methods for food preservation, which contributes to their flavor, appearance and texture (Mazahreh et al., 2008; Quasem et al., 2009). Hence fermented products are generally more attractive to the consumer than non-fermented ones.

However, due to the ever escalating human population size, and the advent of commercial starter cultures, commercial *amasi* is now produced from pasteurized milk, under controlled processing conditions. Commercially, *amasi* products are pasteurized before distribution and consumption, and the shelf life of the refrigerated beverages is 21 days (Beukes et al., 2001; Dlamini and Buys

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Table 1. Definition of attributes constituting the sour milk (*amasi*) products.

Attribute	Definition
Sourness	Fresh sour smell, for example, fresh sour cream
Sweetness	Sweet sensation of sugar
Texture (thickness)	Resistance to flow or motion
Smoothness	Absence of detectable solid particles
Astringency	Dry feeling in the mouth, similar to mouth-feel after drinking dry red wine
Colour	Whiteness
Overall acceptability	How good is all combined attributes

2008; Burger, 2010). Examples of 2008; Burger, 2010). Examples of *amasi* products in the study location include Limpopo dairy maas, Clover Inkomazi, Save Mor*amasi*/ Spar maas, Moordriftmaas and Hygienik (*amaziakgomo*).

Sour milk is one of the products in which microorganisms are used during its production; therefore in cases where hygienic practices are not properly applied, microbial contamination may occur during processing, distribution of product and retail outlets. The presence of *Escherichia coli* and coliform groups of microorganisms in sour milk could pose a potential health problem. Monitoring the quality of dairy product is important, considering the safety of the consumers. Since milk and milk products are good vehicles for both beneficial and harmful microorganisms, some of which may have health and subsequent financial consequences to the consumer, it is important to independently monitor quality. This study, therefore, examined those physical, chemical, microbial and sensory parameters that determine quality of sour milk product.

MATERIALS AND METHODS

Five commercial sour milk (*amasi*) products were bought from shops in Thohoyandou, Limpopo Province, South Africa. These products were coded: AoA, AoB, AoC, AoD and AoE. The samples were refrigerated at 4°C at the Department of Food Science and Technology, University of Venda (Univen). The sensory evaluation, and the chemical, physical and microbial analyses were carried out in the laboratory. Titration for lactic acid concentration, pH determination and microbial count, colour and viscosity analysis were done.

Physical analysis

The viscosity was determined using DV-E Viscometer (Brookfield Engineering Laboratories model No. RVDVE230). Spindle no. DV-E 4 was used at a speed of 10 rpm and the reading on the viscometer was taken after 3 min for each sample (Kip et al., 2006). The colour was determined as outlined in HunterLab (2008), using a Hunter Lab, ColourFlex model No. 45/0 (Hunter Associates Laboratory, Inc 11491 Sunset Hills Road, Reston, VA 20190-5280).

Physicochemical analysis

The pH of the samples was determined using pH meter, (METTLER TOLEDO, Education Line model No. EL20). The pH probe was immersed inside the 50 ml beaker of sample. Determinations were done in triplicates.The instrument was calibrated using standard buffer solutions at pH 4 and 7. The lactic acid concentration was determined by weighing 10 g of *amasi* sample in a conical flask; 30 mL of distilled water were added to the sample and mixed well by shaking. Phenolphthalein indicator (5 drops) was added and 0.1 M NaOH from the filled burette was used to titrate the solution until it retained a very slight pink tinge after mixing. The volume of NaOH used was recorded. Each sample was titrated three times and the percentage of lactic acid was calculated using the equation (NZIFST, 2010):

$$\text{Acid (\%)} = \frac{\text{(ml of NaOH used) (concNaOH) (0.090 milli equivalent weight of lactic acid) (100)}}{\text{Weight of sample}}$$

Enumeration of lactic acid bacteria (LAB) and *E. coil*

Samples (1 mL) of each sour milk were serially diluted (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}) in 9 mL of buffered peptone water (BPW) and appropriate dilution was surface plated onto Sorbitol MacConkey agar (SMAC) (Oxoid) for *E. coil* and de Man Rogosa and Sharp (MRS) agar was prepared from the ingredients for Lactobacillus. MRS plates were incubated anaerobically at 37°C for 48 h for the enumeration of mesophilic lactobacilli and leuconostocs and at 42°C for the enumeration of thermophilic lactobacilli and streptococci, while SMAC plates were incubated aerobically for 24 h at 37°C (Dlaminiand Buys, 2008). Total viable counts were performed using plate count agar (Merck) incubated at 25°C for 24 h (Lefoka and Viljoen, 2009).

