Probiotic Enrichment and Reduction of Aflatoxins in a Traditional African Maize-Based Fermented Food

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Abstract: Fermentation of food products can be used for the delivery of probiotic bacteria and means of food detoxification, provided that probiotics are able to grow, and toxins are reduced in raw materials with minimal effects on consumer acceptability. This study evaluated probiotic enrichment and detoxification of kwete, a commonly consumed traditional fermented cereal beverage in Uganda, by the use of starter culture with the probiotic Lactobacillus rhamnosus yoba 2012 and Streptococcus thermophilus C106. Probiotic kwete was produced by fermenting a suspension of ground maize grain at 30 ºC for a period of 24 h, leading to a decrease of the pH value to ≤ 4.0 and increase in titratable acidity of at least 0.2% (w/v). Probiotic kwete was acceptable to the consumers with a score of ≥ 6 on a 9-point hedonic scale. The products were stable over a month’s study period with a mean pH of 3.9, titratable acidity of 0.6% (w/v), and Lactobacillus rhamnosus counts >10^8 cfu g⁻¹. HPLC analysis of aflatoxins of the water-soluble fraction of kwete indicated that fermentation led to an over 1000-fold reduction of aflatoxins B₁, B₂, G₁, and G₂ spiked in the raw ingredients. In vitro fluorescence spectroscopy confirmed binding of aflatoxin B₁ to Lactobacillus rhamnosus with an efficiency of 83.5%. This study shows that fermentation is a means to enrich with probiotics and reduce widely occurring aflatoxin contamination of maize products that are consumed as staple foods in sub-Saharan Africa.

Keywords: fermented cereal beverage; kwete; maize; probiotics; Lactobacillus rhamnosus; aflatoxins; binding

1. Introduction

The sustainable production of traditional foods in sub-Saharan Africa offers a viable opportunity to fight increasing hunger and malnutrition [1]. Cereals such as millet, sorghum, and maize are important sources of food in Africa [2], and are predominantly cultivated for human nutrition, in particular, for children [3–5]. The maize-based African fermented kwete is a fermented beverage, which is traditionally produced by Luo communities, but now commercially available in many...
rural and urban areas in Uganda [6,7]. Kwete is a slightly alcoholic, with a thick consistency and a sweet–sour taste [8]. Consumers use these beverages as social drinks, a source of energy, thirst quenchers, and weaning foods [6,8,9]. However, cereals are highly susceptible to aflatoxin contamination. This could be attributed to their rich nutrient composition and relatively high humidity that favors fungal growth [10,11]. Aflatoxin contamination in cereals, such as maize, has been reported to be as high as 46 mg kg$^{-1}$ and 19 mg kg$^{-1}$, in Kenya and Uganda, respectively [11]. These levels of aflatoxin contamination are of great concern considering that maize is used in all pre-primary, primary, and post-primary schools in Uganda for the preparation of breakfast (porridge) and lunch (posho or pap, which is a solid gelatinized product comprising of maize flour and water). The schools get the maize through parental in-kind contributions, direct procurement from the open markets and, to a small extent, from school gardening [12]. None of these sources of maize are subject to quality control and could, therefore, be contaminated with aflatoxins.

There is an urgent need to decrease the risk of aflatoxins due to concerns over human health, food safety, and economic losses. Aflatoxins have created a lot of havoc, particularly in sub-Saharan Africa, where acute and mostly chronic aflatoxicosis has been reported. In 2004, approximately 317 cases of aflatoxicosis and 125 deaths were reported in Kenya [13]. A pilot study in Uganda, which evaluated aflatoxin exposure in rural populations, reported that all 100 adults included in the study and 92 out of 96 children contained detectable levels of aflatoxin–albumin adducts, including five babies who were exclusively fed via breastfeeding [14]. This could explain the high number of cases of liver cancer (estimated at 6.5 and 5.5 age-standardized incidence rate per 100,000 people annually for males and females, respectively) in Uganda [15]. Furthermore, aflatoxins suppress the activity of the human immune system by significantly lowering the levels of perforin, perforin-expressing, and granzyme A-expressing CD8+ T cells. This results in impaired CD$^+$ T cells which, in turn, affects cellular immunity against infectious diseases [16,17]. Aflatoxins also affect absorption of nutrients through alteration of intestinal integrity [18], thus affecting child growth and development.

Therefore, the mitigation of aflatoxins in food is of great importance, and methods such as high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA) have been developed to monitor their levels. However, these methods can only be operated in laboratories by well-trained personnel [19,20]. As a drive to reduce exposure to contaminated food, we developed an on-site detection method [21] and validated this method for the analysis of maize flour from markets and households in Kampala [22]. Using this innovative detection methodology, we found that the average aflatoxin contamination at household level was higher (22.2 ± 4.6 µg kg$^{-1}$) than at the markets (7.6 ± 2.3 µg kg$^{-1}$). About three out of four samples from households tested positive for aflatoxins and nearly half the samples contained higher levels than the East Africa acceptable limit of 10 µg kg$^{-1}$ [23]. This is a strong indication that high levels of aflatoxins are consumed daily by the Ugandan population. A combinatory approach aimed at prevention of contamination, lowering the amount of aflatoxins in the food chain as well as decreased uptake after human ingestion, could be most effective in lowering the burden of aflatoxins in cereals.

Fermentation has been appreciated as means to bring down the concentration of aflatoxins in cereal-based foods [24]. However, variations in product quality and safety associated with the undefined nature of starter cultures in traditional fermentations create a barrier for this approach. The application of defined starters with lactic acid bacteria could offer an easy and inexpensive method that could be adopted for detoxifying aflatoxins in food in a controlled and reproducible manner [25,26]. The potential of probiotic strains to reduce the risk of aflatoxins has been studied, both in vitro and in vivo [27,28]. Probiotics do not only mitigate aflatoxins but also convey other health benefits to consumers, such as the decrease of intensity and duration of diarrhea, which is a major disease burden, especially in developing countries [29]; a promising candidate in this respect is the probiotic model strain Lactobacillus rhamnosus GG [30].

