Nutrition and Food Sciences Research Vol 8, No 4, Oct-Dec 2021, pages:45-52

Original Article

Stabilization and Preservation of a Traditional Sorghum-based Fermented Beverage

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Received: November 2020 **Accepted:** March 2021

ABSTRACT

Background and Objectives: Traditional fermented cereal beverages such as *Obushera* from sorghum and/or millet are commercialized owing to their popularity among consumers. However, *Obushera* separates into two phases after processing, which could be mistaken for spoilage. Additionally, *Obushera* has a limited shelf life of 4–7 days at room temperature. The objective of this study was to assess the potential of various stabilizers and preservatives for the production of acceptable shelf-stable *Obushera*.

Materials and Methods: Effects of seven treatments of 1) 0.4% xanthan gum, 2) 0.4% carboxymethyl cellulose, 3) *Lactobacillus rhamnosus* yoba, 4) 0.4% xanthan gum and *Lactobacillus rhamnosus* yoba, 5) 0.4% carboxymethyl cellulose and *Lactobacillus rhamnosus* yoba, 6) 0.4% xanthan gum and 0.4% carboxymethyl cellulose and *Lactobacillus rhamnosus* yoba, and 7) a control (no stabilizers) on sedimentation rate were assessed within 120 h at room temperature. Effects of four treatments of 1) pasteurization (90 °C for 10 min) and 0.25% xanthan gum, 2) pasteurization and 0.25% xanthan gum and 0.2% potassium sorbate and 3) pasteurization and 0.25% xanthan gum and 0.1% sodium-benzoate and 4) control (pasteurized with no additives) on shelf stability and consumer acceptability were investigated.

Results: Treatments with xanthan (sedimentation index of 0–25%) for stabilizing *Obushera* were significantly ($p < 0.05$) more effective than those with no xanthan (sedimentation index of 49–67%). Xanthan (0.25%) significantly ($p < 0.05$) improved consumer acceptability of *Obushera.* All preservation treatments ($p > 0.05$) prolonged the shelf life of the beverage up to four months. No microbial growth was detected in the products during storage while pH (3.7–4.0) and acidity (0.5–0.6%) did not change significantly ($p > 0.05$). All products were acceptable during storage.

Conclusions: *Obushera* and related products can be stabilized and preserved using xanthan (0.25%) and pasteurization (90 °C for 10 min) with no added preservatives.

Keywords: Sorghum, Fermented beverage, Obushera, Stabilization, Preservation, *Lactobacillus rhamnosus*

Introduction

Obushera refers to a group of traditionally fermented or unfermented alcoholic or non-alcoholic cereal beverages popularly consumed in southwestern Uganda (1). The beverages are used as common drinks, social drinks, energy drinks or weaning foods (2, 3). One of the common types of *Obushera* is *Enturire* (2). *Enturire* is produced by adding nearly 15% honey to a malted sorghum slurry and fermenting for four or more days (2, 4). It is an alcoholic effervescent beverage with a sweet-sour taste. This beverage is commonly consumed at social functions such as weddings (2, 3). Depending on the type, *Obushera* has pH, acidity and alcohol values of 3.2–4.42, 0.13–1.33% and 0–4.5%, respectively (4, 5). The shelf life of *Obushera* at room temperature is 4–7 days (2). Byaruhanga and Ndifuna (6) reported that refrigeration and pasteurization could extend the shelf life of *Obushera* by eight days or up to a month, respectively. However, most (90%) of the *Obushera* in the market in Kampala, Uganda, is packaged with no pasteurization and nearly 50% of this do not meet microbiological criteria for safety (7). Researchers have used preservatives such as calcium propionate, benzoic acid, sodium benzoate and potassium sorbate to extend the shelf life of fermented cereal products (8–11). Generally, no reports have been published on the use of chemical preservatives in extending the shelf life of *Obushera.*