Sensory analysis of sour milk products

A panel of 10 judges familiar with the *amasi* from the Department of Food Science and Technology at Univen was constituted and used to evaluate the five products. The panelists were asked to score for sourness, sweetness, texture (thickness), smoothness, astringency, colour and overall acceptability of the products (Table 1) on a 5-point hedonic scale ranging from 5 to 1, where 1 is dislike very much, 3 is neither like or dislike and 5 like very much (Bille et al., 2002).

Statistical analysis

Products were analyzed in triplicates. Analysis of variance (ANOVA) was applied to the entire data set to determine the significance of the differences ($p < 0.05$) between the sour milk products using a Microsoft Excel for windows 7.

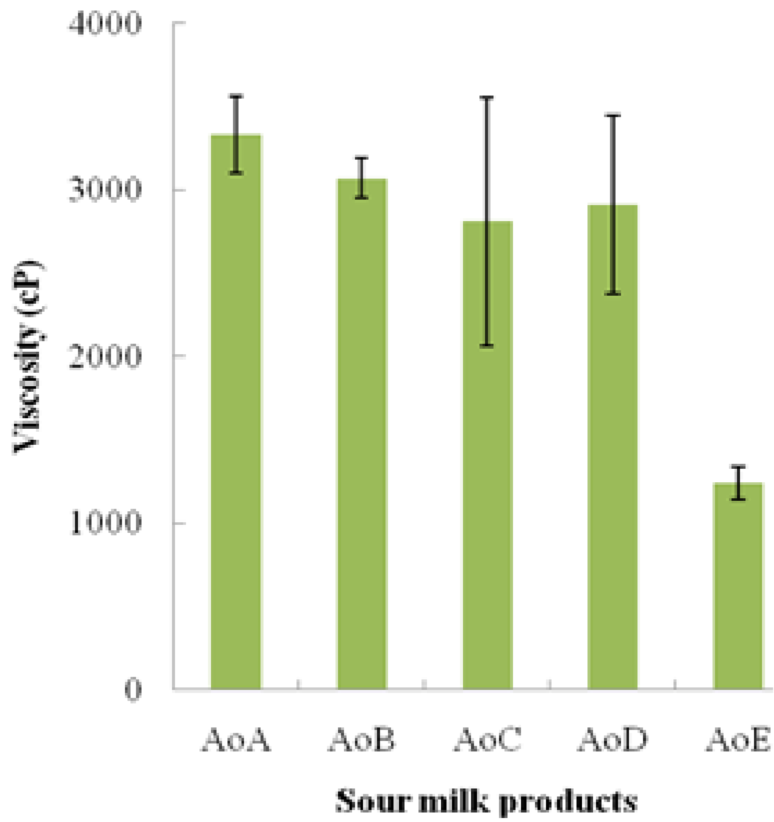

Figure 1. The viscosity of commercial sour milk (*amasi*) products.

RESULTS AND DISCUSSION

Physical analysis

The results presented are from the following physical analysis: viscosity and colour. The results for viscosity are shown in Figure 1. It seems that sample AoA had a higher viscosity ($p < 0.05$) as compared to sample AoE which had a low viscosity. Significant differences at $p < 0.05$ existed for the viscosity between the samples (*amasi*). Sample AoA showed the highest viscosity of 3330 cP, while sample AoE showed the lowest viscosity of 1240 cP (Figure 1). There were differences ($p < 0.05$) between sample AoA and sample AoB and AoE, whereas there was no difference in samples AoC and AoD. Sample AoB differed from sample AoC, AoD and AoE. Sample AoB, AoC and AoD had values of 3070, 2810 and 2915 cP, respectively. There was no difference in viscosity between sample AoC and AoE but there was difference in sample AoD. More work has been done to improve the *amasi* using either enrichment of the milk with 2.5% (w/v) skim milk or by dry matter ultra-filtration (Narvhus et al., 1998).

Ozerand and Atamer (1994) reported that acid development is required for fine curd formation. The study by Mustafa et al. (2001) on yoghurt recorded a viscosity value of 100 to 2825 cP. The LAB that produce exopolysaccharides are often used to increase the viscosity of stirred fermented milks, such as yoghurt and to decrease the susceptibility to syneresis (Ruas-Madiedo et al., 2002). Sample AoE had lowest viscosity and not surprising it also recorded lowest on consumer acceptance on texture and smoothness sensory attributes.