The probiotic L. rhamnosus GG is now accessible in East Africa, under the name L. rhamnosus yoba 2012, following the introduction of the concept of ‘generic probiotics’ [31,32]. The L. rhamnosus yoba
2012 strain has been previously applied for the preparation of African traditional products like uji (fermented maize), mutandabota (fermented pulp of the baobab fruit and milk), zomkom (fermented wheat), and the naturally fermented milk lait caillé [1,31,33]. Recently, Di Stefano et al. [34] reported the growth parameters, organoleptic characteristics, and acceptability of fermented millet by use of \textit{L. rhamnosus} GR-1 and \textit{S. thermophilus} C106, which provided a good reference point for this study. The inclusion of probiotic starters in a product may affect the products’ sensory properties and, hence, acceptability [35–37]. It is therefore not only essential to ensure growth of the probiotic, but to also compare sensory characteristics and consumer acceptability of the probiotic-enriched, traditional fermented products.

In this paper, we evaluated the ability of \textit{L. rhamnosus} yoba 2012 and \textit{S. thermophilus} C106 to propagate in \textit{kwete}, a traditional maize-based drink. In addition, we studied survival of the probiotic during storage and compared consumer acceptability with commercial traditional products previously introduced on the market. We also monitored the effect of the probiotic starter culture on the levels of aflatoxins during fermentation, and confirmed the ability of the probiotic to bind aflatoxin B$_1$. The results of this study demonstrate the potential of utilizing widely consumed locally available traditional foods as carriers for probiotics, which adds health benefits and improves product safety.

2. Materials and Methods

2.1. Ingredients for Probiotic Kwete

The dried probiotic starter culture comprising \textit{L. rhamnosus} yoba 2012 and \textit{S. thermophilus} C106 [31] was obtained from the Yoba for Life Foundation (Amsterdam, The Netherlands) and stored at $-40\degree$C prior to use. Hullled maize flour for preparation of probiotic \textit{kwete} was purchased from Maganzu millers, Kampala, Uganda, and kept at room temperature in a dry place prior to use.

2.2. Preparation of Probiotic Kwete

Probiotic \textit{kwete} was prepared using a modification of the traditional method described by Muyanja and Namugumya [8]. To prepare one liter of probiotic non-alcoholic \textit{kwete}, 150 g of hullled maize flour was mixed with 100 mL of water to form dough. The dough was roasted to golden brown on a saucepan over a hotplate with continuous turning to prevent burning. The roasted dough was subsequently mixed in one liter of boiled water to form a slurry. The slurry was boiled for 15 minutes to form porridge and to inactivate all unfavorable microorganisms. The porridge was subsequently cooled down to 45 $\degree$C followed by inoculation with prefermented maize porridge of 2% (v/v). The porridge was left to ferment at 30 $\degree$C for 24 h, with the acidity and pH monitored at $t = 0, 3, 6, 9, 12, \text{and } 24\text{ h}$. Bacterial counts were taken at $t = 0, 12, \text{and } 24\text{ h}$. The experiment was carried out in triplicate on separate days. The shelf stability of probiotic \textit{kwete} was determined by physicochemical, microbiological, and consumer acceptability tests. Samples were fermented for 24 h and stored in sterile bottles under refrigeration at 4 $\degree$C. Analyses were done at weekly intervals for a period of four weeks.

2.3. Inoculation Approaches

In this study, we evaluated the production of probiotic \textit{kwete} by the use of three inoculation approaches for fermentation at 30 $\degree$C. These approaches included (i) prefermented milk (ii) direct inoculation with the dried starter culture, and (iii) prefermented maize porridge. For the first procedure with prefermented milk, one gram of probiotic dried starter culture was used for one liter of milk and incubated overnight at 37 $\degree$C, as described previously [38]. Subsequently, the \textit{kwete} base was inoculated with 2% (v/v) of the prefermented milk. The second inoculation was performed by direct addition of one gram of the dried starter culture to one liter of \textit{kwete} base. The third inoculation procedure includes 2% prefermented maize porridge. This porridge was obtained by 50 g of hullled maize flour mixed with one liter of water to a final concentration of 5% (w/v). The mixture was boiled to obtain
a thick porridge followed by cooling down to 45 °C and inoculation with one gram of dried starter culture. Fermentation was carried out at 30 °C for 24 h. Samples during fermentation after all three inoculation procedures were taken at t = 0 and 24 h for analysis of pH, acidity, and colony forming units of \( L. \text{rhamnosus} \) yoba 2012 and \( S. \text{thermophilus} \) C106.

2.4. Enumeration of Colony Forming Units

Serial dilutions of probiotic \( kwete \) samples were prepared by using four-times-diluted Ringer’s solution. Counts of lactic acid bacteria (LAB) were determined by pour plating of selected serial dilutions in sterile MRS agar for \( L. \text{rhamnosus} \) yoba 2012 and M17 agar for \( S. \text{thermophilus} \) C106, followed by incubation anaerobically at 30 °C for 48 h. Yeast counts were determined by surface spreading of selected dilutions in potato dextrose agar and incubating at 30 °C for 2–5 days. Coliform counts were determined by pour plating selected serial dilutions in violet red bile lactose agar and incubation at 30 °C for 48 h. All media were obtained from CONDA Laboratories (Madrid, Spain).

2.5. Determination of Titratable Acidity and pH

Titratable acidity was determined by weighing 10 mL of the \( kwete \) sample. The sample was subsequently filtered through Whatman (Whatman International Ltd, Maidstone, England) number 1 filter paper. The filtrate was titrated against a standardized solution of 0.1 N NaOH with phenolphthalein as the indicator [39]. The experiment was performed in triplicate. The pH was determined using a bench top FiveGo digital pH meter (Mettler Toledo, Greifensee, Switzerland) which was calibrated using DKD-certified buffers (Mettler Toledo) of pH 4.00, 7.00, and 9.20 prior to analysis. The pH of the samples was determined in duplicate.