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Obushera can naturally separate into two phases of a clear liquid upper phase and a lower phase with precipitated solid particles. Separation of cereal beverages is attributed to differences in densities of the particles and supporting liquids (12). Producers mask the separation by packaging the beverage in opaque containers and advising that the product must be shaken before consumption. However, new consumers of the product may confuse this separation with spoilage. Separation of the beverages may result in product rejection (13) and thus necessitating stabilization of such products. Hydrocolloids, including several microbial exopolysaccharides, are widely used to stabilize food products (13–15). Xanthan and carboxymethyl cellulose (CMC) are among the most commonly used hydrocolloids. Xanthan is an extracellular heteropolysaccharide widely used for thickening and stabilizing products (16). The compound is fast-hydrating, water-soluble and can be dissolved at room temperature (16). Xanthan demonstrates stability to various temperatures, ions, pH, enzymes and chemical reactivity (16,17). Carboxy methyl cellulose is a linear polymer, which is water soluble, negatively charged and shows moderate stability to acid (18). Lactic acid bacteria (LAB), including several species such as *Lactobacillus (L) rhamnosus*, produce exopolysaccharides (EPS) which can contribute to product stability and viscosity (19, 20). *L. rhamnosus* yoba was selected for this study since this probiotic strain has successfully been used in fermentation of *Obushera* (21). Despite their wide use in food products, xanthan, CMC and bacterial EPS have not been assessed as stabilizers for *Obushera*. The objective of this study was to investigate potential use of two hydrocolloids (xanthan and CMC) and an exopolysaccharide-producing starter culture (*L. rhamnosus* yoba) in preventing separation of *Obushera*. Moreover, the study assessed effects of preservatives (potassium sorbate and sodium benzoate) and pasteurization on the shelf life of *Obushera*. Stabilization and preservation of the fermented cereal beverages such of *Obushera* can improve their consumer appeal and marketability.

Materials and Methods

Sorghum malt flour, honey and food additives

Sorghum grains (SESO 3 variety) were provided by the National Semi-Arid Resources Research Center (NaSARRI), Serere, Uganda. Grains were washed thoroughly with pressurized potable water and steeped in water containing 2% of sodium hydroxide for 24 h to remove tannins and soften the grains for germination. Then, water was drained and grains rinsed with clean potable water. Grains were transferred onto trays and allowed to germinate at ambient temperature. Germination was halted when the rootlets of the grain were nearly 1 cm long by drying at 60 ˚C for 24 h using cabinet drier to achieve a moisture content of 14%. The malt was ground into flour using a wonder mill (Grote Molen Inc., Pocatello, USA) set at coarse output and sieved using a 500-µm sieve. Malt flour was stored in air tight containers. Purified black wattle honey from Arua District, Uganda, was purchased from Green and White Enterprises Ntinda, Kampala. Honey was stored in air tight containers at room temperature. Xanthan gum, CMC, potassium sorbate and sodium benzoate (Norbright Investments, China) were purchased from Desbro Uganda Limited, Uganda.

Starter cultures

In this study, *L. plantarum* MNC 21 and *Saccharomyces (S.) cerevisiae* MNC 21Y previously isolated from *Obushera* and stored in 15% glycerol at -40 °C were used (1). A co-culture of *L. rhamnosus* yoba and *Streptococcus thermophilus* was provided by Yoba for Life Foundation, Amsterdam, the Netherlands. Stock cultures were separately sub-cultured for three successive generations by transferring 1 mL of the culture into 100 mL of broth. de Man Rogosa Sharpe (MRS) broth (Laboratorios Conda, Madrid, Spain) was used for the LAB and yeast mold broth (YMB) (Laboratorios Conda, Madrid, Spain) was used for the yeasts. The LAB cultures were incubated at 30 °C for 24 h and the yeasts were incubated at 30 °C for 72 h. Then, broth was centrifuged at 7500× g for 10 min. Pellets were washed twice in sterile quarter strength Ringer's solution. Then, cells were suspended in 10 mL of sterile Ringer's solution containing 15% of glycerol and stored at -40 ˚C. Purity of the cells was checked using a microscope (020-518.500 DM/LS I/98, Leica, Germany). Enumeration was carried out after plating serial dilutions of the culture on MRS agar and incubating at 30 ˚C for 24 h. The stored suspensions included nearly 9 log cfu mL^{-1} of each LAB and nearly 7 log cfu/mL of the yeasts.