Large curd in sample such as AoA was observed as compared to sample AoE that suggests that viscosity could have been influenced by the size of the particles and whey proteins from denaturation caused by pH (Masson et al., 2010). This can be caused by unavoidable variations in factors such as temperature, pH and mechanical vibration during production, resulting in grainy texture and varying viscosities (Anonymous, 2010).

Eissa et al. (2010) stated that prolonged metabolic activity of microflora in yogurt causes changes in the micro-structure of the media and hence affecting viscosity. This may suggest that high values on the standard deviation were caused by the length of time each set of collected samples were stored and the number of microflora of the sour milk during analysis. The viscosity is affected by the state and concentration of fats, protein, temperature, pH and milk age (Park, 2007).

Results for colour analysis of sour milk samples are shown in Table 2. There was no significant difference ($p < 0.05$) between the L* values of the sour milk samples, with all the values ranging from 34 to 40 (Table 2). The L*

Table 2. The pH and colour parameters of commercial sour milk (*amasi*) products.

Sample	pH	Colour		
		L*	a*	b*
AoA	4.22 ± 0.18[a]	33.77 ± 4.54[a]	6.26 ± 3.29[a]	16.70 ± 1.19[a]
AoB	4.34± 0.30[a]	35.29 ± 6.03[a]	6.43 ± 0.08[ab]	18.77 ± 0,71[b]
AoC	4.23± 0.12[a]	38.67 ±0.58[a]	3.08 ± 0.49[ac]	13.71 ± 0.30[ac]
AoD	4.30± 0.14[a]	38.04 ±1.84[a]	5.10 ± 0.26[a]	16.02 ± 0.42[ad]
AoE	4.34± 0.12[a]	40.19 ±1.13[a]	3.78 ± 0.31[ac]	16.58 ± 0.52[a]

Mean values with their standard deviations. L* = Lightness (ranging from 0 = black to 100 = white); a* = redness and b* = yellowness. Values in the same column with different letters differ significantly from each other ($p < 0.05$).

indicate the black and white colour which ranges from L = 0 (black) to L = 100 (white) colour, and white is the predominant colour in sour milk. Again, there was no significant difference between the a* values except the significant difference between sample AoB and sample AoC and AoE ($p < 0.05$). The a* values represent the redness colour intensity and were below 6 in all the samples. The b* values indicate the yellowness and were at high level. The yellow is one of the properties of sour milk that appears to be yellowish in colour (Burger, 2010).

The study showed that, L* values ranged from 34 to 40 among the three batches, confirming that the sour milk has relatively dark colour when compared with standard white colour (L* = 100). Phillips et al. (1995) reported that L* value increased with the increase in fat content in milk. Whiteness in fluid milk is as a result of the presence of colloidal particles, such as milk fat globules and casein micelles, capable of scattering light in the visible spectrum (Fox and McSweeney, 1998).

Moreover the a* and b* values of sour milk were observed to be on the positive side, indicating that the product was slightly reddish yellow and the values ranged from 3.08 to 6.26 and 13.71 to 18.77, respectively.

Physicochemical analysis of *amasi* products

The pH of the samples shown in Table 2 ranged from 4.22 to 4.34 with an average of 4.29. Mean values in the same column with different letters differ significantly from each other ($p < 0.05$). Generally there was no significant difference between the samples in terms of pH values obtained (Table 2). The pH values of all the samples were between the range of 4.22 and 4.34; this is almost in the range of 3.6 and 4.2 discussed by Burger (2010). Dlamini and Buys (2008) also mentioned the pH of commercial *amasi* to be pH 4.4.

The use of pure cultures enables different acid flavors to be developed leading to a range of fermented (cultured) products (Kurmann et al., 1992). The pH values obtained from the samples make *amasi* drinkable since the ideal values are between 4.0 and 4.5 because bitter and acid sensations are not present at this interval (da Cruz et al., 2009). The low pH in the fermented milk offers a selective environment for yeast growth, but is unfavorable for most bacteria (Fleet, 1990). However, spoilage becomes evident when the yeast population reaches 10^5 to 10^6 cells/g (Fleet, 1990).