2.6. Consumer Acceptability of Probiotic Kwete

A panel of 62 Ugandan students (equal ratio males and females) evaluated the acceptability of the probiotic \( kwete \) one day after fermentation in a double-blind study. The acceptability of the probiotic was compared with non-probiotic, traditionally prepared \( kwete \) [8]. Panelists ranked their acceptability of various attributes using a 9-point hedonic scale [40]. Water bottles were provided to rinse the palate in between tasting of samples. The group means differences between probiotic and traditionally made \( kwete \) (control) were analyzed using a \( t \)-test. All statistical analyses were performed using XLSTAT software (version 2012.4.03, Addinsoft, Paris, France).

2.7. Aflatoxin Analysis

2.7.1. Total Aflatoxin Concentration in Kwete

To evaluate the potential of \( L. \text{rhamnosus} \) yoba 2012 in mitigating the risk effect of aflatoxins in a maize-based traditional food, 20 mL of \( kwete \) base was spiked with 1.25 mL of 120 ng mL\(^{-1}\) total aflatoxins (40.0 ng mL\(^{-1}\) B\(_1\), 40.0 ng mL\(^{-1}\) G\(_1\), 20.0 ng mL\(^{-1}\) B\(_2\), and 20.0 ng mL\(^{-1}\) G\(_2\)). The aflatoxin standards were obtained from Bioo Scientific (Austin, Texas, USA). Control experiments were set as follows: unfermented \( kwete \) without aflatoxins, fermented \( kwete \) without aflatoxins, unfermented \( kwete \) base with aflatoxins, unfermented \( kwete \) base with 0.92% lactic acid (pH 4.4) with aflatoxins, and \( kwete \) spiked with aflatoxins after fermentation. Incubations were carried out for 24 h at 30 °C with samples taken at t = 0, 12, and 24 h for aflatoxin B\(_1\), B\(_2\), G\(_1\), and G\(_2\) quantification. The water-insoluble phase of \( kwete \) was removed by centrifugation at 3000g for 20 minutes at room temperature. The supernatant was applied to an immunoaffinity column according to the instructions of the manufacturer (AFLASTAR\textsuperscript{TM} R Romer Labs Inc, Union, Missouri, USA). Briefly, 0.5 mL of the extract containing aflatoxins (B\(_1\), B\(_2\), G\(_1\), and G\(_2\)) was diluted to 2.5 mL with deionized water prior to clean-up using the immunoaffinity column. The column was then washed with 4 mL of 16% methanol to remove any unbound aflatoxins, and the bound aflatoxins were eluted using 2 mL absolute methanol. Aliquots of 100 µL of the extract were injected into the HPLC column equilibrated with methanol. The aflatoxins were eluted
using a methanol/acetonitrile/water (8:27.65 (v/v)) solution at a flow rate of 0.7 mL/min. Detection and quantification were performed by a fluorescence detector operated at excitation and emission wavelengths of 365 nm and 450 nm, respectively.

2.7.2. Aflatoxin B₁ Binding to Lactobacillus rhamnosus

In order to further substantiate the mechanism for the reduction of aflatoxins during the controlled fermentation of kwete, the binding affinity of L. rhamnosus yoba 2012 to aflatoxin B₁ was determined. Briefly, the probiotic L. rhamnosus yoba 2012 was cultured in de Man, Rogosa, Sharpe (MRS) broth with 0.1% (v/v) Tween 80 at 37 °C in an atmosphere of air containing 5% CO₂ for 24 h. The cell pellet was collected by centrifugation at 3200g for 10 minutes at room temperature, and washed twice with physiological saline to remove excess MRS broth. The cell pellet was resuspended and serially diluted with physiological saline to generate nine different concentrations in optical densities ranging from 0 to 1 using Ultrospec 2100 pro spectrophotometer (Amersham Biosciences, Piscataway, New Jersey, USA) set at 600 nm. These dilutions were prepared in a microtiter plate and centrifuged to remove supernatant prior to aflatoxin B₁ binding. An aflatoxin B₁ solution in physiological saline of 1.0 µg mL⁻¹ was added to bacterial cell pellets in a microtiter plate, and the cell suspension was incubated at 37 °C for 30 minutes, followed by centrifugation at 3200g for 10 minutes at room temperature. The residual aflatoxin B₁ in the supernatant was analyzed by fluorescence spectroscopy using the Fluostar Omega microplate reader (BMG Labtech, Ortenberg, Germany) operating with a 390 nm excitation filter and a 480 nm emission filter. The fluorescence of the residual aflatoxin B₁ was plotted versus the cell concentrations. Curve fitting was carried out with GraphPad Prism version 7 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Fermentation of Kwete Using the Yoba Starter Culture

The L. rhamnosus yoba 2012 and S. thermophilus C106 bacteria propagated well in the kwete base with notable changes in pH and acidity (Figure 1). Bacterial growth resulted in lactic acid production and an increase in titratable acidity from 1.8‰ to 7.0‰, and a decrease of the pH from 6.2 to 3.9 after 24 h of fermentation (Figure 1). Fermentation of the probiotic cereal-based kwete with a pH of 4.4 and an acidity of 4.5‰ after twelve hours is relatively slow compared to milk fermentation with the same bacterial starter culture [38].

![Figure 1](image-url)  
**Figure 1.** Changes in pH and acidity during fermentation of kwete with L. rhamnosus yoba 2012 and S. thermophilus C106 at 30 °C. Data points represent means of three independent fermentations. Error bars represent standard deviations.
3.2. Acceptability of Probiotic Kwete

The consumer acceptability scores of probiotic kwete in comparison to traditionally made kwete assessed by a panel of 62 university students are shown in Table 1. The acceptability scores for color, aroma, and overall acceptability of probiotic kwete were generally comparable \((p > 0.05)\) to the local (traditional) kwete on the market. Although the taste of probiotic kwete was highly acceptable, it was quite different from the locally made type, which had very sour and slightly alcoholic flavors with a pH of \(3.4 \pm 0.1\). The latter pH value was similar to those reported by Muyanja and Namugumya [8]. The acceptability scores for probiotic kwete ranged from 3 to 9 (‘dislike moderately’ to ‘like extremely’).

