Lactic acid fermentation of sour porridge and *mahewu*, a non– alcoholic fermented cereal beverage

C. Simango

Department of Medical Laboratory Sciences, University of Zimbabwe Medical School, P.O.Box A 178, Avondale. Harare. Zimbabwe.

The microbiological and acidic changes during the natural fermentation of *mahewu*, a non–alcoholic cereal beverage, and sour porridge were investigated. The presence of pathogenic yeasts in both products was also investigated. The study was carried out over a period of six months in the year 2000. The pH and total acidity as well as microbiological analysis were carried out at intervals of time during the fermentation period. There was a sharp decrease in pH in the *mahewu* and sour porridge broths in the first 12 to 24 hours of fermentation. Very little titratable acids were produced in the first 6 to 12 hours which was followed by a steady increase during the rest of the fermentation period. Enteric bacteria increased slightly in the first 12 hours but decreased sharply afterwards and could not be detected when the pH was around 3.5 whereas lactic acid bacteria predominated during the fermentation period. Yeasts increased in numbers as the pH dropped and were detected in lower numbers than lactic acid bacteria throughout the fermentation period.

The yeasts isolated in both *mahewu* and sour porridge broths were *Saccharomyces cerevisiae, Candida* species which included the pathogenic species, *C.glabrata, C.kefyr*, and *Zygosaccharomyces* species. The other pathogenic *Candida* species isolated in sour porridge broth only were *C.inconspicua* and *C.guilliermondii. Klebsiella pneumoniae, Enterobacter* species, *Escherichia coli and Serratia ficaria* were the enteric bacteria isolated in *mahewu* broth whereas *Klebsiella* and *Enterobacter* species were the enteric bacteria isolated in sour porridge. The lactic acid bacteria isolated in *mahewu* broth were *Lactobacillus* species, *Pediococcus pentosaceus, Lactococcus lactis* and *Leuconostoc lactis* whereas *Lactobacillus coprophilus* and *Leuconostoc lactis* were isolated in sour porridge broth.

Keywords: Fermentation, *mahewu*, sour porridge, pathogenic yeasts, bacteria.

Introduction

Lactic acid fermentation of cereal foods is one of the oldest methods of food processing and in Zimbabwe, *mahewu* and sour porridge are products of cereal lactic acid fermentation. There is growing interest in the use of traditional fermented

cereal foods for weaning with the view of increasing their energy and nutrient density and reducing pathogen contamination of the foods. *Mahewu* is a non–alcoholic fermented sour beverage popular among the Bantu people of Southern Africa. In Zimbabwe, *mahewu* is prepared in the traditional way at the household level by many families by fermenting a broth mixture of gelatinized maize meal flour with malt flour. The malt flour used can either be purchased from retail shops or can be prepared at home using cereal grains. *Mahewu* is produced at household level as well as on an industrial scale in Zimbabwe whereas sour porridge is only prepared at the household level mainly in rural areas. *Mahewu* is the most common traditional fermented food prepared and consumed by rural communities in Zimbabwe followed by sour porridge (Simango, 1997).

Traditional *mahewu* fermentation is a natural process and the malt flour added to the thin porridge provides the inoculum for the spontaneous fermentation process, brought about by lactic acid bacteria and yeasts. The main end product after fermentation of *mahewu* has been shown to be lactic acid, which results in the reduction of the pH of this beverage after fermentation (van Noort and Spence, 1976). *Mahewu* is a beverage that is consumed cold without further boiling to kill the microbes present after fermentation.

Traditional sour porridge fermentation is also a natural process brought about by lactic acid bacteria and yeasts which are present in the cereal flour used. Organic acids produced during the fermentation process result in the reduction of the pH of the porridge (Simango, 1995). *Mahewu* and sour porridge have antimicrobial substances which include organic acids such as lactic and acetic acids, produced during the fermentation process, which are inhibitory to many diarrhoea–causing bacteria (Simango and Rukure, 1991; 1992). The present study was performed to investigate microbiological and acidic changes that occur during the traditional fermentation processes of *mahewu* and sour porridge. The presence of opportunistic pathogenic yeasts in the fermenting broths was also investigated.

