

DOI: 10.18697/ajfand.74.15305

**STANDARDIZATION OF CASSAVA *MAHEWU* FERMENTATION AND
ASSESSMENT OF THE EFFECTS OF IRON SOURCES USED FOR
FORTIFICATION**

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ABSTRACT

Cassava root is the main staple for 70% of the population in Mozambique, particularly in inaccessible rural areas, but is known to be low in iron. Anaemia is a public health problem in mothers and preschool children in Mozambique and up to 40% of these cases are probably due to dietary iron deficiency. The World Health Organization (WHO) and Food and Agriculture Organization of the United Nations (FAO) recognize the fortification of foodstuff as an effective method to remedy dietary deficiencies of micronutrients, including iron. Cassava *mahewu*, a non-alcoholic fermented beverage is prepared at subsistence level from cassava roots using indigenous procedures. The aim of the study was to standardize *mahewu* fermentation and investigate if the type of cassava fermented, or the iron compound used for fortification affected the final product. Roots of sweet and bitter varieties of cassava from four districts (Rapale, Meconta, Alto Molocue and Zavala) in Mozambique, were peeled, dried and pounded to prepare flour. Cassava flour was cooked and fermented under controlled conditions (45°C for 24 h). The fermentation period and temperature were set, based on the findings of a pilot study which showed that an end-point pH of about 4.5 was regularly reached after 24 h at 45°C. Cassava *mahewu* was fortified with ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) or ferrous fumarate ($\text{C}_4\text{H}_2\text{FeO}_4$) at the beginning (time zero) and at the end of fermentation (24 h). The amount of iron added to the *mahewu* was based on the average of the approved range of iron used for the fortification of maize meal. The mean pH at the endpoint was 4.5, with 0.29% titratable acidity. The pH and acidity were different to those reported in previous studies on maize *mahewu*, whereas the solid extract of 9.65% was found to be similar. Lactic acid bacteria (LAB) and yeast growth were not significantly different in *mahewu* fortified with either of the iron compounds. There was no significant difference between cassava *mahewu* made from bitter or sweet varieties. A standard method for preparation and iron fortification of cassava *mahewu* was developed. It is recommended that fortification occurs at the end of fermentation when done at household level.

Key words: Cassava *mahewu*, fermentation, ferrous fumarate, ferrous sulfate, iron fortification, Mozambique

INTRODUCTION

Cassava is consumed by more than 70% of the population in Mozambique and is considered the staple food [1]. A popular form in which cassava is consumed is as *mahewu*. *Mahewu* is a non-alcoholic fermented beverage consumed traditionally in African nations and Arabian Gulf countries [2]. In Africa, it is estimated that an adult black person consumes between 12 and 14 L of commercial *mahewu*. However, people across the continent provide for their needs by making home-made *mahewu* [2]. It is made by fermenting maize, sorghum and millet malt or wheat flour [2]. Spontaneous fermentation is frequently due to lactic acid bacteria (LAB) and yeast, with the former being the predominant fermentation agent [2]. Maize *mahewu* has been produced at commercial levels in African countries such as Botswana, South Africa and Zimbabwe [3-5]. At commercial level, maize *mahewu* has been enriched with minerals and vitamins to improve the nutritional value [6]. Fermentation promotes the bioavailability of minerals such as calcium, iron and zinc [2]. No published information on cassava *mahewu* fermentation could be found at the time of this study, but it is likely that, as for maize, fermentation would increase the protein concentration and the bioavailability of amino acids and minerals [7].

Anaemia is considered to be a serious public health problem in Mozambique and it is estimated that about 40% of cases are due to dietary iron deficiency [8]. Fortification of maize *mahewu* has proved effective in providing dietary iron. Ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) and ferrous fumarate ($\text{C}_4\text{H}_2\text{FeO}_4$) have been used for iron fortification of several food staples including maize flour in countries such as Brazil, Mexico and Venezuela [9]. Ferrous sulfate is a water soluble iron source, recommended for the fortification of de-germed maize flour and flours with a short shelf life [10]. Ferrous fumarate is poorly soluble in water and is also used for maize flour fortification [11]. The absorption of iron from fortified food depends on the chemical composition of the iron compounds used and the presence of enhancers [11]. Iron fortification of food with ferrous sulfate has been shown to significantly increase serum ferritin and hemoglobin levels and decrease dietary anaemia in women of reproductive age [12]. According to the WHO, iron compounds recommended for fortification of wheat and maize include ferrous sulfate, ferrous fumarate, and sodium iron EDTA (NaFeEDTA) [13]. Only ferrous fumarate and ferrous sulfate were commercially obtainable and were, therefore, used in this study.