Table 1. Comparison of the consumer acceptability scores of probiotic kwete produced by \(L.\) \textit{rhamnosus} yoba 2012 and \(S.\) \textit{thermophilus} C106 with a commercial brand on 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. Values are means and standard deviations \((n = 62\) respondents).

| Sample       | Acceptability Scores | Appearance | Color | Aroma | Taste | Overall |
|--------------|----------------------|------------|-------|-------|-------|---------|
| Probiotic    |                      | 6.2 ± 1.1  | 6.6 ± 1.9 | 6.7 ± 0.8 | 6.8 ± 1.4 | 6.4 ± 1.7 |
| Commercial brand |                    | 6.8 ± 2.2  | 6.1 ± 1.2 | 6.3 ± 2.1 | 6.5 ± 1.6 | 6.5 ± 2.0 |
| \(p\)-value  |                      | >0.05      | >0.05   | >0.05   | <0.05  | >0.05   |

3.3. Shelf Stability of Probiotic Kwete

Shelf stability for probiotic kwete was evaluated by monitoring changes in pH, acidity, viability of \(L.\) \textit{rhamnosus} yoba 2012, \(S.\) \textit{thermophilus} C106, coliforms, yeasts, and consumer acceptability during refrigerated storage for a period of four weeks (Figure 2). The pH of the products ranged between 3.2 and 4.0, and the acidity ranged between 0.6% and 0.7% \((p < 0.05)\) during four weeks of storage. \(Lactobacillus\) \textit{rhamnosus} yoba 2012 cell counts remained above \(10^8\) cfu g\(^{-1}\) during the entire storage period, but \(S.\) \textit{thermophilus} C106 dropped 3 log units after 2 weeks to \(10^4\) cfu g\(^{-1}\) in week 4. Coliforms, yeasts, and molds were not detected (<1 cfu g\(^{-1}\)) in the samples. Probiotic kwete remained acceptable during the four weeks of storage with overall acceptability scores ranging, on average, between 6.0 to 7.7 (equivalent to ‘like moderately’ to ‘like very much’). Overall acceptability scores did not vary significantly \((p > 0.05)\) during storage.
3.4. Effect of Inoculation Method

The preparation of fermented milk with the freeze-dried starter yoba starter culture bacteria has been widely applied throughout Uganda in areas with access to milk. However, fermented milk as starter culture to ferment kwete base would not be helpful for people living in areas with limited milk availability. Therefore, two alternative approaches for initiating the fermentation of probiotic kwete were evaluated (Table 2). Both inoculation approaches (direct use of the dried starter culture and fermented maize porridge) have been used to produce probiotic kwete at room temperature for 24 h. The changes during fermentation in pH, titratable acidity, L. rhamnosus yoba 2012, and S. thermophilus C106 counts are shown in Table 2. The probiotic bacterium L. rhamnosus yoba 2012 performed well for all three fermentations with an increase of two to three log units. The bacterium S. thermophilus C106 propagated well in kwete inoculated with fermented milk, showing an increase of more than two log units. However, in the absence of milk, in the case of the dried starter and prefermented kwete inoculation methods, the counts only increased slightly and remained at seven log units. For all fermentations, the pH dropped from 5.6–6.3 to 4.2–3.9, and the acidity increased from 0.2% to 0.3%–0.5%. The highest pH and acidity differences were observed for the kwete fermentation inoculated with prefermented maize porridge.
Table 2. Comparison of inoculation methods for initiating the fermentation of *kwete* with *L. rhamnosus* yoba 2012 and *S. thermophilus* C106. Values are means of two independent fermentations (no variations observed in pH values; variation in counts <20%; variation in acidity <10%). Fermentations were carried out at 30 °C.

| Parameter             | Time (hours) | Prefermented Milk | Dried Starter | Prefermented Maize |
|-----------------------|--------------|-------------------|---------------|-------------------|
| *L. rhamnosus* (log cfu g⁻¹) | 0            | 6.5               | 6.3           | 5.7               |
|                       | 24           | 8.4               | 8.7           | 8.9               |
| *S. thermophilus* (log cfu g⁻¹) | 0          | 6.8               | 7.1           | 7.3               |
|                       | 24           | 9.1               | 7.8           | 7.9               |
| pH                    | 0            | 5.6               | 6.3           | 6.2               |
|                       | 24           | 3.9               | 4.2           | 3.9               |
| Acidity (% acid)      | 0            | 0.2               | 0.2           | 0.2               |
|                       | 24           | 0.3               | 0.4           | 0.5               |

3.5. Reduction Aflatoxins B₁, B₂, G₁, and G₂ by Fermentation

The reduction of aflatoxins B₁, B₂, G₁, and G₂, spiked into the maize *kwete* base, was assessed during fermentation for 24 h with the yoba starter culture by HPLC analysis. The chromatogram in Figure 3 shows the concentration of aflatoxins from the immunoaffinity-purified water-soluble fraction at t = 0, 12, and 24 h. A notable decrease was recorded in the concentration of aflatoxins B₁, G₁, B₂, and G₂ of 92% ± 0.1%, 91.4% ± 0.2%, 91.8% ± 0.2%, and 90.9% ± 0.2%, respectively, after a period of 12 h of fermentation. However, after 24 h of fermentation, no detectable levels of aflatoxins were left in the sample, showing that yoba starter culture bacteria efficiently removed the concentration of all of the four major types of aflatoxins (Figure 3). In order to exclude that the reduction of aflatoxins was a result of other factors than the fermentation by the starter culture, we carried out a number of control experiments. These experiments indicated that no aflatoxins can be detected in case they are not added to the raw ingredients, that fermentation with the starter culture is required for the reduction, and that an incubation of 24 h in an acidic environment (lactic acid, pH 4.4) does not lead to a reduction of the four major aflatoxins (Table 3).