Materials and Methods

Preparation of mahewu *and sour porridge samples*

Mahewu broth was prepared using maize meal flour and sorghum malt flour, produced by a milling company in Zimbabwe. The *mahewu* broth was prepared by boiling a mixture of 100 g of maize meal flour and 2 litres of unchlorinated water for 15 minutes to gelatinize the starch. The cooked broth was cooled to 37°C and 50 g of sorghum malt flour was added to the broth and the contents were mixed thoroughly and transferred to a sterile 3 litre erlenmeyer flask. The *mahewu* broth was left to ferment at 25°C in a cooled incubator for 48 hours. The sour porridge broth was prepared by mixing 300 g of maize meal flour, produced by a milling company in Zimbabwe, and 2 litres of sterile unchlorinated water in a sterile 3 litre erlenmeyer flask. The broth mixture was left to ferment at 25°C in a cooled incubator for 72 hours.

Chemical analysis

The pH and total acidity of both products was determined at intervals of time up to 48 hours for *mahewu* broth and up to 72 hours for sour porridge broth. The pH of *mahewu* and sour porridge broths was measured and total acidity, expressed as lactic acid, was determined by titrating each broth with sodium hydroxide with phenolphthalein as indicator (AOAC., 1984).

Microbiological analysis

The broths were cultured for the enumeration of enteric bacteria, lactic acid bacteria, yeasts at intervals of time up to 48 hours for *mahewu* broth and up to 72 hours for sour porridge broth. Ten–fold serial dilutions of each *mahewu* broth sample were prepared in 0.1 percent peptone water. A glass spreader was used to surface–plate 0.1 millilitres of each serial dilution in duplicate on appropriate media.

MacConkey agar was used for the enumeration of enteric bacteria. The inoculated plates were incubated at 37^{o}C for 24 hours. Both lactose and non–lactose fermenting colonies typical of enteric bacteria, were counted. Lactose and non–lactose fermenting colonies with distinct morphological differences were selected and identified using the API 20E System (API System, France). The API 20E is a standardized identification system for enterobacteriaceae and other non– fastidious Gram–negative rods, which uses 23 miniaturized biochemical tests and a data base. de Man, Rogosa, Sharpe (MRS) agar was used for the enumeration of lactic acid bacteria. The inoculated plates were incubated at 37^{o}C in a candle jar for three days. Colonies of lactic acid bacteria with different morphologies were selected and tested for catalase production and Gram reaction. Colonies which were catalase negative, Gram positive and non–spore–forming were identified using the API 50 CHL System (API System, France). The API 50 CHL is a strip that enables the fermentation of 49 carbohydrates to produce a biochemical profile of the strain that is used in its identification. Yeasts were enumerated on Potato Dextrose agar. The inoculated plates were incubated at 25^{o}C for up to five days. Colonies with distinct morphological differences such as colour, shape and size were selected. Cells of the yeast colonies were examined under the microscope and presumptively identified yeasts were confirmed using the API ID32C System (API System, France). The API ID32C is an identification system for yeasts using standardized and miniaturized carbohydrate assimilation tests.

Results

The pH values and percentages of total acidity as well as the microbial counts of the *mahewu* broth are shown in Figure 1. There was a sharp decrease in pH in the first 12 hours of fermentation from about pH 6.0 to about pH 3.0. Thereafter, there was very little change in pH values up to 48 hours. There was very little titratable acids produced in the first six hours of *mahewu* fermentation but after this period there was a steady increase in titratable acids concentration to about 0.9 percent at 48 hours.

