AJFAND ONLINE
African Journal of Food, Agriculture, Nutrition and Development
Scholarly, Peer Reviewed
Volume 12 No. 6
October 2012
ISSN 1684 5374
Published by African Scholarly Science Communications Trust

CHANGES IN PHYSICO-CHEMICAL PROPERTIES AND FLAVOUR COMPOUNDS DURING FERMENTATION OF DIFFERENT OBUSHERA (SORGHUM AND MILLET) BEVERAGES

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AJFAND ONLINE Scholarly, Peer Reviewed ISSN 1684 5374
African Journal of Food, Agriculture, Nutrition and Development
Volume 12 No. 6
October 2012
Published by African Scholarly Science Communications Trust

ABSTRACT

Four types of Obushera, a fermented millet or sorghum beverage: *Ekitiribita*, *Obuteire*, *Obutoko* and *Enturire* were analyzed for their physico-chemical properties, sugars, organic acids and volatile compounds during fermentation. Standard chemical methods were used to determine the physico-chemical properties of Obushera. The organic acids, carbohydrates, glucose, fructose and maltose were analyzed by high-performance liquid chromatography. The pH of all Obushera varied between 4.00±0.10 and 4.42±0.11 after 4 days of fermentation. The pH of *Obuteire*, *Obutoko* and *Enturire* dropped below 4.5 within one day of fermentation. Acidity ranged between 0.13% (*Ekitiribita*) to 1.33% (*Enturire*) after 4 days of fermentation. Dry matter decreased and varied between 5.4 and 22% after 4 days of fermentation. Fermentation resulted into a 20–40% reduction of dry matter in *Obutoko, Obuteire* and *Enturire. Enturire* had the highest dry matter content and decreased from 22% to 13%. Fermentation resulted in an apparent increase in protein concentration (5.7–12.3%). Flavour compounds identified included sugars, organic acids, aldehydes, ketones, alcohols and esters. *Enturire* contained the highest ($p < 0.05$) concentrations of most flavor compounds. Sugars (maltose, glucose and fructose) were reduced during fermentation. The predominant organic acids detected were lactate, acetate and succinate and increased with fermentation time. Alcohols identified in Obushera were ethanol, 3-methyl-1-butanol, 2-methyl-1-propanol and 2-methyl-1-butanol and increased during fermentation. Ethanol was the predominating alcohol reaching a maximum of 0.8–1.0% (*Obuteire and Obutoko*) and 4.5% in *Enturire* during fermentation. The aldehydes detected in Obushera were acetaldehyde, 2-methyl-1-propanal, 2-methyl-1-butanal and 3-methyl-1-butanal and varied from 0.1 mg kg^{-1} to 6.8 ± 1.2 mg kg^{-1}. Diacetyl, acetoin and 2,3 pentanedione were the ketones identified in Obushera. Esters detected in Obushera included ethyl lactate, ethyl acetate, ethyl butyrate and isobutyl acetate. Significantly higher ($p < 0.05$) amounts of esters were detected in *Enturire* than in the other types of Obushera. Principal Component analysis, grouped Obushera into three categories (*Ekitiribita*, *Obuteire/Obutoko* and *Enturire*) based on flavour profile differences.

Key words: Fermentation, Obushera, Millet, Sorghum, Volatiles

INTRODUCTION

Fermented cereal products are important part of the diet and social life in several parts of Africa and Asia [1]. Fermentation is important in improving nutritional value, sensory diversity and product safety [2]. The past two decades have seen an increased interest towards the development of traditionally fermented products [3,4]. In Uganda, Obushera is one of the fermented cereal beverage that has received particular attention [4]. Obushera is a collective term for a number of traditionally produced sorghum (*Sorghum bicolor* (L.) *Moench*) and/or millet (*Eleucine coracana*) beverages from south-western Uganda. Obushera is consumed *ad libitum* as a source of energy, thirst quencher, weaning food, and social drink [5]. It sold at various points in towns and contributes to the household incomes. Although, Obushera production is becoming increasingly commercialized in urban areas, the processing is still largely an un-optimized artisanal craft and the procedure relies on chance fermentation leading to inconsistent quality and short shelf life. Limited technological control of the process limits large scale industrial production. Many fermented products have been documented in West Africa even some have been commercialized, however Uganda is lagging behind in studying its fermented products, putting into consideration their nutrition benefits.

Earlier studies focused on the microbial diversity of one type of Obushera and evaluation of selected lactic acid bacteria strains as starters [4, 6]. However, four distinct types of Obushera were described as *Ekitiribita, Obuteire, Obutoko* and *Enturire* [6]. Earlier studies revealed that flavor is the most important sensory attribute governing their acceptability[6]. Flavor is influenced by microbial metabolites such as organic acids and volatile organic compounds [3,4]. Raw materials have been shown to influence the flavor of the final product [6]. At present, there is no information on the development of metabolic compounds during fermentation of different type of obushera. To improve the safety and quality of Obushera necessitates the development of starter cultures by selecting the strains that produce desirable sensory characteristics [7]. The objective of this study was to determine the physico-chemical properties and profiles of organic acids and volatile compounds produced in the different types of Obushera during fermentation. The information generated can play an important role in the production of commercial starter cultures for large-scale production of obushera.

MATERIALS AND METHODS

Obushera samples

Four types of Obushera, *Obutoko* and *Enturire* from sorghum, and *Obuteire* and *Ekitiribita* from millet, earlier described [6] were used in this study (Table 1). Obushera, 12 independent fermentations, was prepared with the guidance of 12 experienced local producers from Kabale and Mbarara districts in Uganda. Samples were taken at day zero (day 0) and transported in 3 liter jerry cans on ice. The rest of the actively fermenting Obushera, in 20 liter plastic jerry cans, was transported at ambient temperature to the Department of Food Technology and Nutrition, Makerere University. Samples were withdrawn at intervals of 1, 2 and 4 days and frozen at -

20ºC prior to analysis. Samples for sugars and volatile compounds analysis were kept frozen and transported in Styrofoam boxes with dry ice to Norwegian University of Life Sciences, Biotechnology and Food Science Laboratories, Ås, Norway. Samples were thawed overnight at 4ºC prior to analysis.

Chemical analysis

Dry matter was determined by drying at 100ºC for 24 hours following AOAC method 967.03 [8] The dried samples were then subjected to ash determination following AOAC method 923.03 [9]. Crude protein was determined by the Kjeldahl method, AOAC 920.87 [10]. The conversion factors used were 5.65 for sorghum [11] Obushera and for millet 5.83 [12] Obushera. Total soluble solids content (ºBrix at 20ºC) was determined by a refractometer (Westover, Model RHB- 32, Westover Scientific Inc., Mill Creek, USA). The pH of the samples was determined using digital pH meter (HI 8314 membrane pH meter, Wagtech International Limited, Berkshire, England). Acidity (% lactate) was determined by titrating a 10 mL aliquot of Obushera supernatant against standardized 0.1 M sodium hydroxide according to AOAC method 942.15 [13]. Titratable acidity, total soluble solids and pH determined after centrifuging samples at 5000 g for 10 minutes (Fisher Scientific Centrific, Model 225, Pittsburgh, U.S.A) to obtain a clear supernatant. All samples were analyzed in triplicate.