The TA data are represented in Figure 2. The TA values of the sour milk samples fall within the range 0.80 and 0.84%. These values were lower as compared to the TA of *amasi* discussed in the study by Dlamini and Buys (2008) which had the TA value of 1.4% with the pH of 4.0 at the initial stage. The organic acids (predominantly lactic acid) cause the pH of the sour milk to drop, killing the pathogenic bacteria and inhibiting the growth of many of the common spoilage micro-organisms (Gadaga et al., 2004).

Lactic acid bacteria (LAB) and *E. coil* in *amasi* products

The number of lactic acid bacteria (LAB) shown in Table 3 was high in all the sour milk samples (Table 3), the number of total plate count was less, while in all the sour milk samples, the number of *E. coli* ranged from 1.22×10^4 to 1.78×10^5. The survival of *E. coli* O157:H7 cells for up to several weeks in fermented dairy products, specifically cheese, sour cream, yoghurt, kefir and buttermilk, illustrates the potential health risks associated with post-processing contamination of even low levels of this organism in various dairy foods (Dineen et al., 1998). In the study by Dlamini and Buys (2008), *E. coli* O157:H7 was detectable in commercial *amasi* after 3 days at 7°C but not in traditional *amasi* processed at ambient temperature over the same period.

The number of LAB ranged between 1.25×10^5 and 1.97×10^6 cfu/mL with sample AoA and AoB being the highest by 1.96×10^6 and 1.97×10^6 cfu/mL, respectively. Beukes et al. (2001) reported that the lactic acid bacteria predominated the microbial population and numbers between 4.7×10^5 and 2.03×10^9 cfu/mL were recorded with mean of 108 cfu/mL on MRS, M17 and Rogosa agar in South African traditional fermented milks. In a study by Savadogo et al. (2004) on Fulani traditional fermented milk in Burkina Faso, they obtained almost similar results where the mean count of thermophilic bacteria (42°C) on MRS agar, 8.04×10^5 cfu/mL was less than the mean mesophilic count (35°C), 7.80×10^7 cfu/mL indicating the predominance

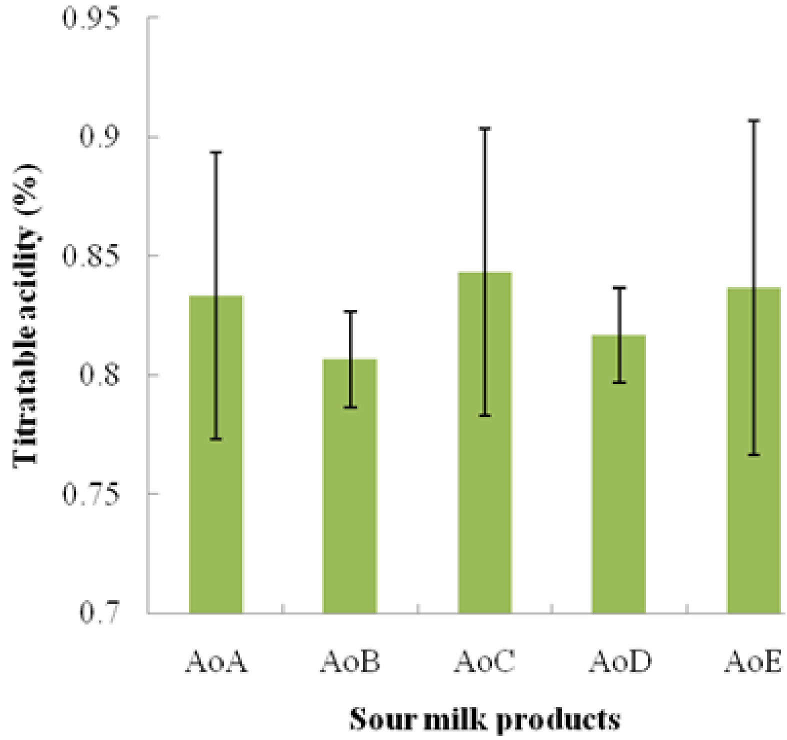

Figure 2. Titratable acidity (TA) of commercial sour milk (*amasi*) products.

Table 3. Microbial count of commercial sour milk (*amasi*) products.