![Figure 3](image-url)
**Table 3.** Reduction of spiked aflatoxins B$_1$, B$_2$, G$_1$, and G$_2$ after 24 h of incubation of *kwete* base. Values are means and standard deviations of analyses from three independent experiments. * Counts were performed by use of selective de Man, Rogosa, Sharpe (MRS) nutrient agar plates and indicate numbers for *L. rhamnosus* yoba 2012.

| Experimental Runs (spike, starter) | Initial pH | Final pH | Initial Counts * (log cfu g$^{-1}$) | Final Counts * (log cfu g$^{-1}$) | Aflatoxin Concentration (ng mL$^{-1}$) |
|-----------------------------------|------------|----------|-------------------------------------|-----------------------------------|--------------------------------------|
|                                   |            |          |                                     |                                   | B$_1$      B$_2$    G$_1$   G$_2$     Total |
| Controls                          | no spike, no starter | 6.3 ± 0.1 | 6.3 ± 0.1 | 0 | 0 | 0 | 0 | 0 | 0 |
|                                   | no spike, starter | 6.3 ± 0.3 | 4.2 ± 0.1 | 6.5 ± 0.2 | 9.4 ± 0.3 | 2.4 ± 0.1 | 1.1 ± 0.0 | 2.4 ± 0.1 | 1.1 ± 0.0 | 7.0 |
|                                   | spike (t = 0 h), no starter | 6.3 ± 0.0 | 6.1 ± 0.3 | 0 | 0 | 2.4 ± 0.1 | 1.1 ± 0.0 | 2.4 ± 0.1 | 1.0 ± 0.5 | 6.9 |
|                                   | spike (t = 0 h), lactic acid, no starter | 6.3 ± 0.1 | 4.4 ± 0.2 | 0 | 0 | 2.4 ± 0.1 | 2.4 ± 0.1 | 1.1 ± 0.0 | 1.1 ± 0.0 | 6.8 |
|                                   | spike (t = 24 h), starter | 6.3 ± 0.5 | 3.9 ± 0.2 | 6.2 ± 0.4 | 9.0 ± 0.2 | 2.4 ± 0.3 | 0.9 ± 0.1 | 2.4 ± 0.1 | 1.1 ± 0.0 | 6.9 |
| Experiment                        | t = 0 h | 6.3 ± 0.5 | 6.1 ± 0.4 | 6.1 ± 0.5 | 6.5 ± 0.2 | 2.4 ± 0.2 | 1.2 ± 0.1 | 2.4 ± 0.3 | 0.9 ± 0.1 | 6.9 |
|                                   | t = 12 h | 6.3 ± 0.1 | 4.7 ± 0.2 | 6.1 ± 0.5 | 7.5 ± 0.2 | 0.2 ± 0.0 | 0.1 ± 0.0 | 0.2 ± 0.1 | 0.1 ± 0.0 | 0.6 |
|                                   | t = 24 h | 6.3 ± 0.1 | 4.1 ± 0.1 | 6.3 ± 0.6 | 8.9 ± 0.1 | 0 | 0 | 0 | 0 | 0 |
3.6. Binding of Aflatoxin B1 by Lactobacillus rhamnosus

The binding of the major aflatoxin B1 to the probiotic bacterium \textit{L. rhamnosus} yoba 2012 was studied by monitoring the residual aflatoxin after incubation of a dilution series of cell suspensions with aflatoxin B1 at a concentration of 1.0 µg mL\(^{-1}\). The fluorescence aflatoxin B1 was plotted as a function of OD\(_{600}\) (Figure 4). The residuals for the curve fit at low optical density values (0.0–0.3) of \textit{L. rhamnosus} yoba 2012 were relatively high, probably resulting from high experimental errors at relatively low bacterial cell concentrations (Supplementary Material). The bacterial cell concentration at OD\(_{600}\) of 0.5 showed binding of 83% of the aflatoxin.

![Graph showing binding of aflatoxin B1 by Lactobacillus rhamnosus](image)

**Figure 4.** \textit{Lactobacillus rhamnosus} yoba 2012–aflatoxin B1 binding curve.

4. Discussion

Introduction of bacterial probiotic strains in traditional fermented foods can be used as a means to convey their health benefits [41]. In this study, we used the probiotic model bacterium \textit{L. rhamnosus} GG, since there is a wealth of scientific evidence showing its beneficial effects in the prevention and treatment of gastrointestinal diseases, including rotavirus and \textit{Clostridium difficile}-associated diarrhea, and travelers’ and antibiotic-associated diarrhea (AAD) [42–45]. In addition, this strain is readily accessible in its generic form, \textit{L. rhamnosus} yoba 2012, packed in a lyophilized state together with \textit{S. thermophilus} C106 in a sachet as the yoba starter culture [31].

The yoba starter culture bacteria successfully fermented the traditional maize-based food \textit{kwete}, as evident from the production of lactic acid shown by a decrease in pH and simultaneous increase in titratable acid. As required for microbiological safety and stability of lactic acid-fermented beverages [46–49], the observed pH values of probiotic fermented \textit{kwete} were ≤4.3, and the amount of titratable acid was at least 0.6% after 24 h of fermentation at 30 °C. It should be noted that in case of natural \textit{kwete} fermentations, it can take between 24 to 120 h to attain these pH and acidity values, while—in line with our findings—with defined starter cultures, these values are reached within 12 to 24 h of fermentation [6]. The maximum acidity levels observed during storage of probiotic \textit{kwete}, of 0.7%, corresponded to the maximum levels of acid production previously observed with starters containing \textit{L. rhamnosus} GG for fermentation of maize porridge with added barley [50].

In this study, \textit{kwete} was used as a substrate to enhance growth of the probiotic \textit{L. rhamnosus} yoba 2012, reaching a maximum of 1.0 \times 10^9 cfu g\(^{-1}\) after 24 h fermentation of \textit{kwete} at a temperature of 30 °C. These counts of colony forming units were similar to those reported for other traditional products serving as a substrate for the same starter culture, including \textit{mutandabota} (a dairy product containing baobab pulp), \textit{ujji} (fermented maize and sorghum beverage), and \textit{zomkom} (a fermented sorghum beverage [1,31]. Maximum counts of \textit{L. rhamnosus} yoba 2012 in \textit{kwete} were also comparable to those reported for other starter cultures with lactic acid bacteria, such as \textit{L. reuteri}, \textit{L. acidophilus}
We speculate that aflatoxin degradation is a specific property of our starter culture, as other studies reported less than 100% removal by L. rhamnosus strains [59–61]. However, under the experimental conditions used so far, we have not been able to confirm degradation of aflatoxins by pure cultures of bacterial strains in the yoba starter culture.