Organic acids, sugars and volatile organic compounds

Organic acids, carbohydrates, glucose, fructose and maltose levels in Obushera were analyzed by high-performance liquid chromatography (HPLC) as described earlier [14]. Organic acids and sugars were detected with a UV detector set at 210 nm and refractive index detector (series 200 from Perkin Elmer, Norwark, CT, USA), respectively. Organic acids were identified basing on comparison of their retention times with standard solutions of citrate, orotic acid, pyruvate, succinate, DL-lactate, uric acid, DL-pyroglutamate, proprionate, acetate and formate. Identification of sugars was also based on retention times. All compounds were obtained from Merck KGaA, Darmstadt, Germany. Quantification was done using external calibration curves of mixed standards in de-ionized water.

The volatile organic compounds were determined by headspace gas chromatography (HS-GC) as described earlier [14]. The samples were analyzed using a Hewlett Packard HP 7694 automatic headspace sampler (Agilent, Santa Clara, CA, USA), a 6890 series GC system (Agilent Technologies) fitted with a Flame Ionisation Detector, a 900 Series Interface (Perkin Elmer, Shelton, CT, USA) and Turbocrom version 4.1 LC terminal (Perkin Elmer). Identification and quantification was based on external calibration with standard solutions of acetaldehyde, 2-pentanone, 2-butanone, ethylacetate (Fluka, Buchs SG, Switzerland); ethanol (Arcus, Oslo, Norway); 2-methyl-1-propanol, 2-methyl-1-butanol, 2-methyl-1-butanal, 3-methyl-1-butanol, 2-methyl-1-butanol, 2-methyl-1-propanol, 3-methyl-1-butanal, 3-methyl-1-butanol, 2-methyl-1-butanol, 2-methyl-1-propanal, diacetyl, 2-propanol, 1- butanol, 2-butanol, acetoin, iso-butylacetate, acetone, 2,3-pentanedione, ethylacetate, acetone, isobutyl acetate and ethyl butyrate (Merck KGaA, Darmstadt, Germany).

Statistical analyses

Data were subjected to one way analysis of variance (ANOVA) to test for significant differences ($p < 0.05$). Comparisons of means were done using the Fisher's LSD test. Principal Component Analysis (PCA) was performed on peak area data for volatile organic compounds, organic acids and sugars. The statistical package used was XLSTAT software (version 2010.5.02, Addinsoft, Paris, France).

RESULTS

Dry matter, protein, ash and total soluble solids

The changes in the physiochemical properties of Obushera are shown in table 2. The initial dry matter content of Obushera ranged from 5.4 to 22 %. *Enturire* had a significantly higher initial dry matter content ($p<0.05$) than the other types of Obushera. Dry matter contents of *Obutoko* (8.8%) and *Obuteire* (7.7%) were comparable ($p>0.05$) but higher ($p<0.05$) than in *Ekitiribita*. A reduction of 20 – 40% in dry matter was observed and more pronounced in *Enturire.* Initial protein content ranged from 5.7 to 7.1% and showed a near 2-fold increase ($p < 0.05$) in *Enturire*, *Obutoko* and *Obuteire* after four days of fermentation. The ash content of different Obushera varied between 2.6 and 3.1% and no significantly changes were observed during 4-day fermentation. Total soluble solids decreased except in *Ekitiribita* (Table 2).

Acidity and pH

The initial pH of Obushera observed in this study was lower than 5 (Table 2), except for *Ekitiribita* (pH 6.0), A drop in pH was observed with increasing titratable acidity (Table 2) in Obushera attributed to concomitant increase in lactate, acetate and succinate (Table 3). Apart from *Ekitiribita*, the pH of the other types of Obushera fell to 3.5 – 4.0 within 24 to 48 hours. The pH of *Ekitiribita* gradually decreased to 4.42 during the period of fermentation. The initial titratable acidity of Enturire (0.63%) was higher ($p < 0.05$) than the other types of Obushera. The lowest titratable acidity was noted in *Ekitiribita* (0.13%) during 4 days fermentation.

Sugars

The initial amounts of maltose (26 g kg^{-1}), glucose (62 g kg^{-1}) and fructose (46 g kg^{-1}) were significantly higher ($p < 0.05$) in *Enturire* than those of the other types of Obushera (Table 3). *Ekitiribita*, the type of Obushera from un-malted millet flour, had very low initial amounts of maltose (0.1 g kg^{-1}), glucose (0.1 g kg^{-1}) and fructose (0.2 g kg^{-1}). At the start of fermentation *Obutoko* (16 g kg^{-1}) had significantly higher (p <0.05) amounts of maltose than *Obuteire* (6.2 g kg^{-1}); however, their glucose and fructose levels were similar. Sugars decreased during fermentation, with glucose and fructose disappearing faster than maltose. However, *Ekitiribita*, showed a slight increase in maltose at the end of fermentation concurrent with an increase in total soluble solids (Table 2).

Organic acids

The organic acids identified in Obushera included: citrate, lactate, succinate acetate, pyruvate and propionate. Lactate, acetate and succinate (Table 3) were the predominant acids and increased as fermentation progressed. *Enturire* had the highest amounts of lactate (7,400–12,000 mg kg^{-1}), acetate (900–3,600 mg kg^{-1}) and succinate (900–1,350 mg kg^{-1}). Both *Obutoko* and *Obuteire* had amounts of lactate ranging from 2,200 to 6,000 mg kg^{-1} and acetate from 200 to 800 mg kg^{-1}. *Ekitiribita* had the lowest amounts of lactate (280–1680 mg kg^{-1}), acetate (140 – 250 mg kg^{-1}) and succinate (10–60 mg kg^{-1}). *Enturire* had significantly higher ($p<0.05$) levels of acetate than the other types of Obushera. Citrate decreased with progression of fermentation. Citrate was detected in higher amounts in millet-based Obushera (*Ekitiribita* and *Obuteire*), However, it was not detectable in a *Enturire*. The raw materials, both malted and un-malted millet flours contained considerable amounts of citrate (1000-1200 mg kg^{-1}) but not detectable in sorghum flour. Levels of pyruvate in Obushera were below 23 mg kg^{-1} whereas propionate ranged between 22 to 35 mg kg^{-1} in *Enturire*. Less than 5 mg kg^{-1} of pyruvate was detected in both *Obutoko* and *Ekitiribita*.

Volatile organic compounds

Results for volatile compounds detected in Obushera are shown in tables 4 and 5.

