

## Abstract

The rising demand for *Obushera*, a traditional fermented sorghum/millet beverage originally from south western Uganda, is increasing its commercialization in cities such as Kampala. Haphazard commercial production of this beverage is currently a trend across the city owing to the lax enforcement of food safety regulations in the country. This situation may result in consumers being exposed to unsafe products. This study evaluated the safety (bacterial pathogens and total aflatoxincontamination) of *Obushera* on the Kampala market. The criteria and methods used to evaluate the probiotic potential of microorganisms were reviewed. Consequently, the probiotic potentials of *Obushera* starter cultures: *Lactobacillus plantarum* MNC 21, *Lactococcus lactis* MNC 24 and *Weisella confusa* MNC 20 with regard to improving the safety of the beverage were examined. *Lactobacillus rhamnosus* yoba 2012, a known probiotic was used as a reference. *Obushera* (n=59), sorghum flour (n=20) and millet flour (n=20) from processors (n=47) and common trade points were analyzed for *E. coli*, total and thermo-tolerant coliforms, *Staphylococcus* spp., *Salmonella* spp., molds and aflatoxins. Titratable acidity and pH of *Obushera* were also determined. The microbiological and physicochemical data were obtained using standard procedures. Data on the processors' food safety knowledge, attitudes and practices (KAP) were collected using researcher administered questionnaires. *Obushera* contained 0.0 – 7.3 log cfu/mL total and thermo-tolerant coliforms, 0.0 – 6.2 log cfu/mL *E. coli*, 0.0 – 8.1 logcfu/mL *Staphylococcus* spp. and 2.0 – 5.5 log cfu/mL molds. Flours contained 3.2 – 7.3 log cfu/g total and thermo-tolerant coliforms, 1.0 – 4.9 log cfu/g *E. coli*, 2.4 – 7.3 logcfu/g *Staphylococcus* spp., and 0.0. – 6.5 log cfu/g molds. *Salmonella* spp. was not detected in the *Obushera* and flours. Fifty six of 59 *Obushera* samples contained coliforms and *Staphylococcus* spp. above the recommended values for safety. Total aflatoxin levels in *Obushera*, sorghum and millet were  $10.4 \pm 6.1$  ppb,  $22.3 \pm 21.2$  ppb and  $9.9 \pm 10.0$  ppb, respectively. Processors had fairly good knowledge ( $63.0\% \pm 2.3$ ) and attitudes ( $52.2\% \pm 3.0$ ), but fairly poor food safety practices ( $38.7\% \pm 2.4$ ). There was no correlation between the KAP and the microbiological safety of *Obushera*. Literature review revealed that there are guidelines for evaluating candidate probiotic microorganisms. However, some of the proposed screening methods are outdated, biased or irrelevant. Therefore, the screening approaches, their relevance, strengths and weaknesses were discussed. The screening process should involve three major categories of tests: tolerance to the harsh gastric conditions, safety and probiotic benefit(s).

The *Obushera* cultures tolerated pH=3, 1% bile and displayed Bile Salt Hydrolase activity. MNC 20 and MNC 21 produced agmatine, a desirable biogenic amine. The cultures did not degrade gastric mucin and were resistant to 10 of 21 antibiotics tested. Cultures were also slightly hydrophobic (4.3– 15.9% in xylene and 6.8 – 18.3% in toluene), auto-aggregated (75.4 – 100% after 5 h and 100% after 24 h) and co-aggregated with *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Salmonella enterica*. They had ileal binding abilities ( $7.3 - 8.0$  log cfu/cm<sup>2</sup>) and inhibited growth of *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Salmonella enterica*. The cultures inhibited growth of virulent, acid tolerant and antibiotic resistant *E. coli* isolated from *Obushera*. They bound 19.3 – 69.4% aflatoxin B1 in physiological saline spiked with 1000 ppb of the toxin. The binding efficiency was in the order of *Lb. rhamnosus* yoba 2012 = MNC 21 > MNC 20 = MNC 24. Commercially produced *Obushera* in Kampala may serve as a source of human pathogens and aflatoxins. Fortunately, the starter cultures for this product: *Lb. plantarum* MNC 21, *L. lactis* MNC 24 and *W. confusa* MNC 20 could significantly improve its microbiological safety whilst also contributing to improving human health.