Stabilization studies

The first part of the study included assessing effects of various treatments on the stability (prevention of separation) of *Obushera*. The *Enturire* type of *Obushera* was produced by mixing 52.5 g of sorghum malt flour and 150 g of honey in 1 L of potable water (4). The mixture was heated to 90 °C using a batch pasteurizer with constant stirring and held at this temperature for 10 min. The slurry was cooled down to 30 °C and inoculated with nearly 6 log cfu/mL of *L. plantarum* MNC 21 and 4 log cfu/mL of *S. cerevisiae* MNC 21Y. The slurry was then incubated at room temperature (25–28 °C) for 24 h to produce *Enturire* (Figure 1). Seven treatments were assessed for their effects on sedimentation rate of *Obushera*, including (i) 0.4% xanthan gum, (ii) 0.4%

CMC, (iii) *L. rhamnosus* yoba, (iv) 0.4% xanthan gum and *L. rhamnosus* yoba, (v) 0.4% CMC and *L. rhamnosus* yoba, (vi) 0.4% xanthan gum and 0.4% CMC and *L. rhamnosus* yoba, and (vii) a control (no stabilizers). For the treatments with *L. rhamnosus* yoba, the strain was added at a rate of 6 log cfu/mL at the start of fermentation. In previous studies, inoculation of *L. rhamnosus* was carried out at 7.0–8.5 log cfu/ml and the maximum EPS production was observed at 8.0–9.0 log cfu/ml after 10–20 h of fermentation (22, 23). In a previous study, *L. rhamnosus* was inoculated into *Obushera* at nearly 6 log cfu/ml and grew to 8.8 log cfu/ml within 24 h at 21–25 °C (21). Therefore, *L. rhamnosus* inoculated at 6 log cfu/ml in this study was sufficient to achieve the necessary cell concentration for EPS production. Moreover, the product was stored for 120 h allowing for further microbial activity. Product stability to sedimentation was assessed by calculating the sedimentation index (IS %). A follow-up experiment was used to assess effects of various concentrations of xanthan (0.25, 0.3 0.35 and 0.4%) since xanthan was verified as a better treatment than other treatments in stabilizing *Obushera*.

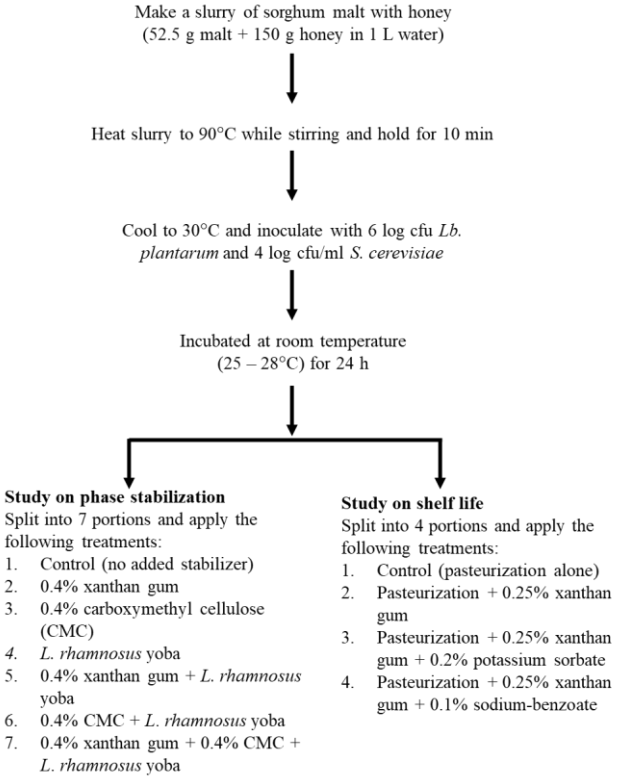

Figure 1. Procedures followed in the production of *Obushera* and assessment of the effects of stabilizers and preservation regiments

