Fumonisin B_1 Reduction in Lactic Acid Bacteria Fermentation of Maize Porridges

Nyamete, F.A[1]., B. Mourice[2] and J.K. Mugula[1]

[1]Sokoine University of Agriculture
Department of Food Technology, Nutrition and Consumer Sciences
P.O. Box 3006
Morogoro, Tanzania

[2]Michigan State University
Department of Food Science and Human Nutrition
204 g.m.trout fshn building
East lansing, Michigan 48824-1224, USA

Corresponding author e-mail: *nachep@yahoo.com*

Abstract

Fumonisins are natural contaminants of maize and other cereal crops. They are heat-stable and highly toxigenic metabolites produced by Fusarium spp, of which fumonisin B_1 is the most toxic to both humans and animals. The toxin has been linked to oesophagus cancer, neural tube defects, and stunting in children. In Tanzania, maize-based porridge is a popular weaning food for infants. Typically, the porridges are homemade and are never tested for mycotoxin levels. Thus, control measures, such as processing methods that may significantly reduce fumonisin B_1 levels in foods is therefore warranted. This study investigated how fermentation can promote fumonisin B_1 reduction in maize-based porridges. Four starter culture of lactic acid bacteria (Lactobacillus plantarum, Pediococcus pentosaceus, Lactobacillus casei and Lactobacillus fermentum) were used, porridge samples was spiked with fumonisins B1 and allowed to ferment in an incubator set at 30°C for 24 h. Overall, lower pH (below 3.50, achieved by longer fermentation time) favoured greater fumonisin B_1 reduction, indicating that pH and time were intricately involved in fumonisins elimination. Lactic acid (related to sample pH) produced by the Lactic Acid Bacteria (LAB) used in this study, ranged from 3.30 – 3.95 mg/mL, and was also considered a significant factor in fumonisin B_1 reduction. Fumonisin B_1 reduction by LAB cultures ranged between 14.0 – 30.0%, after 24 hours was more pronounced using in the back-slopping fermentation method (30% reduction) than in the natural fermentation process (20% reduction), indicating fumonisin elimination by LAB could be strain specific. This suggests that, careful selection of LAB strains is therefore critical. Overall, results from this study suggest that back-sloping technique was the most economical, feasible and effective method to detoxify mycotoxins and reduce fumonisins levels in homemade weaning foods such as maize-based fermented porridges.

Keywords: Mycotoxins, Fumonisins, Lactic acid fermentation, food safety, maize porridge, Tanzania.

Introduction

Fumonisins are toxigenic metabolites produced by *Fusarium* spp., of which *Fusarium verticillioides* and *Fusarium proliferatum* (Fandohan *et al.,* 2003) cause the most concern to human and animal health. *Fusarium verticillioides*, for example, constitute up to 95% of all Fusarium strains recovered from maize fields in Africa (Ncube *et al.,* 2011) Not only are these fungi able to grow within maize tissues without causing visible symptoms of disease infection but are also directly responsible for several animal diseases. Horses and pigs, for instance, have been shown to suffer from

leukoencephalomalacia and pulmonary oedema syndrome ((Milićević *et al.,* 2010), when they consume fumonisin contaminated feeds.

Fumonisins are known hazards to human health and in some cases may cause serious illnesses and even death. Increased incidences of neural tube defects in infants of mothers consuming maize-based foods contaminated with fumonisins have been reported (Kimanya, 2015). In adults, however, fumonisins contamination is reportedly associated with oesophageal and liver cancers in China (Sun *et al.,* 2007). Around the world, many communities rely on maize as a dietary staple food which has been associated with fumonisin B_1 and some disease symptoms upon their contamination by mould and consumption. For instance, in India, a severe outbreak of abdominal pain and diarrhoea was associated with consumption of mouldy maize contaminated with fumonisin B_1 (Peraica *et al.,* 2001). It is therefore not only sufficient to discourage consumption of mouldy crops but also to find better processing methods that can eliminate or reduce the toxic effects of fumonisins in human foods.

Since fumonisins are natural contaminants of maize, it becomes an unavoidable vehicle in introducing these toxins into the human food chain, with fumonisin B_1 being the most toxic (Murphy *et al.,* 2006). Unfermented maize based porridge, a popular beverage in Tanzania, that is one such vehicle. Unfortunately however, since these foods are never formally packaged, then they are never tested for the presence of mycotoxins (especially fumonisins B_1), and thus are potentially fatal. This is due to the fact that there is general lack of public awareness about their existence, weak regulatory mechanisms, poor pre- and post-harvest management practices, and the economic pressure during chronic food shortages (Rheeder *et al.,* 2016).

Fortunately, industrial processing methods, such as cleaning, washing, nixtamalization, dehulling and milling, have been shown to be effective in reducing mycotoxins in cereal based products (Scudamore *et al.,* 2008). These processes are usually performed on cereal products meant for export market or formal trade. However, traditional African maize products are found in various forms, such as porridges and pastes, and the operations maize goes through during processing may not be sufficient to eliminate fumonisins. Unfortunately, little information is available on efforts to reduce fumonisins contamination in maize based foods using traditional processing methods such as fermentation in Africa, although maize-based food products are very popular.

In Tanzania, where weaning infants with maize based porridges is very popular, determination of the fate of fumonisin B_1 toxin during fermentation and identification of factors that give a significant reduction in fumonisin B_1 levels during the process is therefore warranted. Infants are known to be highly susceptible to mycotoxin contamination, and stunting in Tanzanian children has even been linked to fumonisin contamination (Kimanya *et al.,* 2009). This study investigated the ability of various lactic acid bacteria to reduce fumonisin B1 during fermentation in maize based porridges typically used for weaning infants. The use of three different fermentation methods (natural fermentation, back-sloping and the use of various starter cultures) were investigated in this study, and their effect on fumonisin B_1 reduction were determined.