Samples	LAB (cf/mL)	*E. coli* (cf/mL)	Total plate count (cf/mL)
AoA	$1.03 \times 10^6 \pm 0.98$	$7.80 \times 10^4 \pm 0.09$	$4.20 \times 10^3 \pm 2.40$
AoB	$1.97 \times 10^6 \pm 1.19$	$7.20 \times 10^4 \pm 1.10$	$9.09 \times 10^4 \pm 0.80$
AoC	$1.80 \times 10^6 \pm 0.74$	$1.78 \times 10^5 \pm 1.02$	$8.20 \times 10^3 \pm 0.90$
AoD	$4.81 \times 10^5 \pm 3.94$	$2.09 \times 10^4 \pm 0.41$	$2.66 \times 10^4 \pm 0.44$
AoE	$1.27 \times 10^6 \pm 0.09$	$1.22 \times 10^4 \pm 1.28$	$7.55 \times 10^4 \pm 1.75$

LAB = lactic acid bacteria.

of lactic acid bacteria. The mean count of coliforms was 0.98 x 10^4 cfu/mL for 25 samples. Dlamini and Buys (2008) also found that LAB counts in commercial *amasi* inoculated with AA and NA *E. coli* O157:H7 increased from 7.0 to 8.1 $\log_{10}$ cfu/mL. Differences in experimental conditions such as different fermentation temperatures and storage could have caused this difference.

High viable counts of LAB are necessary to get the desired acid production, which affects the product shelf-life (Helland et al., 2004). LAB also plays a major role in the development of flavour and aroma through the production of flavouring compounds such as diacetyl, acetoin and acetyaldehyde (Oberman and Libudzisz, 1998).

Consumer acceptability of *amasi* products

The mean scores for sensory properties of the sour milk samples are shown in Table 4. Five samples were evaluated for sourness, sweetness, texture, astringency, colour and overall acceptability. There were no significant differences observed between the samples in terms of texture, colour and overall acceptability (Table 4). However, there were some significant differences in sweetness, smoothness and astringency properties of the samples. Sample AoA was the most liked by the consumers than sample AoE. Sensory properties of foods offer quality control criteria.

The sensory score indicate that consumers did like the sour milk, as the overall acceptability ranges from 3.8 to 4.4 where 5 is like very much, and 1 being dislike very much (Table 4). The annual consumption of *amasi* is considerable in South Africa and an estimated 104 million liters were consumed in 2003 by black miners alone (McMaster et al., 2005). In this study, the most liked

Table 4. Sensory properties of commercial sour milk (*amasi*) products.

Sample	Sensory attributes						
	Sourness	Sweetness	Texture	Smoothness	Astringency	Colour	Overall acceptability
AoA	4.5± 0.97[a]	3.8± 1.23[ac]	4.0± 0.94[a]	4.3± 1.06[a]	4.3± 1.06[a]	4.4± 0.97[a]	4.4± 0.70[a]
AoB	3.7 ± 0.95[a]	3.1± 0.57[a]	3.8± 1.03[a]	3.7± 1.34[ab]	3.6± 1.51[ab]	4.2± 1.03[a]	4.0± 0.67[a]
AoC	3.7 ± 1.25[a]	2.7± 0.67[b]	3.8± 0.79[a]	3.6± 1.07[b]	4.0± 1.05[b]	4.3± 0.67[a]	3.8± 0.92[a]
AoD	3.7 ± 1.34[a]	4.0± 1.25[c]	3.9± 0.74[a]	4.3± 0.48[a]	3.8± 1.03[a]	4.5± 0.71[a]	4.3± 0.67[a]
AoE	3.3 ± 1.25[b]	3.3± 0.95[ad]	3.6± 1.07[a]	3.2± 0.79[bc]	3.1± 1.29[c]	4.0± 0.82[a]	3.8± 1.14[a]

Sensory characteristics were graded on the scale 1 = dislike very much; 5 = like very much. Mean values in the same column with different letters differ significantly from each other (p< 0.05).

sensory attribute was the colour with the scores between 4.0 and 4.5. The analysis of the aroma of dairy products is complex due the heterogonous nature of milk.

Sample astringency increased with storage time (Irigoyen et al., 2005). In contrast, Katsiari et al. (2002) found that storage did not significantly affect the sensory attributes of yoghurt samples. This can be true because the sour milk products examined in this study had different expiring dates. Moreover, their astringency was not affected by the storage period as the panelists did not judge the products based on the influence of storage period. However, significant differences existed between the examined sour milk products.

Conclusion

The outcome of this study showed the availability of *E. coli* in the sour milk products which can be of health concern to consumers. Lactic acid bacteria dominated the count in all the products in MRS media and the total plate count showed low bacteria count. However, the significant differences which existed on other parameters tasted did not affect the consumer acceptability as it was predicted.

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