The aim of the study was, therefore, to standardize *mahewu* fermentation and compare whether fermenting sweet or bitter cassava, the variety of cassava fermented, or the iron compound used for fortification affected the final product.

MATERIALS AND METHODS

Cassava

Cassava roots were collected from four different geographical regions in Mozambique where sweet or/and bitter types were predominant. Sweet cassava roots (two different varieties *Calamidade* and *Munhaça*) were collected from Meconta District in Nampula Province and Zavala District in Inhambane Province, respectively. Bitter cassava roots

(*Tomo* and *Incirricano* varieties) were collected from Rapale District in Nampula Province and Alto Molocue District in Zambézia Province, respectively.

Cassava flour preparation

Indigenous methods and implements were used to make cassava flour. Roots were peeled, washed with tap water, grated with a plastic grater and sun-dried for two to three days. The dried roots were manually pounded with a traditional wooden pestle and mortar and then sieved into flour, which was boiled with water to make porridge prior to fermenting. The proportions are elaborated on below.

Mahewu preparation

Cassava flour was fermented using the method described by Mutasa and Ayebo [3] and Bvochora *et al.* [4] for maize *mahewu*, with minor changes. Initially 20 g cassava flour was mixed with 49 mL of distilled water and added to 150 mL of boiling water. The mixture was boiled for 10 min to gelatinize the starch then cooled to a temperature of 25°C. The porridge was transferred to a 250 mL Erlenmeyer flask and 1.25 g of starter culture (freeze-dried cassava *mahewu* previously prepared traditionally), was added and mixed thoroughly.

Modifications of the methodology suggested by Mutasa and Ayebo [3] and Bvochora *et al.* [4] included the type of substrate used (20 g cassava flour instead of 15 g of maize or sorghum meal); starter culture (1.25 g of freeze dried cassava *mahewu* instead of 7.5 g of sorghum malt); temperature and fermentation period (24 h at 45°C and not 36 h at 30°C); and, iron fortification (no fortification was done with the maize *mahewu*).

A pilot study had determined that an end-point of approximately pH 4.5 was consistently reached after 24 h at 45°C, after which the pH stabilized [14]. Thus, the broth was fermented at 45°C for 24 h. During the fermentation period, changes in pH titratable acidity (acid concentration) and total solids were measured at hour 0 and hour 24. At the same time (hour 0 and hour 24) samples were collected for microbial analysis (aerobic mesophylic bacteria, LAB and yeast). The schematic flow of cassava *mahewu* preparation is presented in Figure 1.