Our in vitro fluorescence experiments did confirm binding of aflatoxin B1 to a cell suspension of L. rhamnosus yoba 2012 at OD600 of 0.5, which reduced the aflatoxin B1 concentration of 1.0 µg/mL to 17%. Preliminary results indicated that the binding of aflatoxin B1 to S. thermophilus C106 was...
less efficient, with a reduction of aflatoxin B₁ to 86% at the same cell density. Aflatoxin binding to lactic acid bacteria was previously suggested as a safe means to reduce the bioavailability and enhance excretion of the toxin from the body [62,63]. Although the mechanism of binding is still poorly understood, cell surface polysaccharide, peptidoglycans, and teichoic acids have been suggested as the binding sites [59,64,65]. Here, we show that the yoba starter, including *L. rhamnosus* yoba 2012 and *S. thermophilus* C106, were able to remove 100% of 120 µg kg⁻¹ total aflatoxins spiked in the water-soluble fraction of *kwete*, which is highly relevant considering the range of aflatoxin concentrations we previously found in maize flour in households in Uganda [22].

5. Conclusions

This study showed that yoba starter culture bacteria were able to produce *kwete* products with comparable acceptability to commercially available traditional products. The yoba starter culture bacteria were able to ferment *kwete*, reducing the pH to below 4.0 in 24 h at room temperature. The products remained stable during refrigerated storage for a month. This study demonstrated that yoba starter culture bacteria can reduce aflatoxins during fermentation to non-detectable levels. Accordingly, fermentation with this starter culture can positively contribute to reduction of the risk of aflatoxins in maize-based foods widely used in schools in Uganda.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/11/2/265/s1, Supplementary file (fit through the data points, residuals, calculated $r^2$).

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Conflicts of Interest: The authors declare that the Yoba for Life foundation is a non-profit organization, with accreditation from the Dutch Tax Authorities as a Public Benevolent Institution (PBI). The Yoba for Life foundation aims at the promotion of local production and consumption of fermented products in Africa through the distribution and sales of ready-to-use sachets with dried bacterial starter cultures through a network of partners and volunteers.

References

1. Mpofu, A.; Linnemann, A.R.; Sybesma, W.; Kort, R.; Nout, M.; Smid, E.J. Development of a locally sustainable functional food based on mutandabota, a traditional food in southern Africa. *J. Dairy Sci.* 2014, 97, 2591–2599. [CrossRef] [PubMed]
2. Bruinsma, J. *World Agriculture: Towards 2015/2030: An FAO Perspective*; Routledge: London, UK, 2003.
3. Awika, J.M.; Piironen, V.; Bean, S. *Advances in Cereal Science: Implications to Food Processing and Health Promotion*; ACS Publications: Washington, DC, USA, 2011.
4. Kikafunda, J.; Tumwine, J. Diet and socio-economic factors and their association with the nutritional status of pre-school children in a low income suburb of Kampala city, Uganda. *East Afr. Med. J.* 2006, 83, 565–574. [CrossRef]
5. Acham, H.; Tumuhimbise, G.A.; Kikafunda, J.K. Simple food group diversity as a proxy indicator for iron and vitamin a status of rural primary school children in Uganda. *Food Nutr. Sci.* 2013, 4, 1271. [CrossRef]
6. Mukisa, I. Sensory characteristics, microbial diversity and starter culture development for obushera, a traditional cereal fermented beverage from Uganda. Ph.D. Thesis, Norwegian University of Life Sciences, As, Norway, 2012.
7. Muyanja, C.; Kikafunda, J.; Narvhus, J.; Helgetun, K.; Langsrud, T. Production methods and composition of bushera: A Ugandan traditional fermented cereal beverage. *Afr. J. Food Agric. Nutr. Dev.* 2003, 3, 10–19. [CrossRef]
8. Muyanja, C.; Namugumya, B. Traditional processing, microbiological, physicochemical and sensory characteristics of kwete, a Ugandan fermented maize based beverage. *Afr. J. Food Agric. Nutr. Dev.* 2009, 9, 1046–1059. [CrossRef]

9. Mukisa, I.; Muyanja, C.M.; Byaruhanga, Y.; Langsrud, T.; Narvhus, J. Changes in physico-chemical properties and flavour compounds during fermentation of different obusheera (sorghum and millet) beverages. *Afr. J. Food Agric. Nutr. Dev.* 2012, 12, 6665–6685.

10. Pratiwi, C.; Rahayu, W.P.; Lioe, H.N.; Herawati, D.; Broto, W.; Ambawati, S. The effect of temperature and relative humidity for *Aspergillus flavus* BIO 2237 growth and aflatoxin production on soybeans. *Int. Food Res. J.* 2015, 22, 82–87.

11. Makun, H.; Dutton, M.; Njobeh, P.; Gbodi, T.; Ogbadu, G. Aflatoxin contamination in foods and feeds: A special focus on Africa. In *Trends in Vital Food and Control Engineering*; InTech Open Access Publisher: Rijeka, Croatia, 2012; pp. 187–234.

12. Najjumba, I.; Bunjo, C.; Kyaddondo, D.; Misinde, C. Improving learning in Aganda volume i: Community-led school feeding practices and issues for consideration; World Bank Publications: Washington, DC, USA, 2013; Volume 1, pp. 1–85.

13. Centers for Disease Control and Prevention (CDC). Outbreak of aflatoxin poisoning—eastern and central provinces, Kenya, January–July 2004. *MMWR. Morb. Mortal. Wkly. Rep.* 2004, 53, 790–793.