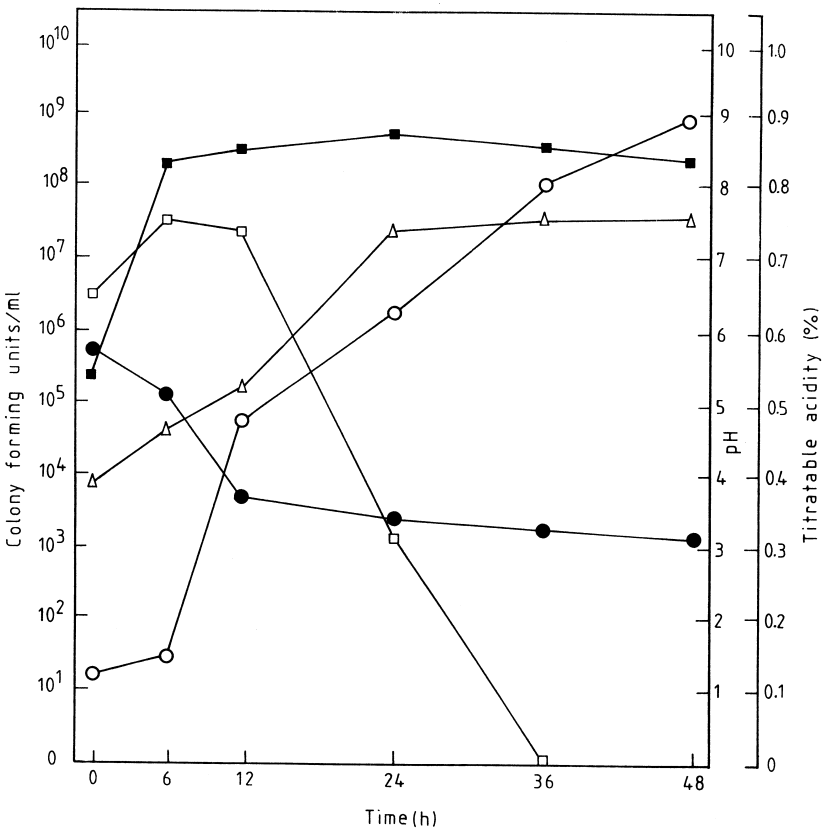

Figure 1: Fermentation profile of *mahewu* beverage □, Enteric bacteria; ■, Lactic acid bacteria; Δ, Yeasts; ○, percent titratable acidity; ●, pH.

There was a slight increase in numbers of enteric bacteria of about ten fold c.f.u. per ml of *mahewu* broth in the first six hours of fermentation. This was followed by a sharp decline in numbers of enteric bacteria from 12 hours onwards from very high cell counts of about ten million c.f.u. per ml and no enteric bacteria were detected at 36 hours where the pH of the *mahewu* broth was around pH 3.5. There was a sharp increase in numbers of lactic acid bacteria in the first six hours of fermentation of about one thousand fold c.f.u. per ml of *mahewu* broth after which their numbers remained constant. There was also a sharp increase in numbers of yeast cells of about one thousand fold c.f.u. per ml in the first 24 hours, followed by very little change in yeast cell numbers in the last 24 hours. Yeast cell counts were less than those of lactic acid bacteria in the *mahewu* broth during the fermentation period.

The pH values and percentages of total acidity as well as the microbial counts of the sour porridge broth are shown in Figure 2. There was a sharp decrease in pH of the sour porridge broth in the first 24 hours of fermentation from about pH 6.5 to about pH 3.5. Thereafter, there was very little change in pH values up to 72 hours. There was very little titratable acids produced in the first 12 hours of fermentation but after this period there was a steady increase in titratable acids concentration to about 0.5 percent at 72 hours.