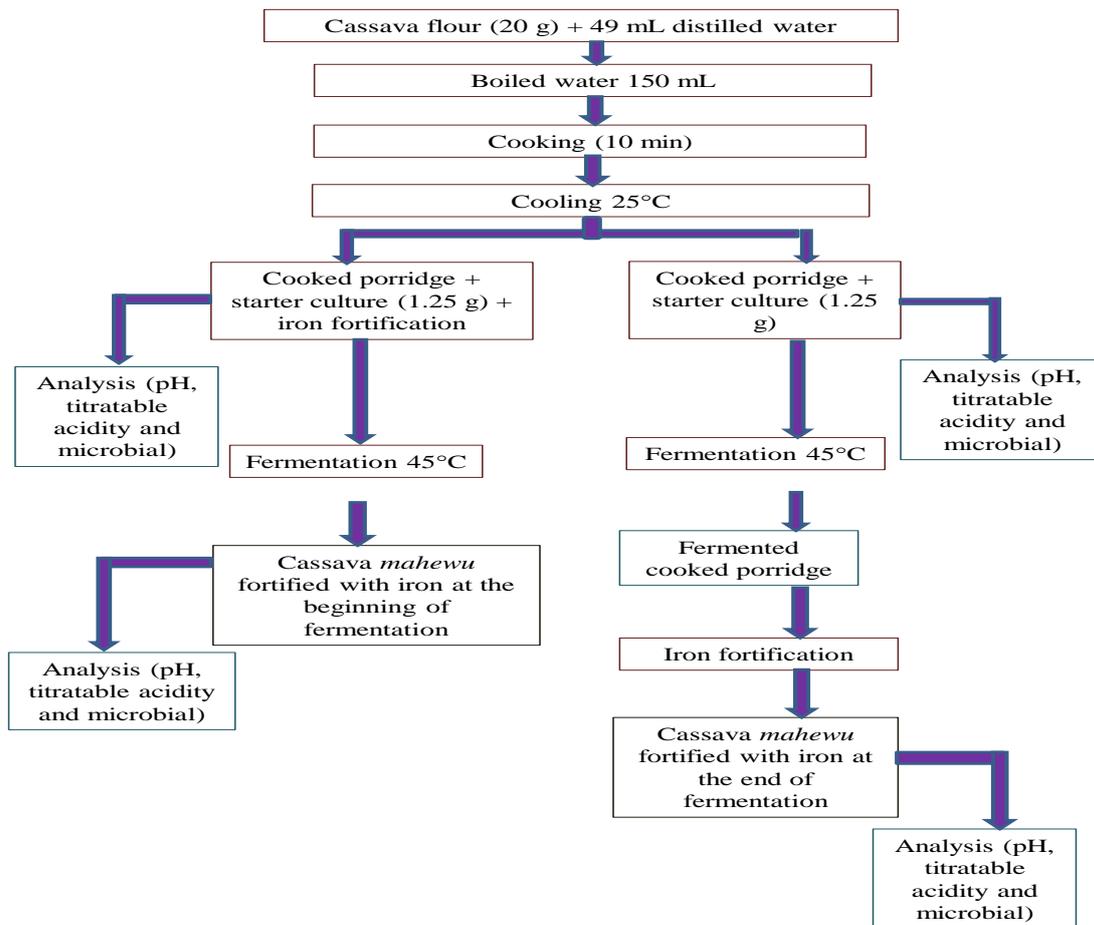


Figure 1: Schematic presentation of the preparation of cassava *mahewu* and indication of the stage at which iron is added

pH changes, titratable acidity and total solids

The pH of *mahewu* was measured just after the addition of the starter culture (time zero) and at hour 24 (end of the fermentation). The titratable acidity was determined by titration of 9 mL samples using 0.1 M NaOH with phenolphthalein as indicator. The concentration of acid was expressed as a percentage of lactic acid, according to the formula:

$$\% \text{ Lactic acid} = \frac{\text{Volume of 0.1M NaOH (mL)} \times \text{normality of NaOH} \times \text{Mol wt of acid}}{\text{Volume of sample (mL)} \times 10}$$

Total solids were determined using a freeze drying technique (Freezone 6.1, Labconco). Before freeze drying, the freeze drier cups were washed, dried and put into a desiccator overnight. The mass of the freeze drier cups was determined, after which *mahewu* was transferred and the cups were reweighed. The *mahewu* in the cups was freeze-dried over a period of 5 days, and then reweighed. To express the results on a dry weight basis the following formula was used:

$$\% \text{ Total solids} = \frac{\text{Mass of dry sample}}{\text{Mass of sample before freeze dry} \times 100}$$

Microbial analysis

Serial dilutions of 1 mL fermented *mahewu* were prepared and plated on to Nutrient Agar (NA), Malt extract Agar (MEA) and de Man, Rogosa & Sharpe (MRS) Agar (Merck Germany). The NA plates were incubated at 37°C for 24 h, to assess presence of mesophylic aerobic bacteria. The MRS agar plates were incubated at 37°C for 48 h under anaerobic conditions, to assess growth of LAB. The MEA plates were incubated at 25°C for 5 days and checked daily for presence of yeast colonies. Colony counts were done at the end of the incubation period.

Mahewu iron fortification

Cassava *mahewu* was fortified with two different iron sources, ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) and ferrous fumarate ($\text{C}_4\text{H}_2\text{FeO}_4$), at two different stages of preparation. These were at the beginning (time zero when the starter culture was added) and at the end of fermentation (hour 24).