14. Asiki, G.; Seeley, J.; Srey, C.; Baisley, K.; Lightfoot, T.; Archileo, K.; Agol, D.; Abaasa, A.; Wakeham, K.; Routledge, M.N. A pilot study to evaluate aflatoxin exposure in a rural Ugandan population. *Trop. Med. Int. Health* 2014, 19, 592–599. [CrossRef]

15. Kew, M.C. Epidemiology of hepatocellular carcinoma in sub-Saharan Africa. *Ann. Hepatol.* 2015, 12, 173–182.

16. Jiang, Y.; Jolly, P.E.; Ellis, W.O.; Wang, J.-S.; Phillips, T.D.; Williams, J.H. Aflatoxin B1 albumin adduct levels and cellular immune status in Ghanaians. *Int. Immunol.* 2005, 17, 807–814. [CrossRef] [PubMed]

17. The Foodborne Disease Burden Epidemiology Reference Group (FERG) WHO Initiative to Estimate the Global Burden of Foodborne Diseases. 2008. Available online: https://www.who.int/foodsafety/foodborne_disease/FERG2_report.pdf (accessed on 24 January 2019).

18. Gong, Y.Y.; Turner, P.C.; Hall, A.J.; Wild, C.P. Aflatoxin exposure and impaired child growth in West Africa: An unexplored international public health burden. *Mycotoxins Detect. Methods, Manage. Publ. Health Agri. Trade* 2008, 53–66.

19. Wacoo, A.P.; Wendiro, D.; Vuzi, P.C.; Hawumba, J.F. Methods for detection of aflatoxins in agricultural food crops. *J. Appl. Chem.* 2014, 1, 1–15. [CrossRef]

20. Wacoo, P.A. Development of a cysteine based electroless silver biosensor platform for electrochemical detection of aflatoxin B1, Master’s Thesis, Makerere University, Kampala, Uganda, 2016.

21. Wacoo, P.A.; Ocheng, M.; Wendiro, D.; Vuzi, P.C.; Hawumba, F.J. Development and characterization of an electroless plated silver/cysteine sensor platform for the electrochemical determination of aflatoxin B1. *J. Sens.* 2016, 2015, 1–8. [CrossRef]

22. Wacoo, A.P.; Wendiro, D.; Nanyonga, S.; Hawumba, J.F.; Sybesma, W.; Kort, R. Feasibility of a novel on-site detection method for aflatoxin in maize flour from markets and selected households in Kampala, Uganda. *Toxins* 2018, 10, 327.

23. East African Community. East African Standard. Milled maize (corn) products—specification. Arusha, Tanzania, 2011; pp. 1–11. Available online: https://law.resource.org/pub/eac/ibr/eas.44.2011.html (accessed on 29 December 2018).

24. Assouhoun, M.C.; Djeni, T.N.; Koussénon-Camara, M.; Brou, K. Effect of fermentation process on nutritional composition and aflatoxins concentration of doklu, a fermented maize based food. *Food Nutr. Sci.* 2013, 4, 1120–1127.

25. Ahlberg, S.H.; Joutsjoki, V.; Korhonen, H.J. Potential of lactic acid bacteria in aflatoxin risk mitigation. *Int. J. Food Microbiol.* 2015, 207, 87–102. [CrossRef] [PubMed]

26. Mokoena, M.; Chelule, P.; Gqaleni, N. The toxicity and decreased concentration of aflatoxin B1 in natural lactic acid fermented maize meal. *J. Appl. Microbiol.* 2006, 100, 773–777. [CrossRef] [PubMed]

27. Verheecce, C.; Liboz, T.; Mathieu, F. Microbial degradation of aflatoxin B1: Current status and future advances. *Int. J. Food Microbiol.* 2016, 237, 1–9. [CrossRef] [PubMed]
28. Gnonlonfin, G.J.B.; Hell, K.; Adjovi, Y.; Fandohan, P.; Koudande, D.; Mensah, G.; Sanni, A.; Brimer, L. A review on aflatoxin contamination and its implications in the developing world: A sub-Saharan African perspective. *Crit. Rev. Food Sci. Nutr.* 2013, 53, 349–365. [CrossRef]
29. Allen, S.J.; Martinez, E.G.; Gregorio, G.V.; Dans, L.F. Probiotics for treating acute infectious diarrhoea. *Cochrane Database Systematic Rev.* 2010. Available online: https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD003048.pub3/full (accessed on 24 January 2019).
30. Segers, M.E.; Lebeer, S. In Towards a better understanding of *Lactobacillus rhamnosus* GG-host interactions. *Microb. Cell Factories* 2014, 13, 1–16. [CrossRef] [PubMed]
31. Kort, R.; Westerik, N.; Serrano, L.M.; Douillard, F.P.; Gottstein, W.; Mukisa, I.M.; Tuin, C.J.; Basten, L.; Hafkamp, B.; Meijer, W.C. A novel consortium of *Lactobacillus rhamnosus* and *Streptococcus thermophilus* for increased access to functional fermented foods. *Microb. Cell Factories* 2015, 14, 195. [CrossRef] [PubMed]
32. Kort, R.; Sybesma, W. Probiotics for every body. *Trends Biotechnol.* 2012, 30, 613–615. [CrossRef] [PubMed]
33. Parker, M.; Zobrist, S.; Donahue, C.; Edick, C.; Mansen, K.; Hassan Zade Nadjari, M.; Heerikhuisen, M.; Sybesma, W.; Molenaar, D.; Diallo, A. Naturally fermented milk from northern senegal: Bacterial community composition and probiotic enrichment with *Lactobacillus rhamnosus*. *Front. Microbiol.* 2018, 9, 2218. [CrossRef] [PubMed]
34. Di Stefano, E.; White, J.; Seney, S.; Hekmat, S.; McDowell, T.; Sumarah, M.; Reid, G. A novel millet-based probiotic fermented food for the developing world. *Nutrients* 2017, 9, 529. [CrossRef] [PubMed]
35. Gomes, A.M.; Malcata, F.X. *Bifidobacterium* spp. and *Lactobacillus acidophilus*: Biological, biochemical, technological and therapeutical properties relevant for use as probiotics. *Trends Food Sci. Technol.* 1999, 10, 139–157. [CrossRef]
36. Nyanzi, R.; Jooste, P.; Abu, J.O.; Beukes, E.M. Consumer acceptability of a symbiotic version of the maize beverage mageu. *Dev. South. Afr.* 2010, 27, 447–463. [CrossRef]
37. Rathore, S.; Salmerón, I.; Pandiella, S.S. Production of potentially probiotic beverages using single and mixed cereal substrates fermented with lactic acid bacteria cultures. *Food Microbiol.* 2012, 30, 239–244. [CrossRef]
38. Westerik, N.; Wacoo, A.P.; Sybesma, W.; Kort, R. Novel production protocol for small-scale manufacture of probiotic fermented foods. *JoVE (J. Visual. Exp.)* 2016, 115, e54365. [CrossRef]
39. Garner, D.; Crisosto, C.; Wiley, P.; Crisosto, G. Measurement of pH and titratable acidity. In *Quality Evaluation Methodology;* Kearney Agricultural Center: Parlier, CA, USA, 2005.
40. Wichchukit, S.; O’Mahony, M. The 9-point hedonic scale and hedonic ranking in food science: Some reappraisals and alternatives. *J. Sci. Food Agric.* 2015, 95, 2167–2178. [CrossRef]
41. Franz, C.M.; Huch, M.; Mathara, J.M.; Abriouel, H.; Seney, S.; Reid, G.; Galvez, A.; Holzappfel, W.H. African fermented foods and probiotics. *Int. J. Food Microbiol.* 2014, 190, 84–96. [CrossRef] [PubMed]
42. De Roos, N.M.; Katan, M.B. Effects of probiotic bacteria on diarrhea, lipid metabolism, and carcinogenesis: A review of papers published between 1988 and 1998. *Am. J. Clin. Nutr.* 2000, 71, 405–411. [CrossRef] [PubMed]
43. Nout, M.R.; Rombouts, F.; Havelaar, A. Accelerated natural lactic fermentation of maize porridge with added malted barley. *Int. J. Food Microbiol.* 2004, 91, 305–313. [CrossRef]
51. Byakika, S. Evaluation of sorghum malt as a growth and carrier medium for Lactobacillus plantarum MNC 21 biomass. Master’s Thesis, Makerere University, Kampala, Uganda, 2015.