Assessment of shelf life

Obushera was prepared as described previously using a combination of *L. plantarum* MNC 21 and *S. cerevisiae* MNC 21Y starter cultures (Figure 1). After fermentation, products were pasteurized at 90 °C for 10 min. Then, effects of four treatments on the beverage shelf stability and consumer acceptability were investigated. Treatments included (i) pasteurization alone (control), (ii) pasteurization and 0.25% xanthan gum, (iii) pasteurization and 0.25% xanthan gum and 0.2% potassium sorbate, and (iv) pasteurization and 0.25% xanthan gum and 0.1% sodium benzoate. All samples were stored in a dark place at room temperature (25–28 °C) for four months. Samples were analyzed at weeks 0, 1, 2, 4, 8 and 16 for microbial growth, pH, titratable acidity and consumer acceptability.

Analyses

Sedimentation index: To assess stability, samples (200 ml) were transferred into transparent crown capped glass bottles and set at room temperature for 120 h. The total height of the liquid head (H_0) and upper clear liquid phase (H_t) were measured in mm at Hours 0. 12, 24, 48, 72, 96 and 120. Furthermore, IS (%) was calculated as $100 \times H_t / H_0$ (24). Figure 3a shows stored *Obushera* samples and dimensions of H_0 and H_t.

Microbiological analyses: To assess microbial stability during storage, total plate counts and fungal counts were carried out. Total plate counts were carried out by pour plating 1 mL of selected serial dilutions of *Obushera* in sterile plate count agar (Laboratorios Conda, Madrid, Spain) and incubating the plates at 37°C for 24 h according to ISO 4833-1 (25). Yeasts and molds were enumerated by surface plating 0.1 mL of each selected dilution on potato dextrose agar (PDA) (Laboratorios Conda, Madrid, Spain) acidified with 1% lactic acid, followed by incubation at 25 °C for 5 days based on standards of ISO 21527-2:2008 (26).

Titratable acidity and pH: Titratable acidity was assessed by titrating 10 mL of *Obushera* against a standardized solution of 0.1 M NaOH with phenolphthalein as an indicator based on the AOAC official method no. 942.15 (27). The pH was measured using digital pH meter (pH 98107, USA).

Consumer acceptability: **An untrained consumer panel (*n* = 50) comprising of** regular consumers of *Obushera* was used to assess product acceptability. Panelists ranked the overall acceptability of *Obushera* using a 9-point hedonic scale (9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely). Commercial bottled water was used to rinse the mouth between the sample tasting sets.

Statistical analysis*:* All experiments were carried out in triplicate. Means of IS, titratable acidity, pH, alcohol content, microbial counts and consumer acceptability were

analyzed using one-way analysis of variance to report significance differences at an α level of 5%. Tukey's HSD test was used to separate the means. Data were analyzed using XLSTAT Software 2017 (Addinsoft, New York, USA).

Results

Stabilization of Obushera

Figures 2 and 3a show effects of various stabilizers on the IS of *Obushera* during storage. Xanthan was significantly (p < 0.05) more effective than CMC at controlling separation of *Obushera* during storage. The highest IS was observed in samples containing *L. rhamnosus* as the stabilizing agent. Sedimentation was not detected in two treatments of (i) 0.4% xanthan gum and (ii) 0.4% xanthan gum and *L. rhamnosus* yoba (e.g., IS 0%). The IS increased during storage for all other treatments reaching a maximum of 25% for 0.4% xanthan gum, and 0.4% CMC and *L. rhamnosus* yoba. Other treatments such as the control had maximum IS of 49–67%. Since xanthan was more effective at preventing separation of *Obushera*, a follow-up experiment was carried out to assess effects of various concentrations of xanthan (0.25, 0.3, 0.35 and 0.4%). Sedimentation was not observed for any of the four concentrations of xanthan (Figure 3b).