The “overage”, which is the additional amount of the fortificant added to the flour, to compensate for storage and processing losses, was calculated as 1.2 mg [15]. The amount of fortificant (ferrous sulfate or ferrous fumarate) added was calculated by taking the amount of iron present in each fortificant into consideration, which was according to the chemical formula. This resulted in 6.8 g ferrous sulfate or 3.8 g ferrous fumarate being dissolved in 100 mL of distilled water of which 1 mL of the solution was added to the *mahewu*. For fortification at the end of the fermentation process (24 h), 6.2 g of ferrous sulfate or 3.45 g of ferrous fumarate, was dissolved in 100 mL distilled water and 1 mL of the solution added to the *mahewu*.

Samples taken to compare the effects of adding iron at the beginning or end of fermentation were in each case taken after the addition of the iron compound. That is to say sampling at time zero followed addition of iron and sampling at 24 h, was also done after iron fortification, using each iron compound (Figure 1). This was the reason why the amount of iron added at the beginning differed from the amount of iron added at the end of the fermentation process.

Statistical analysis

Mahewu was prepared in duplicate for each variety of cassava (bitter or sweet). Samples for each repeat of bitter or sweet variety of cassava were taken in duplicate, to test for pH changes and titratable acidity. During microbial analysis serially diluted samples (10^{-1} to 10^{-6}) of each repeat were plated in duplicate on each medium (NA, MRS and MEA). All data were analyzed using STATA version 12 with one-way analysis of variance (ANOVA) at 95% confidence level.

RESULTS

Table 1 shows the variation in pH, titratable acidity and total solids during fermentation of sweet and bitter cassava. *Mahewu* was fortified using two different sources of iron, added either before or after fermentation. During the fermentation process, as the pH decreased, the acidity increased and the microorganisms multiplied. When *mahewu* made from bitter and sweet cassava were compared, there was no statistically significant difference in pH changes and titratable acidity at the 95% confidence level. There was also no significant difference in pH changes and titratable acidity between *mahewu* fortified at the beginning of fermentation or fortified after 24 h ($p > 0.05$). The mean pH, with and without fortification, at the start of the fermentation process was 6.08 (range 5.87-6.28) and at the end it was 4.52 (range 4.5-4.58). The average total solids in both *mahewu* made from bitter and sweet cassava, with and without iron fortification, was 9.65% (range 8.90-10.22%). The mean acidity at the beginning of fermentation was 0.07% (range 0.05-0.09%) and after 24 h was 0.29% (range 0.24-0.35%). The type of iron used for fortification did not significantly affect pH, titratable acidity or total solids.

The microbial counts at time zero (beginning) and hour 24 (end) for the respective mediums, after the incubation periods, are presented in Table 2. There was a significant increase in microbial counts of aerobic mesophylic bacteria, LAB and yeasts from 0 to 24 h. There was no statistically significant difference ($p > 0.05$) between *mahewu* made from bitter cassava and sweet cassava, although microbial counts seemed to be higher in *mahewu* fortified at the beginning of fermentation with either ferrous sulfate or ferrous fumarate, (Table 2). Microbial counts appeared to be higher in the *mahewu* fortified with ferrous sulfate compared to *mahewu* fortified with ferrous fumarate, although there was no significant statistical difference. Fortified *mahewu* had slightly higher microbial counts when compared to the control (unfortified).

The parameters for the fermentation process are shown in Table 3. It can be seen that the mean pH of fortified cassava *mahewu* was 4.5 while the acidity was 0.29%. The mean solid content of standardized fermented cassava *mahewu* was found to be 9.65%.

DISCUSSION

The increase in LAB and yeast during fermentation from time zero to hour 24 (Table 2), suggested that these microorganisms were mainly responsible for the fermentation of the cassava *mahewu*. The importance of LAB and yeast in the fermentation of traditional foods, including cassava, has been described previously [5]. The association of LAB and yeast as fermentation agents, found in cassava *mahewu* is also in agreement with that which was previously reported for maize *mahewu* [16] and other fermented traditional drinks [17]. The LAB and yeast facilitate the breakdown of complex compounds, into simple and easily digestible substances that improve nutrient quality [18]. Yeast has also been related to the fermentation of other cassava products such as *agbelima* [19] and *fufu* [20]. Previous work has reported the influence of LAB and yeast in the development of typical characteristics including smell, taste, visual appearance, and consistency of fermented cassava foods [19].