Aldehydes

The aldehydes were detected in Obushera were acetaldehyde, 2-methyl-1-propanal, 2-methyl-1-butanal and 3-methyl-1-butanal (Table 4). These compounds were initially higher ($p<0.05$) in *Enturire* than the other types of Obushera. Acetaldehyde decreased in *Enturire* (11 to 6.8 mg kg^{-1}) but increased in the other types of Obushera (0.1 – 0.4 to 2 – 6 mg kg^{-1}) during fermentation. Fermentation resulted in a significant rise ($p<0.05$) of 2-methyl-1-propanal (0.02 – 0.26 mg kg^{-1}) in *Obutoko* and *Obuteire*. A significant decrease in 3-methyl-1-butanal was observed in *Enturire* (0.05 – 0.01 mg kg^{-1}) but this compound did not significantly vary in *Obutoko* and *Obuteire*. In *Ekitiribita*, 3-methyl-1-butanal was not detected. Levels of 2-methyl-1-butanal and 3-methyl-1-butanal were below 0.03 mg kg^{-1} and 0.05 mg kg^{-1} respectively, after 4 days fermentation.

Ketones

The key ketones identified in Obushera were diacetyl, acetoin and 2,3 pentanedione and their evolution followed a similar trend during fermentation (Table 5). While diacetyl in sorghum Obushera decreased (1.7 – 0.2 mg kg^{-1}), an increase was observed in millet Obushera (0.2 – 2.4 mg kg^{-1}). Significant amounts of diacetly higher ($p<0.05$) were detected in millet at end of the fermentation. Acetoin levels were initially higher in *Obutoko* (11 mg kg^{-1}) and *Enturire* (16 mg kg^{-1}) than in *Ekitiribita* (3.3 mg kg^{-1}) and *Obuteire* (not detectable). Acetoin in *Obutoko* and *Enturire* decreased to values close to 5 mg kg^{-1} after four days of fermentation. An increase in acetoin was noted in millet Obushera with *Ekitiribita* showing the highest amounts (3.3 – 21 mg kg-1). Levels of 2,3 pentanedione were less than 0.35 mg kg-1 throughout the fermentation period.

Alcohols

The alcohols identified in Obushera, in order of decreasing quantity, included: ethanol, 3-methyl-1-butanol, 2-methyl-1-propanol and 2-methyl-1-butanol (Table 5).

Levels of the different alcohols were highest in *Enturire*, followed by *Obutoko* and *Obuteire*, and almost negligible in *Ekitiribita*. Significant alcohol production in *Obutoko* and *Obuteire* occurred after one day of fermentation with ethanol values reaching a maximum of 0.8–1.0% (w/w). Ethanol in *Enturire* ranged from 1.9–4.5% (w/w) during fermentation. The maximum amounts of 3-methyl-1-butanol, 2-methyl-1-propanol and 2-methyl-1-butanol detected in Obushera between 2 and 4 days of fermentation were in the range of 30–84 mg kg^{-1}, 11–26 mg kg^{-1} and 7.5–15 mg kg^{-1}, respectively.

Esters

Ethyl lactate, ethyl acetate, ethyl butyrate and isobutyl acetate were the esters identified in Obushera (Table 5). Esters were initially higher ($p < 0.05$) in *Enturire* than in the other types of Obushera. Ethyl lactate in *Enturire* increased from 110 to 330 mg kg^{-1} with a significantly increase noted after one day of fermentation. Ethyl acetate declined from 26 mg kg^{-1} to undetectable levels in *Enturire* at end of fermentation. However, it significantly increased ($p < 0.05$) after one day of fermentation in *Obutoko* (7.1 mg kg^{-1}) *and Obuteire* (13 mg kg^{-1}). Maximum amounts of ethyl butyrate and isobutyl acetate detected were 0.26 mg kg^{-1} and 0.14 mg kg^{-1}, respectively.

Principal Component Analysis of Obushera sugars, organic acids and VOC

Plots of principal component analysis carried out on sugars, organic acids and volatile organic compounds of Obushera are shown in Figures 1 and 2. In Figure 1, most of the variation (77.89%) was explained by the first two principal components with the first component (PC1) accounting for 64.31% of the variation. Principal Component One (PC1) in full divided Obushera into three groups, mainly on the basis of progress of fermentation (days 0 to the left with day 2 and 4 more to the right). Group 1 comprised - *Ekitiribita* (C) plus days 0 and 1 of both *Obutoko* (A) and *Obuteire* (D). These samples were characteristically low in concentrations of metabolites such as lactate, acetate, alcohols, aldehydes and esters. Group 2 comprised: days 2 and 4 of *Obutoko* (A) and *Obuteire* (D) which had moderate amounts of the above mentioned metabolites. Group 3 included *Enturire* with the highest amounts of most of the metabolites. Separation of day 0 samples of *Enturire* from the other three types of Obushera, along both PC 1 and PC 2, was due to the high starting amounts of sugars, acetaldehyde, 3-methyl-1-butanal, 3-methyl-1-butanal and ethyl butyrate.

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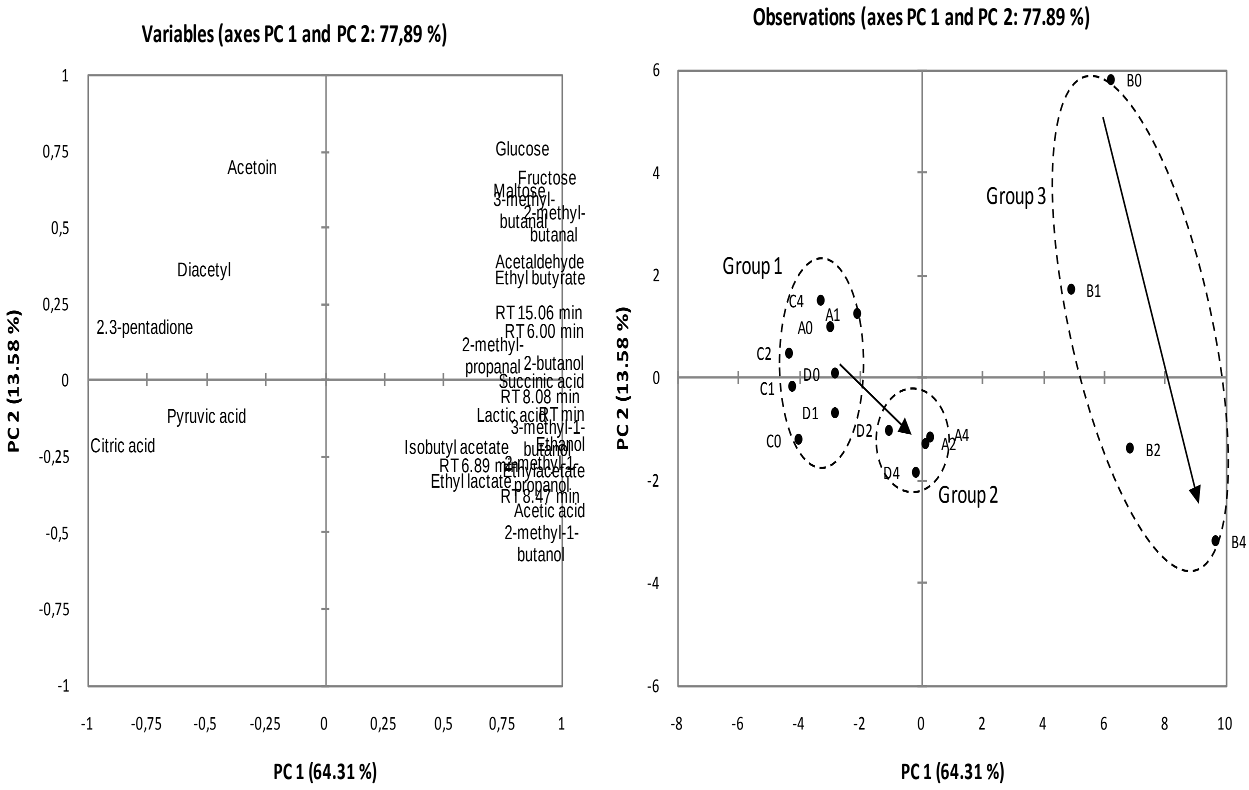