There was a sharp increase in numbers of lactic acid bacteria of about one million fold c.f.u. per ml of sour porridge broth in the first 24 hours of fermentation. This was followed by very little change in numbers of the bacteria and there was a

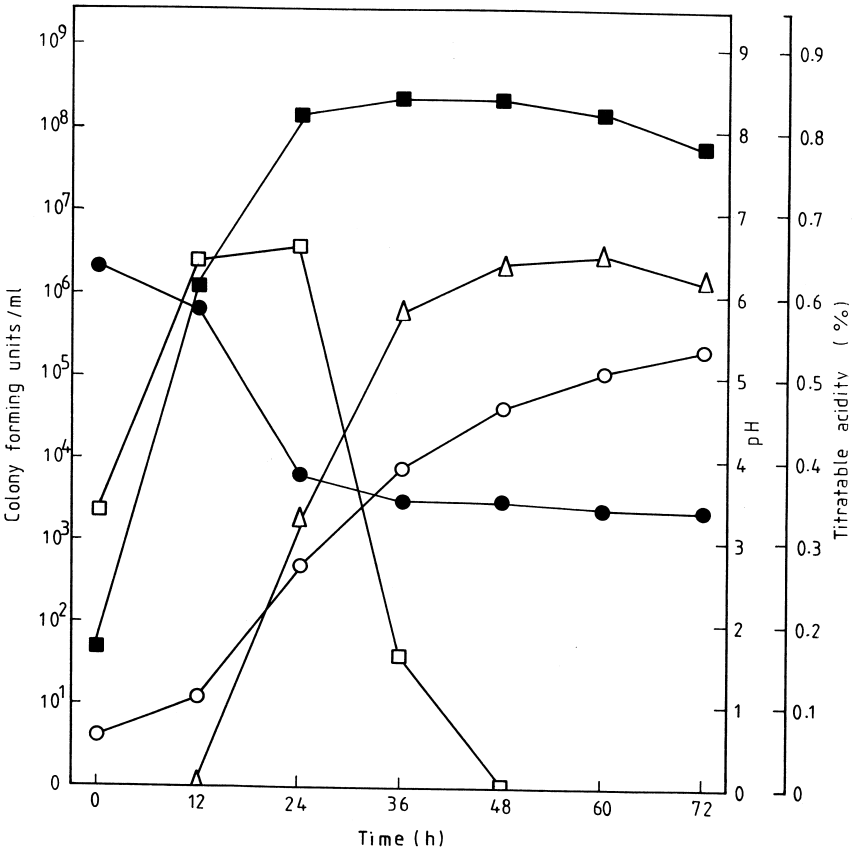

Figure 2: Fermentation profile of sour porridge broth. ■, Lactic acid bacteria; □, Entric bacteria; Δ, Yeasts; ●, pH; ○, percent titratable acidity.

slight decrease in numbers of lactic acid bacteria in the last 24 hours of fermentation. Yeast cells could not be detected in the first 12 hours of fermentation but there was a sharp increase in numbers of yeast cells of about one million fold c.f.u. per ml of sour porridge broth between 12 and 36 hours of fermentation. Thereafter, there was very little increase in numbers of yeast cells followed by a slight decrease in numbers of yeast cells in the last 12 hours of fermentation.

Enteric bacteria increased sharply from about three thousand to about three million c.f.u. per ml of sour porridge broth in the first 12 hours of fermentation but there was a sharp decline in numbers of surviving cells after 24 hours. No enteric bacteria were detected at 48 hours of fermentation when the pH value of the broth was around pH 3.5.

Table 1 shows the species of yeasts and bacteria isolated from *mahewu* broth during the fermentation process. The yeasts isolated were *Saccharomyces cerevisiae, Candida* species including the opportunistic pathogenic species *C.glabrata, C.kefyr*, and *Zygosaccharomyces* species. *Klebsiella pneumoniae*, *Enterobacter* species, *Escherichia coli* and *Serratia ficaria* were the species of the enteric bacteria present in the *mahewu* broth. The lactic acid bacteria isolated were *Lactobacillus* species, *Pediococcus pentosaceus, Lactococcus lactis* and *Leuconostic lactis*.