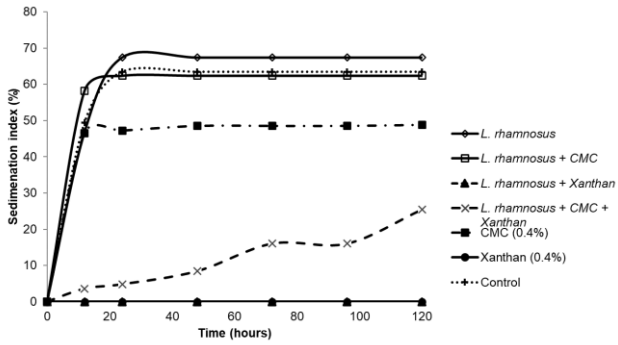

Figure 2. Effects of various treatments on the sedimentation index of *Obushera* at room temperature (25–28°C). Values are means of three independent fermentations

Shelf-life studies

No microbial growth was detected in *Obushera* samples subjected to the various preservation treatments through four months of the shelf-life study (results not shown). The pH (3.7–4.0) and titratable acidity (0.5–0.6%) of *Obushera* were constant (p > 0.05) through the storage (Table 1). Addition of preservative did not significantly affect pH and acidity.

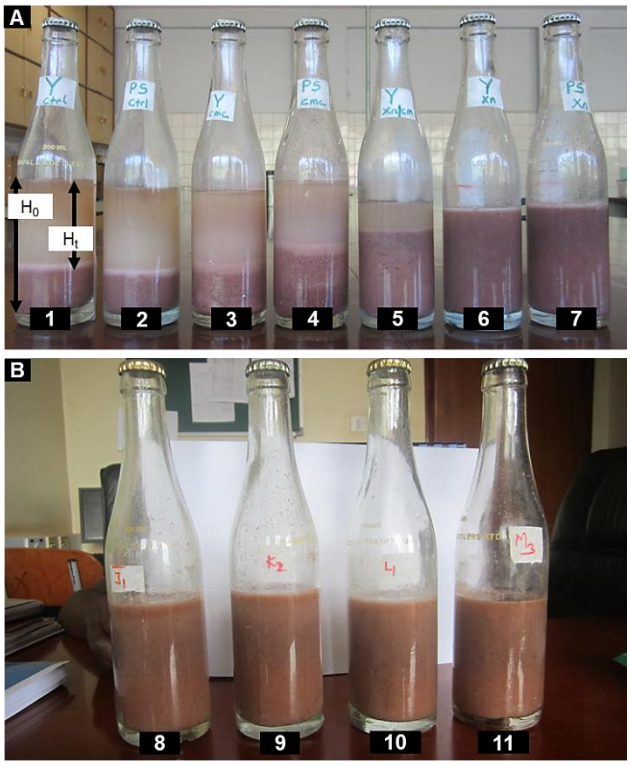

Figure 3. Effects of various treatments on the stability of *Obushera* stored at room temperature (25–28 °C) for 120 h. Figure 2a: 1, *L. rhamnosus* yoba; 2, control (no stabilizer); 3, 0.4% CMC and *L. rhamnosus* yoba; 4, 0.4% CMC; 5, 0.4% xanthan gum and 0.4% CMC and *L. rhamnosus* yoba; 6, 0.4% xanthan gum and *L. rhamnosus* yoba and 7, 0.4% xanthan gum. Figure 2b: 8, 0.25% xanthan; 9, 0.3% xanthan; 10, 0.35% xanthan and 11, 0.4% xanthan

Results of changes in overall acceptability scores of the preserved *Obushera* are shown in Figure 4. The control (pasteurized with no additives) had the lowest (p < 0.05) overall acceptability scores ranging between 4 (dislike slightly) and 5 (neither like nor dislike). Addition of stabilizers and preservatives increased the overall consumer acceptability scores. With the exception of the control, all *Obushera* samples were acceptable through 16 weeks of storage with scores above 5 (neither like nor dislike). *Obushera* with potassium sorbate had higher acceptability scores than that *Obushera* with sodium benzoate.