Figure 1: Principal component plot of sugars, organic acids and volatile organic compounds in the different types of Obushera: *Obutoko* (A); *Enturire* (B); *Ekitiribita* (C); *Obuteire* (D). The numbers 0, 1, 2 and 4 represent days of fermentation. Data points are a mean of 3 independent observations

Figure 2 shows the PCA output which eliminated the strong influence of *Enturire* (B) on the separation of the different Obushera. In this plot, PC1 (43.9% of the variation) separated samples according to the progress of fermentation. Principal Component 2 (PC2) separated the samples on the basis of raw materials into: *Ekitiribita* made from un-malted millet flour; *Obuteire* made from a combination of *Ekitiribita* and malted millet flour; and *Obutoko*, a product from malted sorghum flour. *Obutoko* was associated with having higher amounts of sugars than *Obuteire* and *Ekitiribita* whereas *Obuteire* and *Ekitiribita* had higher levels of citrate acid, 2.3 pentanedione, and diacetyl.

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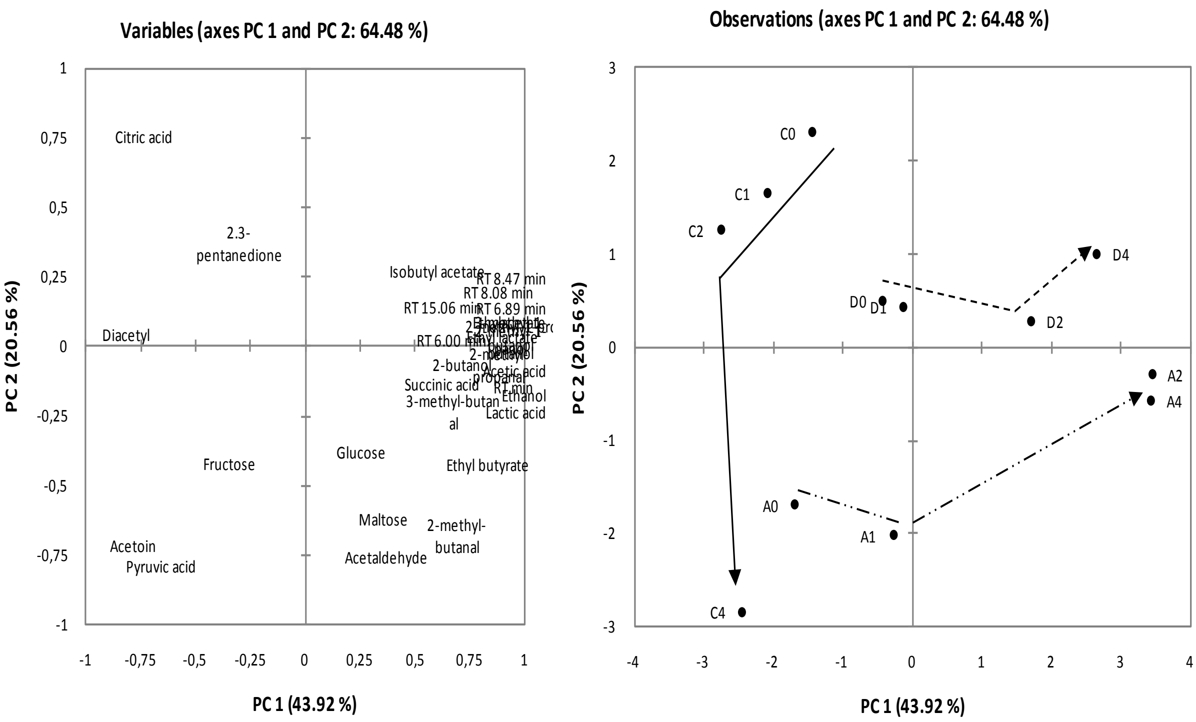

Figure 2: Principal component plot of sugars, organic acids and volatile organic compounds in the different types of Obushera excluding *Enturire*: *Obutoko* (A); *Ekitiribita* (C); *Obuteire* (D). The numbers 0, 1, 2 and 4 represent days of fermentation. Data points are a mean of 3 independent observations.

DISCUSSION

Dry matter, Total Soluble Solids (TSS), Protein, and Ash

The decrease in dry matter of cereal beverages has been reported during fermentation (6). Dry matter and TSS decreased by about 41 and 58%, respectively, during fermentation of o*bushera* [15]. The observed reduction of 20–40% in dry matter can be explained by degradation and oxidation of starch to derive energy required for metabolic activities during fermentation. However, during fermentation, the microrganisms involved may utilise the fermenting medium components such as starch liberation of sugars and hence increasing the energy densities in the diets. In some instance, some microganisms may utilise the available sugars and hence reduce the energy densities. The decrease of the TTS is attributable to the utilization of the fermentable sugars by fermenting flora [15]. The apparent increase in protein content during fermentation can be explained by the loss of dry matter [15,16, 17]. The utilization of fermentable sugars by the fermenting flora lead to loss in dry matter also the loss of some volatiles during the drying of the samples is a contributory factor. *Ekitiribita* is used as weaning beverage, however, cereal porridges with dry matter content of about 50 g kg^{-1}, cereal porridges are generally insufficient for use as weaning foods owing to their low nutrient and energy density [18]. Improvement in

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nutrient and energy density requires preparation of porridges with a dry matter content of at least 200 g kg^{-1} [18]. However, this introduces challenges of highly viscous products that are unsatisfactory for infant feeding unless their viscosity is reduced.

The changes in pH and titratable acidity are similar to findings by other researchers who have studied other fermented foods [4]. The slower pH decrease in *Ekitiribita* may be associated with the low initial numbers of fermenting flora as no malt is added to initiate the fermentation.