The species of yeasts and bacteria isolated from the sour porridge broth during the fermentation process are shown in Table 2. *Saccharomyces cerevisiae, Candida* species, including the opportunistic pathogenic species *C.glabrata, C.inconspicua, C.kefyr, C.guilliermondii,* and *Zygosaccharomyces* species were the yeasts isolated. *Klebsiella* and *Enterobacter* species were the enteric bacteria isolated whereas *Lactobacillus coprophilus* and *Leuconostoc lactis* were the lactic acid bacteria isolated.

Discussion

The fermentation of *mahewu* is a natural process and the malt flour provides the inoculum for the fermentation process as well as amylolytic enzymes which break down the starch into fermentable sugars such as dextrins and maltose. Some of the micro–organisms present in the malt flours may also be amylolytic. *Mahewu* produced in this way is both a fermented and malted product. Sour porridge is another fermented cereal product prepared at the household level in Zimbabwe and is a natural process brought about by lactic acid fermentation. The cereal flour used is the source of the micro–organisms like lactic acid bacteria and yeasts, involved in the fermentation process.

There was a succession of microbial growth in both *mahewu* and sour porridge broths during the fermentation process. Although the enteric bacteria initially increased in numbers in the early stages of the fermentation process, the acidic environment created was not favourable for the growth of enteric bacteria, which disappeared in the *mahewu* broth after 24 hours and after 48 hours in sour porridge broth during the fermentation process, when the pH was a little above 3.0 in both products. The acidic environment created by the lactic acid bacteria in both *mahewu*

Table 1: Species of yeasts and bacteria isolated from *mahewu* broth during the fermentation process.

Yeasts isolated (n = 24)		Enteric bacteria isolated (n = 23)		Lactic acid bacteria isolated n = 22)	
Species i	No. of solates	Species	No. of isolates	Species	No. of isolates
Saccharomyces cerevisiae	12	*Klebsiella pneumoniae*	8	*Lactobacillus brevis*	5
Candida glabrata	5	*Enterobacter cloacae*	7	*Lactobacillus buchneri*	2
Candida kefyr	2	*Enterobacter sakazakii*	2	*Lactobacillus curvatus*	2
Candida pelliculosa	2	*Escherichia coli*	4	*Lactobacillus plantarum*	1
Candida holmii	1	*Serratia ficaria*	2	*Pediococcus pentosaceus*	7
Zygosaccharomyces species	2	TOTAL	23	*Lactococcus lactis*	3
TOTAL	24			*Leuconostoc lactis*	2
				TOTAL	22

Table 2: Species of yeasts and bacteria isolated from sour porridge broth during the fermentation process.

Yeasts isolated (n = 24)		Enteric bacteria isolated (n = 23)		Lactic acid bacteria isolated (n = 21)	
Species	No. of isolates	Species isolates	No. of isolates	Species	No. of
Saccharomyces cerevisiae	13	*Klebsiella pneumoniae*	9	*Lactobacillus coprophilus*	18
Candida glabrata	5	*Klebsiella oxytoca*	3	*Leuconostoc lactis*	3
Candida inconspicua	3	*Enterobacter cloacae*	5	Total	21
Candida kefyr	1	*Enterobacter*			
Candida guilliermondii	1	*agglomerans*	4		
Zygosaccharomyces species	1	*Enterobacter sakazakii*	2		
Total	24	Total	23		

and sour porridge fermentation, which was unfavourable for the growth and survival of enteric bacteria, was favourable for yeast growth. Yeasts started to increase sharply once the pH of both *mahewu* and sour porridge broths had dropped to about pH 3.5. Lactic acid bacteria and enteric bacteria grew together at least in the first 24 hours of fermentation where the enteric bacteria may have contributed to the characteristics of the final product by producing organic acids, and volatile flavour compounds. In the present study, as the enteric bacteria were decreasing in numbers, lactic acid bacteria increased sharply in the early stages of fermentation and they predominated throughout the fermentation period.