52. Sekwati-Monang, B. Microbiological and chemical characterisation of ting, a sorghum-based gluten-free fermented cereal product from Botswana. Ph.D. Thesis, University of Alberta, Edmonton, Canada, 2011.

53. Salmerón, I.; Thomas, K.; Pandiella, S.S. Effect of potentially probiotic lactic acid bacteria on the physicochemical composition and acceptance of fermented cereal beverages. J. Funct. Foods 2015, 15, 106–115. [CrossRef]

54. Tripathi, M.; Giri, S. Probiotic functional foods: Survival of probiotics during processing and storage. J. Funct. Foods 2014, 9, 225–241. [CrossRef]

55. Knorr, D. Technology aspects related to microorganisms in functional foods. Trends Food Sci. Technol. 1998, 9, 295–306. [CrossRef]

56. Aka, S.; Konan, G.; Fokou, G.; Dje, K.M.; Bonfoh, B. Review on African traditional cereal beverages. Am. J. Res. Commun. 2014, 2, 103–153.

57. Oluwafemi, F.; Kumar, M.; Bandyopadhyay, R.; Ogunbanwo, T.; Ayanwande, K.B. Bio-detoxification of aflatoxin B1 in artificially contaminated maize grains using lactic acid bacteria. Toxin Rev. 2010, 29, 115–122. [CrossRef]

58. Pizzolitto, R.P.; Bueno, D.J.; Armando, M.R.; Cavagliere, L.; Dalcero, A.M.; Salvano, M.A. Binding of aflatoxin B1 to lactic acid bacteria and Saccharomyces cerevisiae in vitro: A useful model to determine the most efficient microorganism. In Aflatoxins-Biochemistry and Molecular Biology; InTech Open Access Publisher: Rijeka, Croatia, 2011; Volume 16, pp. 323–346.

59. Haskard, C.A.; El-Nezami, H.S.; Kankaanpää, P.E.; Salminen, S.; Ahokas, J.T. Surface binding of aflatoxin B1 by lactic acid bacteria. Appl. Environ. Microbiol. 2001, 67, 3086–3091. [CrossRef] [PubMed]

60. El-Nezami, H.; Kankaanpaa, P.; Salminen, S.; Ahokas, J. Ability of dairy strains of lactic acid bacteria to bind a common food carcinogen, aflatoxin B1. Food Chem. Toxicol. 1998, 36, 321–326. [CrossRef]

61. Peltonen, K.; El-Nezami, H.; Haskard, C.; Ahokas, J.; Salminen, S. Aflatoxin B1 binding by dairy strains of lactic acid bacteria and bifidobacteria. J. Dairy Sci. 2001, 84, 2152–2156. [CrossRef]

62. El-Nezami, H.; Mykkänen, H.; Kankaanpää, P.; Salminen, S.; Ahokas, J. Ability of Lactobacillus and Propionibacterium strains to remove aflatoxin B1 from the chicken duodenum. J. Food Prot. 2000, 63, 549–552. [CrossRef]

63. Gratz, S.; Täubel, M.; Juvonen, R.; Viluksela, M.; Turner, P.; Mykkänen, H.; El-Nezami, H. Lactobacillus rhamnosus strain GG modulates intestinal absorption, fecal excretion, and toxicity of aflatoxin B1 in rats. Appl. Environ. Microbiol. 2006, 72, 7398–7400. [CrossRef]

64. Lahtinen, S.J.; Haskard, C.A.; Ouwehand, A.C.; Salminen, S.J.; Ahokas, J.T. Binding of aflatoxin B1 to cell wall components of Lactobacillus rhamnosus strain GG. Food Addit. Contam. 2004, 21, 158–164. [CrossRef]

65. Hernandez-Mendoza, A.; Guzman-de-Peña, D.; Garcia, H. Key role of teichoic acids on aflatoxin B1 binding by probiotic bacteria. J. Appl. Microbiol. 2009, 107, 395–403. [CrossRef] [PubMed]

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