The high solubility of ferrous sulfate in water [21] explains the relatively higher microbial counts observed in cassava *mahewu* fortified with this iron source, although the difference was not significant. The WHO has found ferrous sulfate to be more suitable for food fortification [22]. However, no significant difference in fermentation parameters was found between *mahewu* made from bitter or sweet varieties of cassava fortified with either ferrous sulfate or ferrous fumarate. It is important that iron fortification of the bitter variety was equally successful, as this type predominates in Mozambique due to drought and pest resistance [23].

Fermentation may also increase bioavailability of iron after fortification. Previous studies on cassava fermentation have reported an increase in the bioavailability of minerals such as calcium, iron and zinc [24]. Cassava *mahewu* is known to contain an organic acid (lactic acid) which could also potentiate iron uptake. According to Teucher *et al.* [25] fermented foods with high levels of organic acids are appropriate vehicles for iron fortification.

During cassava *mahewu* fermentation, as the LAB multiplied, the pH declined and acidity increased, probably due to the transformation of sugar into lactic acid during fermentation. The latter favours the growth of yeast, which proliferates at a lower pH. This finding is in agreement with Silva and Yang [26] who reported that pH is a key factor for growth of microorganisms. Cassava flour had been reported as being a good substrate for lactic acid production through fermentation [27].

The parameters of cassava *mahewu* in the present study differ from those observed in South African indigenous maize *mahewu*, which has been reported as having a pH between 2.74 and 3.5, with acidity between 0.4 and 0.5% [28]. Bvochora *et al.* [4] produced maize *mahewu* under controlled conditions and obtained an acidity of 0.25 % with a pH 3.5. Indigenous maize *mahewu* has been found to contain a solid substrate of 8 to 10% [3, 4], which was in agreement with the solid substrate found in the present study.

Cassava has a different type and proportion of carbohydrates to maize and may not ferment in the same way, causing a different end point pH and acidity to that described for maize *mahewu*. These may also have been affected by the temperature and time of fermentation, amounts and proportions of flour and water, or type and volume of starter culture used. The variable results reported for maize *mahewu*, may have been due to variations in quality and quantity of microorganisms in the starter cultures. In the current study, starter cultures were freeze dried, resulting in high repeatability during fermentation.

CONCLUSION

A standardized method was developed to prepare and fortify cassava *mahewu* under controlled conditions. It is recommended that fortification occurs at the end of traditional fermentation when done at household level. This should be done by providing consumers with sachets, containing sugar fortified with either of the iron sources investigated, to be added to the beverage just before consumption.

ACKNOWLEDGEMENTS

Financial assistance from the Graca Machel Scholarship Fund, the National Research Foundation and the Department of Food Science, University of Pretoria are gratefully acknowledged. Permission to undertake this study was granted by the Research Ethics Committee of the University of Pretoria (67/2013).

Table 1: pH changes, titratable acidity and total solids of cassava *mahewu*

Hours	Parameters	FeSO ₄ .7H ₂ O Fortification				C ₄ H ₂ FeO ₄ Fortification				Control	
		BTB ^a	SWB ^b	BTE ^c	SWE ^d	BTB ^a	SWB ^b	BTE ^c	SWE ^d	BT ^e	SW ^f
0	pH	6.05 ± 0.02	5.87 ± 0.30	6.18 ± 0.06	5.82 ± 0.05	5.99 ± 0.06	6.22 ± 0.18	6.1 ± 0.12	6.28 ± 0.13	6.13 ± 0.06	6.13 ± 0.00
	Acidity (%)	0.07 ± 0.01	0.08 ± 0.01	0.06 ± 0.00	0.05 ± 0.02	0.06 ± 0.01	0.05 ± 0.00	0.05 ± 0.03	0.06 ± 0.01	0.09 ± 0.01	0.08 ± 0.01
24	pH	4.5 ± 0.01	4.53 ± 0.01	4.58 ± 0.06	4.51 ± 0.13	4.46 ± 0.21	4.42 ± 0.24	4.5 ± 0.04	4.51 ± 0.13	4.57 ± 0.01	4.57 ± 0.01
	Acidity (%)	0.33 ± 0.05	0.35 ± 0.04	0.27 ± 0.00	0.29 ± 0.00	0.26 ± 0.02	0.29 ± 0.04	0.24 ± 0.01	0.27 ± 0.04	0.25 ± 0.00	0.3 ± 0.17
	Total solids (%)	9.71	10.22	8.9	9.95	9.56	9.55	9.52	9.76	9.82	9.51