The sharp increase in number of lactic acid bacteria was paralleled by a sharp decrease in the pH of *mahewu* and sour porridge broths. Since *mahewu* and sour porridge may be prepared under unhygienic conditions in rural settings where some of the ingredients used in its preparation can be contaminated with enteric bacterial pathogens or contamination can occur during storage, the pathogens do not survive long at a pH close to 3.5, making the foods unlikely sources of the bacterial enteric pathogens. *Mahewu* and sour porridge have been shown to have antimicrobial properties which are inhibitory to many diarrhoea causing bacteria (Simango and Rukure, 1991; 1992).

In South Africa the degree of sourness required to make *mahewu* acceptable has been shown to depend on individual preferences but is an average degree of acidity of 0.4 to 0.5 percent titratable acids at which acidity the average pH is 3.5 (Schweigart and Fellingham, 1963). The pH of 3.5 is similar to the pH of *mahewu* broth in the present study at which no enteric bacteria were detected. This suggests that the enteric bacteria, which may be present in the early stages of the fermentation, are no longer viable at the pH at which *mahewu* is ready for consumption. *Mahewu* prepared in rural homes in Zimbabwe had a similar pH of 3.6 (Simango and Rukure, 1991).

Although the growth of lactic acid bacteria stabilized at six hours in *mahewu* broth and at 24 hours in sour porridge broth and thereafter, there was little change in numbers of lactic acid bacteria but there was a steady increase in titratable acidity. There was a mixed population of lactic acid bacteria and yeasts in both *mahewu* and sour porridge broths in the late stages of fermentation. The co–existence of lactic acid bacteria and yeasts has been shown to be a common occurrence in cereal fermentations and this has been attributed to a symbiotic relationship where the lactic acid bacteria provide an acid environment for yeast growth, and the yeasts provide growth factors such as vitamins for lactic acid bacteria (Odunfa and Adeyele, 1985).

Some of the yeasts present in the fermented cereal foods can act as opportunistic pathogens in immunocompromised persons if the foods are consumed without further cooking after fermentation. In the present study, *Candida glabrata* was isolated in both *mahewu* and sour porridge broths. *C.glabrata* has been associated with mucosal and systemic infections in immunocompromised persons, which are difficult to treat and are associated with high mortality rates (Fidel *et al*., 1999).

The presence of this yeast in fermented foods like *mahewu*, which are consumed with live microorganisms without further boiling, is a cause for concern if the food is consumed by immunocompromised persons since the foods can be a source of *C.glabrata*. Since *mahewu* can be a source of opportunistic yeast pathogens such as *C.glabrata*, this beverage should be boiled before consumption by immunocompromised persons. *C.kefyr, C.inconspicua and C.guilliermondii*, which were also isolated in the fermented broths of both products, have been shown to be associated with rare human infections (Prasad *et al.*, 1999; Farina *et al.*, 1999). However, further research work is required to determine the prevalence of opportunistic pathogenic yeasts in *mahewu* or the raw materials such as malt flour, that are used to prepare the fermented food.

More species of lactic acid bacteria were observed in *mahewu* than in sour porridge broth. *Mahewu* fermentation was a faster process than sour porridge fermentation probably due to the amylase enzymes in the malt flour, which break down starch into fermentable sugars. *Lactobacillus coprophilus*, which was predominant in the lactic acid fermentation of sour porridge, may indicate its adaptation to grow in a starch–based broth medium although it is reported as not utilizing starch (Hammes and Vogel, 1995). However, according to Nout (1980) the multiplication of *Lactobacillus* species and other competing micro–organisms like yeasts and enteric bacteria in souring maize is favoured by fermentable sugars released by amylolytic enzymes originating from maize meal.

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