Table 1. Effects of preservation treatments on changes in pH and titratable acidity (TA %) of *Obushera* during storage at room temperature (25–28 °C)

Parameter	Treatment	Storage time (week)					
		0	1	2	4	8	16
pH							
	Control (P)	3.9 ± 0.0aA	3.9 ± 0.1aA	3.9 ± 0.1aA	3.9 ± 0.0aA	3.7 ± 0.1aA	3.7 ± 0.1aA
	P + 0.25% Xanthan	3.8 ± 0.0aA	3.8 ± 0.1aA	3.7 ± 0.1aA	3.7 ± 0.0aA	3.7 ± 0.0aA	3.7 ± 0.0aA
	P + 0.25% Xanthan + 0.2% K-sorbate	4.0 ± 0.1aA	4.0 ± 0.0aA	3.9 ± 0.0aA	3.8 ± 0.0aA	3.9± 0.1aA	4.0 ± 0.0aA
	P + 0.25% Xanthan + 0.1% Na-benzoate	3.9 ± 0.0aA	3.9 ± 0.0aA	4.0± 0.1aA	4.0 ± 0.1aA	3.9 ± 0.2aA	3.9 ± 0.1aA
TA %							
	Control (P)	0.6 ± 0.0aA	0.6 ± 0.0 aA	0.6 ± 0.1aA	0.6 ± 0.1aA	0.6 ± 0.0aA	0.6 ± 0.0aA
	P + 0.25% Xanthan	0.5 ± 0.1aA	0.5 ± 0.0 aA	0.5 ± 0.0aA	0.5 ± 0.0aA	0.5 ± 0.0aA	0.5 ± 0.0aA
	P + 0.25% Xanthan + 0.2% K-sorbate	0.5 ± 0.1aA	0.5 ± 0.0 aA	0.5 ± 0.0aA	0.5 ± 0.0aA	0.5 ± 0.0aA	0.5 ± 0.0aA
	P + 0.25% Xanthan + 0.1% Na-benzoate	0.5 ± 0.0 aA	0.5 ± 0.0 aA	0.5 ± 0.0aA	0.5 ± 0.0aA	0.5 ± 0.0aA	0.5 ± 0.0aA

Values are means ± standard deviations of three independent fermentations. TA: Titratable acidity. Preservatives are K-sorbate: Potassium sorbate and Na-benzoate: Sodium benzoate. P = Pasteurized (90 °C for 10 min). Values in the same row and column for a given parameter with similar superscript letters [a] or [A], respectively are not significantly different.