The changes in flavor compounds during the fermentation of four types of Obushera: *Ekitiribita*, *Obuteire*, *Obutoko* and *Enturire* showed large deviations within a given type of Obushera for most flavor compounds, emphasizing the variability occurring in the uncontrolled natural fermentations. This variability may have contributed to the differences in acceptability of given products[6]. Despite variations, the study has revealed similarities and differences between the four Obushera types. Such findings are important in understanding each products and could form the basis for their improvement in terms of quality**.**

Earlier, *Ekitiribita* was described as bland and possessing cereal and buttery aromas that give way to stale odours within two days of fermentation [6]. Unlike the other three types of Obushera, *Ekitiribita* is produced from flour of un-malted grains, which contains lower amounts of sugars than malted grain [8]. The low amounts of maltose, glucose and fructose in *Ekitiribita* which were below their taste threshold levels (13 g kg^{-1}, 14.4 g kg^{-1} and 7.2 g kg^{-1}, respectively) [19] explain its lack of sweetness. The exclusive use of un-malted millet flour in *Ekitiribita* without addition of malt, which initiates the fermentation, supplies fermentable sugars and amylolytic enzymes slows down the fermentation process [5, 20]. This results in delayed fermentation, slow and minimal development of acidity and flavor compounds that were noticed in *Ekitiribita.* Although the flavor characteristics of *Ekitiribita* may be acceptable to some consumers, the slow acidification (pH 4.22) jeopardizes its safety as an infant food. Fast acidification to pH of 3.5–4.0 is desirable as it inhibits pathogenic and spoilage microorganisms [4]. Inhibition may also be due to antimicrobials such as bacteriocins [21]. Studies on traditional fermented beverages reported coliforms during early fermentation stage and their disappearance as pH drops to 4.0 [4, 22]. In this regard, *Ekitiribita* is a potential vehicle for the transfer of pathogenic microorganisms especially if its prepared under unhygienic practices. This problem is aggravated by lack of post heat treatment prior to consumption. The buttery aroma, earlier reported for *Ekitiribita* [6] may be attributed to diacetyl and 2,3 pentanedione, which were present in amounts close to their odour thresholds of 0.2– 2.8 mg kg^{-1} and 0.03–1 mg kg^{-1}, respectively [23]. Diacetyl is an unstable intermediate in the decarboxylation of pyruvate to 2, 3 butanediol. Diacetyl synthesis is commonly attributed to activity of citrate positive *Lactococci*, *Lactobacilli* and yeasts [14].

In earlier studies, *Obuteire* and *Obutoko* were characterized as two distinct products [6]. However, in this study, these two types of Obushera showed similar properties and trends in the volatiles. From the PCA analysis, their separation was only evident after removing the strong influence of *Enturire* (Fig. 2). *Obuteire* and *Obutoko* deviated from *Ekitiribita* but did not develop strongly in intensity as *Enturire* (Fig. 1). Differences in raw materials and processing procedures could have contributed to the separation. This separation revealed the influence of raw materials on flavor development in Obushera whereas the similarity in trend (Fig. 2) and clustering (Fig. 1) of *Obutoko* and *Obuteire* suggested same flavour profile probably due to similar nature of fermenting microflora.

The sweet taste of *Obutoko* and *Obuteire* reported during the earlier stages of fermentation [6] could be attributed to the use of malt which influenced the liberation of sugars. Maltose, glucose and fructose levels were generally below their taste thresholds [19], but the combined effect of maltose and glucose could have elicited a sensation of sweetness. The malt added initiates fermentation and also introduces amylolytic enzymes which break down residual starch, increasing glucose and maltose in early stages of fermentation [22]. As fermentation progresses, these sugars are metabolized to acids and various flavor compounds [3].

Organic acids enhance the safety and shelf life of fermented products and also lead to the sour tastes [24]. However, excessive amounts of organic acids may have a negative impact on acceptability of the products. Acetate within a range of 700 – 1100 mg kg^{-1}, as observed after 4 days of fermentation, results in pungent vinegary taints [25]. Acetate is produced by lactic acid bacteria via citrate metabolism and oxidation of lactate under anaerobic conditions [25]. The production of acetate is also associated with oxidation of ethanol by acetic acid bacteria [25]. Succinate is known to cause bitter tastes when present in excess amounts and mainly produced from malate by yeasts and leuconostocs [24].

Aldehydes are a group of transient flavor compounds produced during alcoholic fermentation and have low taste thresholds [26]. Acetaldehydes, 2-methyl-1-propanal, 2-methyl-1-butanal and 3-methyl-1-butanal were all within their odour thresholds: 0.015 – 200 mg kg^{-1}, 0.18 mg kg^{-1}, 0.04 mg kg^{-1} and 0.06 mg kg^{-1}, respectively [23]. The aldehydes, 2-methyl-1-propanal, 2-methyl-1-butanal and 3-methyl-1-butanal are products of metabolism of valine, isoleucine and leucine, respectively and are associated with malty tastes [14]. The fruity notes reported in Obushera [6] may be attributed to the presence of acetaldehyde. Production of these malty aldehydes is mainly associated with yeasts and, to a less extent, heterofermentative lactic acid bacteria [24]. Under anaerobic conditions, methyl aldehydes are reduced to their corresponding higher alcohols [26]. The higher alcohols accumulated more than their parent aldehydes since carbon dioxide production during fermentation flushes out oxygen thus favoring their conversion [27]. The higher alcohols exceeded reported odour threshold values after 2 days of fermentation: 2-methyl-1-propanol (5.25 mg kg^{-1}), 2-methyl-1-butanol (5.50 mg kg^{-1}) and 3-methyl-1-butanol (4.75 mg kg^{-1}) [28]. Amounts below 300 mg kg^{-1} impart fruity notes to wine [24].

Ethanol is probably an important flavor compound in *Obuteire* and *Obutoko* since values between 500 and750 mg kg^{-1} enhance other flavors [29]. Ethanol increased beyond the taste threshold, 100–800 mg kg^{-1}, during the first day, confirming the described alcoholic notes [9]. *Obuteire* and *Obutoko* contained ethanol levels of 0.7–1.2 % w/w similar to low alcohol beers.

Esters in *Obuteire* and *Obutoko* significantly rose ($p < 0.05$) after 2 days of fermentation. Esters are products of the esterification of alcohols and organic acids by yeasts [24]. Ethyl acetate and ethyl butyrate increased to levels within their odour thresholds: 0.2–7.5 mg kg^{-1} and 0.001–0.2 mg kg^{-1}, respectively [23, 24]. The increase in esters coincided with an increase in organic acids and alcohols contributing to the fruity notes reported in *Obuteire* and *Obutoko* [6].