^aBitter variety fortified at the beginning;

^bSweet variety fortified at the beginning;

^cBitter variety fortified at the end;

^dSweet variety fortified at the end;

^eBitter variety;

^fSweet variety

Table 2: Microbial counts (CFU/mL) and p values of iron fortified cassava *mahewu* at 0 h and 24 h fermentation

	FeSO ₄ ·7H ₂ O Fortification							C ₄ H ₂ FeO ₄ Fortification					C ₄ H ₂ FeO ₄ Fortification			
	Hour	BTB ^a	BTE ^b	p ^c	SWB ^d	SWE ^e	p ^c	BTB ^a	BTE ^b	p ^c	SWB ^d	SWE ^e	p ^c	BT ^f	SW ^g	p ^c
Aerobic	0	5.68 ± 0.02	5.7 ± 0.01	0.2	5.71 ± 0.06	5.56 ± 0.04	0.07	4.95 ± 0.11	4.8 ± 0.14	0.55	4.55 ± 0.01	4.8 ± 0.10	0.17	4.01 ± 0.00	4.1 ± 0.12	0.4
mesophilic bacteria	24	7.96 ± 0.10	7.67 ± 0.22	0.19	7.69 ± 0.15	7.15 ± 0.15	0	7.83 ± 0.04	7.57 ± 0.07	0.19	7.57 ± 0.60	7.45 ± 0.21	0.87	7.12 ± 0.02	7.59 ± 0.01	0
Lactic acid	0	3.86 ± 0.42	4.06 ± 0.00	0.62	4.21 ± 0.44	3.88 ± 1.05	0.8	3.95 ± 0.17	3.8 ± 0.01	0.46	3.56 ± 0.70	3.3 ± 0.01	0.69	3.59 ± 0.65	3.84 ± 0.04	0.64
bacteria	24	7.72 ± 0.26	7.31 ± 0.01	0.34	7.11 ± 0.25	7.53 ± 0.10	0.34	7.52 ± 0.25	7.51 ± 0.03	0.96	7.51 ± 0.02	7.5 ± 0.12	0.93	7.22 ± 0.05	7.17 ± 0.08	0.61
Yeast	0	3.41 ± 0.00	2.93 ± 0.32	0.28	3.28 ± 0.87	3.48 ± 0.49	0.66	3.86 ± 0.88	3.18 ± 0.80	0.67	3.52 ± 0.32	3.4 ± 0.54	0.59	3.86 ± 0.21	3.76 ± 0.00	0.56
	24	6.84 ± 0.46	6.83 ± 0.30	0.94	7.04 ± 0.19	6.71 ± 0.72	0.69	7.43 ± 0.06	7.46 ± 0.32	0.96	7.51 ± 0.01	7.25 ± 0.45	0.57	5.91 ± 0.51	6.82 ± 0.16	0.14

^aBitter variety fortified at the beginning;

^bBitter variety fortified at the end;

^cp value ($p < 0.05$ values within the same row are significantly different; $p > 0.05$ values within the same row not significantly different)

^dSweet variety fortified at the beginning;

^eSweet variety fortified at the end;

^fBitter variety;

^gSweet variety.

Table 3: Parameters for standardized preparation of iron fortified traditional cassava *mahewu* in Mozambique

	<i>Mahewu</i> fortified with FeSO ₄ ·7H ₂ O	<i>Mahewu</i> fortified with C ₄ H ₂ FeO ₄
Amount of cassava flour	20 g	20 g
Total amount of water	200 ml	200 ml
amount of starter culture	1.25 g	1.25 g
Fermentation temperature	45°C	45°C
Cooking time	10 minutes	10 minutes
Iron added at beginning	6.8 mg	3.8 mg
Iron added at end	6.2 mg	3.45 mg
pH before fermentation	5.98	6.15
pH after fermentation	4.53	4.47
% lactic acid before fermentation	0.065	0.055
% lactic acid after fermentation	0.31	0.27
Total solids (%)	9.7	9.6

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