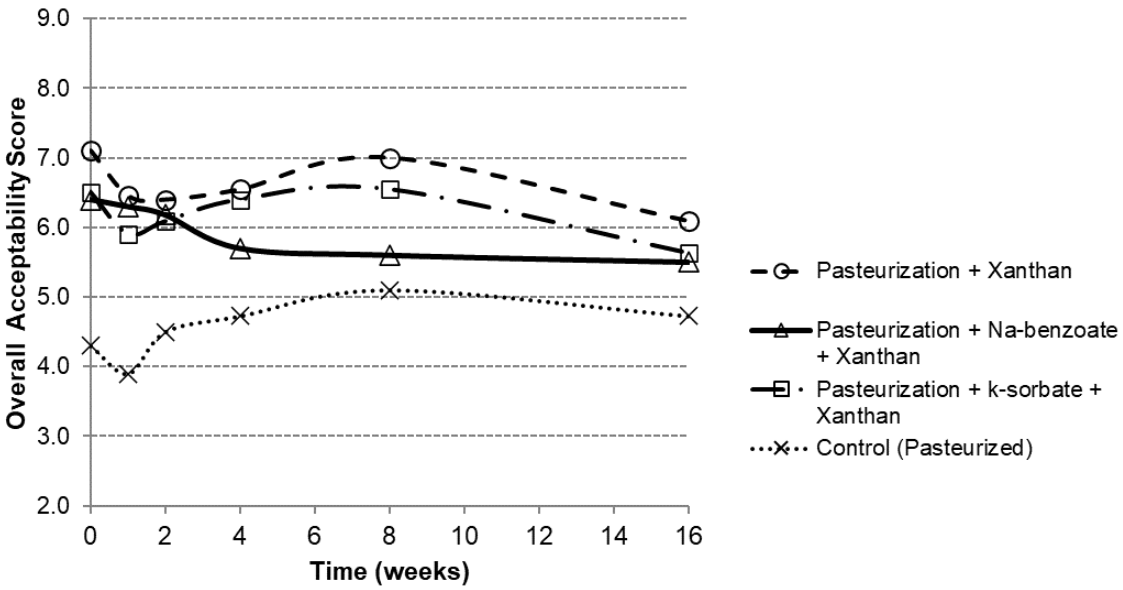

Figure 4. Effects of preservation treatments on the overall consumer acceptability scores of *Obushera.* Values are means of 50 panelists. Preservatives include K-sorbate, potassium sorbate and Na-benzoate, sodium benzoate

Discussion

The aim of this study was to assess effectiveness of two hydrocolloids (xanthan and CMC) and an exopolysaccharide-producing LAB (*L. rhamnosus* yoba) in controlling phase separation in fermented sorghum malt beverages (*Obushera*). Another aim of the study included assessing effects of pasteurization with or without preservatives (potassium sorbate and sodium benzoate) on the shelf stability of *Obushera. Obushera* is a colloidal system; in which, sorghum flour particles are suspended in water. These particles can form sediments resulting in separation of the product (28). Phase separation in food products such as dairy fermented products has been attributed to low pH values of ≤ 4.5 (29). Suspended particles in colloidal systems can be stabilized by electrostatic repulsion forces and increases in viscosity (30). Xanthan and CMC are negatively charged ionic polysaccharides; hence, their addition to *Obushera* is expected to increase electrostatic repulsive forces and viscosity thus stabilizing the product (18). In this study, xanthan was superior to CMC in stabilizing *Obushera.* The superiority of xanthan could be attributed to its branching nature, which is responsible for its excellent rheological properties (14, 31). Xanthan has a linear cellulosic backbone shielded by trisaccharide side chains, which gives the molecule a stiff rod-like structure (32).

This structure causes the molecule's stability to pH, heat, and enzymes (16, 17, 32). High temperatures have minimal effects on the viscosity of xanthan gums (16); however, they irreversibly lower the viscosity of CMC (33). In this study, *Obushera* was pasteurized (90˚C for 10 min) after the addition of stabilizers. High temperature treatment degraded CMC, resulting in its incapability to stabilize the product during storage. Compared to CMC, xanthan is relatively more stable to low acid conditions. Xanthan is stable over a pH range of 2–10 (16) while CMC is majorly used in products with pH greater than 4.2 (34). Khanniri et al. (29) in a study on doogh, an Iranian fermented dairy product (pH ≤ 4.5), reported that CMC (0.6%) was not able to confer stability to this product. Thus, low pH after fermentation (3.7–4.0) could lead to CMC becoming an ineffective stabilizer for *Obushera.*