Obutoko differed from both *Obuteire* and *Obutoko* because it contained higher amounts of sugars and flavor compounds (Fig 2). *Enturire* was described to possess sweet-honey aromas before developing into a more alcoholic product with pungent, sour and bitter characteristics [6]. Addition of crude honey (13% w/w) to *Obutoko* during the processing of *Enturire* raises the dry matter and sugars. Honey contains substantial amounts of fructose (36–39%), glucose (33–38%) and maltose (9.8–11.7%) [30]. These sugars were present in *Enturire* in amounts above their taste threshold throughout the fermentation, accounting for the sweetness [19, 20]. The sugars, organic acids, alcohols, aldehydes and esters as determined, must have contributed to the overall flavor characteristics of *Enturire* since they were in amounts within their taste and aroma thresholds. The loss in sensory appeal of *Enturire* was attributed to over souring, pungency and development of bitter tastes [9]. Amounts of acetic acid observed in *Enturire* in this study (900–3600 mg kg^{-1}) were more than three times the values (700–1100 mg kg^{-1}) reported to cause vinegar taints in wine, which are associated with souring and pungency [23]. The bitter after-taste associated with *Enturire* could have resulted from succinate since it was in amounts 100-fold higher than its taste threshold (47.2 mg kg^{-1}) [23].

CONCLUSION

This study has revealed that there are distinct differences among the types of Obushera on the basis of physicochemical properties, organic acids and volatile organic compounds. The major compounds associated with each product can serve as a basis for further studies in the selection of appropriate starters with desirable flavor characteristics for their improvement. In this respect, the commercial importance can be viewed in the development and production of commercial starter cultures for large scale production of obushera. Controlled production of *Obutoko* and *Obuteire* may require use of strongly acidifying heterolactic lactic starters with ability to produce malty compounds and esters. For *Enturire*, its production requires the selection of strong lactic acid starters as well as yeasts capable of producing high amounts of alcohol and the other flavor compounds identified. The low dry matter content and acidification rate of *Ekitiribita* makes it rather unsatisfactory and unsafe for infant feeding. Its safety could be improved through the use of lactic acid bacteria starters with high acidification rates.

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ACKNOWLEDGEMENTS

We are very grateful to Kari Olsen for the expert technical assistance with the HPLC and HSGC analyses. This research was funded by the Norwegian Universities' Committee for Development Research and Education (NUFU, project 2007/10221) through the Norwegian University of Life Sciences, Ås, Norway and Makerere University, Kampala Uganda.

AJFAND ONLINE Scholarly, Peer Reviewed
African Journal of Food, Agriculture, Nutrition and Development
Volume 12 No. 6
October 2012
ISSN 1684 5374
Published by African Scholarly Science Communications Trust

Table 1: Description of different types of Obushera used in the study

Type	Raw materials	Main uses[a]
Millet Obushera		
Ekitiribita	Un-malted millet flour and water (≈ 1.5 kg/20 L)	• Mainly infant feeding • General consumption
Obuteire	Malted millet flour and fresh *Ekitiribita* (≈ 0.5 kg/20 L)	• General consumption • Infant feeding (when not too sour)
Sorghum Obushera		
Obutoko	Malted sorghum flour and water (≈ 4.5 kg/20 L)	• General consumption • Infant feeding (when not too sour)
Enturire	Honey and *Obutoko* (≈ 3 kg/20 L)	• Mainly a social drink for festivities

All samples were naturally fermented at room temperature for a period of four days. The production of these Obushera types is described in an earlier study [9]. [a]General consumption mainly includes intentions such as quenching thirst and hunger in addition to provision of energy.

Table 2: Changes in physicochemical properties of different types of Obushera during fermentation

Type	Day	Dry matter (% FW)	Protein (% DM)	Ash (% DM)	Soluble Solids (°Brix)	Acidity (% Lactate)	pH
Ekitiribita	0	5.40±0.64[c]	6.74±0.53[a]	2.60±0.51[a]	0.26±0.04[c]	0.03±0.01[a]	6.00±0.36[b]
	1	5.76±0.39[a]	6.99±0.18[b]	2.51±0.62[b]	0.29±0.13[c]	0.05±0.02[c]	5.44±0.29[c]
	2	5.37±0.48[a]	6.73±0.40[b]	2.55±0.39[b]	1.34±1.43[c]	0.12±0.04[c]	4.65±0.00[c]
	4	5.35±0.40[a]	6.92±0.43[c]	2.56±0.40[b]	1.67±1.68[c]	0.13±0.02[c]	4.42±0.11[c]
Obuteire	0	7.71±0.48[a]	7.07±0.79[a]	3.12±0.59[a]	5.27±0.30[a]	0.18±0.10[a]	4.61±0.56[a]
	1	6.71±0.52[a]	4.75±0.71[a]	3.28±0.55[ab]	5.51±0.61[a]	0.24±0.11[a]	4.39±0.20[a]
	2	7.07±0.22[a]	9.50±1.08[a]	3.74±0.61[a]	3.62±0.74[ac]	0.30±0.17[a]	4.16±0.23[b]
	4	6.20±0.06[a]	12.25±2.79[a]	4.47±0.95[a]	3.21±0.86[ac]	0.47±0.21[a]	4.01±0.18[b]
Obutoko	0	8.80±3.46[a]	6.52±0.12[a]	2.72±0.20[a]	6.89±2.90[a]	0.15±0.05[a]	4.81±0.20[a]
	1	7.28±2.98[a]	5.14±0.32[a]	2.88±0.38[ab]	6.20±2.46[a]	0.25±0.08[a]	4.27±0.10[ab]
	2	7.66±3.26[a]	9.32±0.53[a]	3.66±0.17[ab]	5.21±1.91[a]	0.35±0.09[a]	3.95±0.03[a]
	4	6.56±3.18[a]	11.33±1.63[ab]	4.41±0.70[a]	4.11±2.52[a]	0.46±0.18[a]	4.20±0.22[a]
Enturire	0	21.98±3.77[b]	5.65±3.88[a]	2.63±1.15[a]	17.43±6.36[b]	0.63±0.42[b]	4.66±0.72[a]
	1	18.31±4.27[b]	7.58±3.57[b]	3.47±1.29[a]	12.93±5.20[b]	0.84±0.20[b]	4.16±0.11[b]
	2	15.50±4.72[b]	8.93±4.35[a]	4.31±1.82[a]	11.39±5.55[b]	1.06±0.07[b]	3.93±0.02[a]
	4	13.55±3.57[b]	10.04±3.96[b]	4.80±1.83[a]	9.74±4.30[b]	1.33±0.10[b]	4.00±0.10[b]

Values are means ± standard deviation of three independent fermentations. Values for different types of Obushera on the same day with similar superscripts are not significantly different ($p>0.05$). FW- fresh weight; DM- dry matter.