Xanthan is typically used at 0.05–0.5% in foods (16). In the current study, xanthan at 0.25% was sufficient to stabilize *Obushera*. This finding is similar to those of other studies that reported use of 0.3% xanthan for the stabilization of fruit-flavoured beverages containing basil seeds (35) or rice bran milk (13) and 0.26% xanthan for the stabilization of fermented whey (34). Furthermore, addition of xanthan to *Obushera* significantly improved consumer acceptability. This could be attributed to the prevention of separation and enhancement of sensory properties. Xanthan naturally increases viscosity, which improves taste and mouthfeel perception (36). Although microbial EPS could contribute to stability and viscosity of various fermented beverages (19, 20), current fermentation of *Obushera* with *L. rhamnosus* yoba did not improve product stability. Instead, samples fermented by *L. rhamnosus* yoba had the highest sedimentation rates. One major disadvantage of using LAB to stabilize foods is that these bacteria generally produce low quantities of EPS (37). Normally, quantities of EPS produced by *L. rhamnosus* vary with microbial strain, temperature, pH, agitation, oxygen tension and carbon source (19, 37). Gamar- Nourani et *al*. (38) reported a temperature of 25 °C and pH of 6.2–7.2 as the optimum conditions for the EPS production by *L. rhamnosus* C83. In another study, the optimum conditions for the EPS production by *L. rhamnosus* E/N were 37 °C and pH 5.0 (39). Comparing the three strains at 37 °C and controlled pH (pH 6.0) in modified basal media, the maximum production of EPS was achieved in the stationary phase after 48–72 h (19). Oleksy-Sobczak et *al*. (23) observed the highest efficiency of EPS production in *L. rhamnosus* strains LOCK 0943, OM-1 and 0935 at pH 5.7–6.0 and 25 or 37 °C depending on the strain and time (30 or 24 h). In this study, fermentation was carried out at room temperature (25–28 °C) for 24 h, resulting in a final pH of 3.7–4.0. Therefore, failure of *L. rhamnosus* yoba to stabilize *Obushera* could be attributed to the low pH of the product and short fermentation time, which limited EPS production. The higher IS for *L. rhamnosus* with no stabilizers possibly resulted from amylolytic breakdown of the gelatinized starch network since *L. rhamnosus* could produce amylases (40).

Obushera has a shelf life of nearly four days at room temperature (2), which can be extended to eight days by refrigeration or nearly a month by pasteurization (6). In this study, use of pasteurization with or without preservatives (sodium benzoate and potassium sorbate) extended the shelf life of *Obushera* to at least four months. *Obushera* produced in this study had pH values of 3.7–4.0 and acidity of 0.5–0.6%, which were similar to those of a previous study (4). Low pH values (pH ≤ 4.5) and acidity of nearly 0.7% are necessary for inactivating spoilage and pathogenic microorganisms in fermented foods (41–43). However, acid-tolerant spoilage microorganisms such as molds could survive or contaminate food products after pasteurization, resulting in spoilage (10). Surviving or contaminating microorganisms can be inhibited using preservatives such as sodium benzoate and potassium sorbate, extending shelf life of foods (10, 11). In this study, pasteurization at 90 °C for 10 min was sufficient to inactivate all microbes in *Obushera* at the end of fermentation. Absence of microbes in *Obushera* with no preservatives within four months of storage revealed no post-pasteurization contamination of the product. Thus, efficient pasteurization of *Obushera* leading to total inactivation of the microorganisms post-fermentation and prevention of post-contamination may lessen use of chemical preservatives. Similar findings have been documented for *Togwa*, a fermented sorghum beverage from Tanzania (9).

Conclusion

This study has demonstrated that xanthan (0.25%) can be used to improve stability and sensory acceptability of *Obushera.* It has further shown that pasteurization alone (90 °C for 10 min) with no preservatives can be used to extend the shelf life of *Obushera* to at least four months. Therefore, use of xanthan and pasteurization can be adopted for stabilizing and preserving traditional fermented cereal beverages. Stabilization and preservation of these products will contribute to enhancing their consumer appeal and commercial potential.

Financial disclosure

The authors declared no financial interest.

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