AJFAND ONLINE Scholarly, Peer Reviewed
African Journal of Food, Agriculture, Nutrition and Development
Volume 12 No. 6
October 2012
ISSN 1684 5374
Published by African Scholarly Science Communications Trust

Table 3: Changes in sugars and organic acids of different types of Obushera during fermentation

Type	Day	Sugars (x 10^3 mg kg^{-1})			Organic acids			
		Maltose	Glucose	Fructose	Lactate (x 10^3 mg kg^{-1})	Acetate (x 10^3 mg kg^{-1})	Succinic (x 10^1 mg kg^{-1})	Citrate (x 10^1 mg kg^{-1})
Ekitiribita	0	0.1 ± 0.0^c	0.1 ± 0.1^b	0.2 ± 0.2^b	0.3 ± 0.3^b	0.1 ± 0.1^b	5.5 ± 6.8^b	47 ± 7.5^a
	1	0.1 ± 0.1^b	0.1 ± 0.1^b	< 0.1	0.7 ± 0.4^a	0.2 ± 0.1^a	3.6 ± 1.0^a	32 ± 16^a
	2	0.4 ± 0.5^a	< 0.1	nd	0.7 ± 0.6^b	0.1 ± 0.1^b	0.7 ±1.2^b	16 ± 14^a
	4	1.7 ± 2.9^a	0.1 ± 0.1^a	nd	1.7 ± 0.2^c	0.3 ± 0.1^b	0.9 ± 0.8^b	9.9 ± 3.4^a
Obuteire	0	6.2 ± 2.8^{bc}	8.4 ± 5.7^b	0.3 ± 0.0^b	3.2 ± 2.0^{ab}	0.2 ± 0.1^b	20 ± 6.6^b	20 ± 30^{ab}
	1	6.3 ± 2.7^b	7.7 ± 4.8^{ab}	0.2 ± 0.1^a	3.7 ± 1.7^a	0.3 ± 0.1^a	11 ± 6.1^a	19 ± 29^a
	2	3.3 ± 1.6^a	2.8 ± 1.8^a	0.1 ± 0.1^a	4.9 ± 2.1^b	0.4 ± 0.3^b	30 ± 17^b	16 ± 27^a
	4	0.7 ± 0.2^a	0.6 ± 0.3^a	< 0.1	6.0 ± 2.4^b	0.8 ± 0.5^b	44 ± 24^b	9.7 ± 17^a
Obutoko	0	16 ± 11^{ab}	9.7 ± 6.8 ^{b}	1.2 ± 0.3^b	2.2 ± 0.7^{ab}	0.2 ± 0.1^b	36 ± 31^{ab}	6.9 ± 1.2^b
	1	15 ± 11^{ab}	7.2 ± 5.0^{ab}	0.6 ± 0.2^a	3.7 ± 1.0^a	0.3 ± 0.1^a	16 ± 2.2^a	4.7 ± 8.1^a
	2	11 ± 9.8^a	5.4 ± 4.2^a	0.2 ± 0.2^a	5.1 ± 1.3^b	0.5 ± 0.2^b	34 ± 1.4^b	4.2 ± 7.3^a
	4	5.6 ± 6.5^a	1.6 ± 1.4^a	0.2 ± 0.2^a	5.9 ± 1.6^b	0.7 ± 0.5^b	56 ± 33^b	1.5 ± 2.5^a
Enturire	0	26 ± 7.0^a	62 ± 37^a	46 ± 34^a	7.4 ± 5.2^a	0.9 ± 0.5^a	90 ± 58^a	nd
	1	23 ± 12^a	38 ± 37^a	22 ± 24^a	8.6 ± 4.0^b	1.7 ± 1.0^b	104 ± 54^b	nd
	2	16 ± 15^a	27 ± 34^a	17 ± 24^a	10 ± 3.7^a	2.5 ± 1.7^a	128 ± 61^a	nd
	4	9.8 ± 14^a	14 ± 21^a	14 ± 21^a	12 ± 3.3^a	3.6 ± 2.5^a	135 ± 52^a	nd

Values are means ± standard deviation of three independent fermentations. Values for different types of Obushera on the same day with similar superscripts are not significantly different (p>0.05). nd: not detected.

AJFAND ONLINE Scholarly, Peer Reviewed
Volume 12 No. 6
October 2012
ISSN 1684 5374
African Journal of Food, Agriculture, Nutrition and Development
Published by ASSCAT African Scholarly Science Communications Trust

Table 4: Changes in aldehydes and ketones of different types of Obushera during fermentation

Type	Day	Aldehydes				Ketones		
		Acetaldehyde (mg kg^{-1})	2-ME-pro-al (x 10^{-1} mg kg^{-1})	2-ME-but-al (x 10^{-1} mg kg^{-1})	3-ME-but-al (x 10^{-1} mg kg^{-1})	Acetoin (mg kg^{-1})	Diacetyl (mg kg^{-1})	2,3 Pentad (x 10^{-1} mg kg^{-1})
Ekitiribita	0	0.1 ± 0.0^{b}	< 0.1	nd	Nd	3.3 ± 2.2^{a}	0.5 ± 0.6^{a}	0.7 ± 1.2^{a}
	1	0.1 ± 0.0^{b}	0.2 ± 0.4^{b}	nd	Nd	7.7 ± 4.2^{a}	1.3 ± 1.0^{a}	2.5 ± 2.3^{a}
	2	0.2 ± 0.1^{b}	nd	< 0.1	Nd	8.0 ± 7.9^{a}	2.4 ± 1.7^{a}	3.5 ± 2.1^{a}
	4	6.3 ± 6.1^{a}	nd	0.1 ± 0.1^{a}	Nd	21 ± 6.8^{b}	1.3 ± 0.3^{a}	0.5 ± 0.5^{b}
Obuteire	0	0.3 ± 0.1^{b}	0.3 ± 0.1^{b}	nd	0.1 ± 0.0^{b}	nd	0.2 ± 0.0^{a}	0.9 ± 0.1^{a}
	1	0.6 ± 0.5^{b}	0.3 ± 0.1^{b}	< 0.1	0.1 ± 0.0^{b}	3.2 ± 5.6^{a}	0.5 ± 0.4^{a}	1.4 ± 0.9^{a}
	2	2.1 ± 0.6^{b}	1.1 ± 0.2^{b}	0.1 ± 0.1^{a}	0.2 ± 0.1^{a}	5.7 ± 9.9^{a}	0.5 ± 0.5^{b}	1.3± 0.3^{b}
	4	2.7 ± 0.5^{a}	1.7 ± 0.4^{b}	0.1 ± 0.1^{a}	0.1 ± 0.0^{a}	1.3 ± 2.2^{a}	0.8 ± 0.4^{ab}	2.4 ± 1.2^{a}
Obutoko	0	0.4 ± 0.3^{b}	0.2 ± 0.2^{b}	< 0.1	0.1 ± 0.1^{b}	11 ± 17^{a}	1.7 ± 2.6^{a}	2.4 ± 4.2^{a}
	1	5.4 ± 8.9^{b}	0.6 ± 0.5^{b}	< 0.1	0.1 ± 0.1^{b}	11 ± 17^{a}	0.3 ± 0.2^{a}	0.4 ± 0.8^{a}
	2	1.9 ± 1.6^{b}	1.7 ± 1.3^{b}	< 0.1	0.1 ± 0.1^{a}	1.4 ± 2.4^{a}	0.2 ± 0.1^{b}	1.2 ± 1.3^{b}
	4	2.0 ± 1.3^{a}	2.6 ± 1.9^{ab}	0.1 ± 0.0^{a}	0.1 ± 0.1^{a}	4.5 ± 6.8^{a}	0.2 ± 0.2^{b}	1.5 ± 0.3^{ab}
Enturire	0	11 ± 5.6^{a}	5.3 ± 4.2^{a}	0.3 ± 0.2	0.5 ± 0.2^{a}	16 ± 0.8^{a}	1.0 ± 0.5^{a}	1.6 ± 0.8^{a}
	1	5.3 ± 4.3^{a}	4.1± 3.2^{a}	0.1 ± 0.1^{b}	0.3 ± 0.1^{a}	3.9 ± 1.2^{a}	0.9 ± 0.8^{a}	1.1 ±0 .3^{a}
	2	6.6 ± 2.7^{a}	5.4 ± 3.2^{a}	0.1 ± 0.1^{a}	0.2 ± 0.1^{a}	1.2 ± 2.0^{a}	0.3 ± 0.6^{b}	nd
	4	6.8 ± 1.2^{a}	6.1 ± 3.2^{a}	0.2 ± 0.1^{a}	0.1 ± 0.1^{a}	5.1 ± 1.1^{9a}	0.3 ± 0.6^{b}	nd

Values are means ± standard deviation of three independent fermentations. Values for different types of Obushera on the same day with similar superscripts are not significantly different (p>0.05). nd: not detected. 2-ME-pro-al (2-methyl-1-propanal); 2-ME-but-al (2-methyl-1-butanal); 3-ME-but-al (3-methyl-1-butanal); 2,3 Pentad (2,3 pentanedione).

AJFAND ONLINE Scholarly, Peer Reviewed
African Journal of Food, Agriculture, Nutrition and Development
Volume 12 No. 6
October 2012
ISSN 1684 5374
Published by African Scholarly Science Communications Trust

Table 5: Changes in alcohols and esters during fermentation of different types of Obushera

Type	Day	Alcohols				Esters			
		Ethanol (x 10^3 mg kg^{-1})	2-ME-pro-ol (mg kg^{-1})	3-ME-but-ol (mg kg^{-1})	2-ME-but-ol (mg kg^{-1})	ET-lactate (x 10^1 mg kg^{-1})	ET-acetate (mg kg^{-1})	ET-butyrate (x 10^{-1} mg kg^{-1})	BUT- acetate (x 10^{-1} mg kg^{-1})
Ekitiribita	0	< 0.1	< 0.1	< 0.1	< 0.1	Nd	< 0.1	nd	0.2 ± 0.3^a
	1	< 0.1	0.1 ± 0.0^b	< 0.1	< 0.1	Nd	< 0.1	nd	nd
	2	0.1 ± 0.0^b	0.1 ± 0.1^b	< 0.1	< 0.1	Nd	< 0.1	0.5 ± 0.8^a	0.1 ± 0.1^b
	4	0.1 ± 0.1^b	0.1 ± 0.1^b	0.1 ± 0.0^b	< 0.1	0.1 ± 0.1^b	0.1 ± 0.1^b	0.8 ± 1.2^a	0.1 ± 0.1^b
Obuteire	0	0.4 ± 0.4^b	0.4 ± 0.1^{ab}	0.7 ± 0.3a	0.2 ± 0.0^a	0.2 ± 0.0^a	0.7 ± 0.4^a	0.2 ± 0.1^a	nd
	1	0.7 ± 0.6^b	0.4 ± 0.3^b	0.8 ± 0.6^b	0.2 ± 0.1^b	0.2 ± 0.1^b	0.5 ± 0.4^b	0.1 ± 0.1^b	nd
	2	6.6 ± 3.7^b	7.7 ± 3.5^{ab}	22 ± 15^{ab}	4.3 ± 2.7^{ab}	3.0 ± 2.4^a	5.4 ± 4.5^a	0.4 ± 0.2^a	0.2 ± 0.2^b
	4	7.8 ± 7.9^b	13 ± 2.6^{ab}	39 ± 19^{ab}	7.7 ± 3.19^a	Nd	13 ± 9.8^a	nd	nd
Obutoko	0	0.8 ± 0.7^b	0.2 ± 0.2^b	0.5 ± 0.5^a	0.1 ± 0.1^a	0.4 ± 0.4^a	0.6 ± 0.6^a	0.1 ± 0.0^a	nd
	1	2.0 ± 1.1^b	1.3 ± 1.1^b	2.1 ± 1.9^b	0.5 ± 0.4^b	0.5 ± 0.2^b	1.0 ± 1.1^b	0.5 ± 0.5^{ab}	nd
	2	9.7 ± 7.9^b	12 ± 13^{ab}	36 ± 38^{ab}	8.7 ± 10^{ab}	3.3 ± 1.9^a	5.6 ± 3.1^a	1.2 ± 1.3^a	0.2 ± 0.2^b
	4	10 ± 32^b	11 ± 4.5^b	30 ± 13^b	7.5 ± 3.9^a	4.9 ± 1.8^b	7.1 ± 6.1^{ab}	1.5 ± 1.0^a	0.2 ± 0.1^b
Enturire	0	19 ± 18^a	12 ± 11^a	45 ± 43^a	8.7 ± 8.9^a	11 ± 12^a	26 ± 29^a	2.6 ± 2.9^a	0.5 ± 0.3^a
	1	28 ± 16^a	15 ± 13^a	64 ± 54^a	11 ± 9.5^a	13 ± 12^a	29 ± 22^a	1.2 ± 0.9^a	0.5 ± 0.3
	2	37 ± 24^a	21 ± 15^a	77 ± 60^a	15 ± 12^a	20 ± 17^b	9.3 ± 16^a	1.3 ± 0.8^a	0.7 ± 0.2^a
	4	45 ± 16^a	26 ± 9.5^a	84 ± 47^a	15 ± 8.7^a	33 ± 21^a	nd	1.3 ± 0.8^a	1.4 ± 0.6^a

Values are means ± standard deviation of three independent fermentations. Values for different types of Obushera on the same day with similar superscripts are not significantly different ($p>0.05$). nd: not detected. 2-ME-pro-ol (2-methyly-1-propanol); 3-ME-but-ol (3-methyly-1-butanol); 2-ME-but-ol (2-methyl-1-butanol); ET-lactate (Ethyl lactate); ET-butyrate (Ethyl butyrate), BUT-acetate (Isobutyl acetate).

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