Materials and methods

Bacterial Strains, Culture Conditions, and Estimation of Bacterial Concentration

Lactic acid bacteria strains, namely, *Lactobacillus fermentum, Lactobacillus plantarum,* and *Lactobacillus casei* subspecies casei and *Pediococcus pentosaceus* were obtained from United State Department of Agriculture (USDA), Peoria, Illinois, USA. Activation of the lyophilizes strains was done by inoculating into MRS broth (Becton) in screw-capped test tubes and incubated at 30°C for 24 hours (Becton Dickinson incubator, Cockeysville, MD, USA). Single colony isolation of lactic acid bacteria was done by picking from each plate grown in MRS broth and then centrifuged at 655xg for 15 min (Bench top centrifuge, Kubota 2010m Tokyo, Japan).

Peptone physiological salt solution was then used to wash the bacteria cell pellet followed by centrifugation and then re-suspended in physiological salt solution. Final bacteria concentration in the culture was reported as 107 colony forming unit per millilitre (examined as viable count on MRS agar).

Sample preparation and fermentation

Neogen grinder # 9401 (Neogen, USA) was used to grind maize grains collected from Morogoro Municipality in Tanzania. Gruel was prepared according to the procedure described by Mugula *et al.,* (2003). Maize flour slurry (1:9w/v) was boiled (Belling, UK), for approximately 20 minutes to form gruel. Natural fermentation was carried out by complementing a sample of the gruel that has been cooled to 30°C with millet malt alone or with millet malt followed by back–slopping. For controlled fermentation, malt flour was added when the gruel was around 55 – 58°C, allowed to cool, autoclaved (Pelton & Crane Delta 10 Autoclave, USA) at 121°C for 15 minutes and then cooled again to 30°C prior to inoculation.

Sample spiking and inoculation

100 ml of gruel was spiked with 800 ppm solution of fumonisin B_1 for natural fermentation and back-slopping samples (1 ml of sample per 8ppm of fumonisin B_1). For controlled fermentation, 100 ml of the sterile sample was spike with 800ppm fumonisin B_1 solution followed by inoculation with 1 ml of lactic acid starter culture. Homogenous mix was achieved by thorough mixing of the inoculated samples (VortesGene-2, Model G-560E, Scientific Industries, Behemia, New York). Triplicates of the spiked samples were prepared as follows: (i) gruel spiked with fumonisin B_1 and fermented naturally (NF); (ii) gruel spiked with fumonisn B_1 and fermented by back-slopping (BS) and (iii) gruel spiked with fumonisn B_1, fermented with various lactic acid bacteria cultures. Each tube was screwed-capped aseptically, mixed thoroughly and allowed to ferment in an incubator set at 30°C for 24 h. Fumonisin B_1, pH and lactic acid concentrations were monitored at 0, 4 and 24 h. All measurements were taken in triplicate for three replications.

Fumonisin B_1 determination

Fumonisin B_1 was extracted from 5 mL of fermented samples by adding 25 mL of 65% (v/v) ethanol/water and shaken for 3 minutes at 200 rpm shaker. The extracts were then filtered using Whatman #1 filter paper. The concentration of fumonisin B_1 in the extracts was determined by using high performance liquid chromatography (Applied Biosystem, CA, USA) according to the procedures described by Royer *et al.,* (2004) with minor modification: Excitation and emission wavelengths were set at 335 and 450 nm, respectively, and the mobile phase was ethanol/0.05 M citric acid buffer (pH 4.1) (75:25, v/v) at a flow rate of 1.0 mL/min. After derivatization with o-phthalaldehyde OPA reagent (10 mg of OPA in 1 mL of ethanol diluted with 2 mL of 0.1 M sodium borate (pH 9.1) and 14 µL of 2-mercaptoethanol). 20 µL of sample was injected into the HPLC for analysis.

pH determination

The pH of porridge samples at different stages of fermentation was determined using a glass electrode laboratory pH-meter (Model HI 9124, Hanna Instrument Inc, Romania) that was calibrated against standard buffer solutions at pH 4.0, 7.0 and 10.0 prior to the reading.

Lactic acid determination

Lactic acid levels were determined using a method developed by Taylor. (1996) with minor modifications. Approximately 0-60 µg/mL of standard lactic acid (in 15µg increment) was added into borosilicate tubes. Double distilled water was added to bring the total volume to 1 mL. Then, 6 mL of concentrated sulphuric acid was added and vortexed, followed by incubation at 95-100°C for 10 minutes in a steam water bath. The solution was then cooled to room temperature using a water bath(8005 water bath laboratory, Fisher Scientific, USA) and immediately, 100 µL copper sulfate (Sigma Aldrich, USA) was added followed by 200 p-hydroxydiphenyl reagent (Sigma Aldrich, USA), and vortexed(Benchmark Scientific, BV1000-E,UK) for 1 minute. The tubes were kept at room temperature for at least 30 minutes. Samples were filtered using Whatman #1 filter paper (Sigma Aldrich, USA). Collected filtrates

were diluted 1:100 with deionized water and 1 mL from each of the samples was aliquoted and separately filled into individual micro cuvettes, and placed into a spectrophotometer (Thermo Fisher Scientific GENESYS 20 Spectrophotometer), set at 340 nm. Lactic acid concentration was estimated using a standard curve.

Statistical analysis

All experiments were carried out in triplicate, and each sample was analysed in duplicate. The SAS statistical computer package was used to analyse the experimental data (SAS Institute, USA). Analysis of variance was performed and means were separated by Duncan's multiple range test at $p<0.05$. The results were expressed as mean ± SD (standard deviation).

Results

Fumonisins B1 in fermented gruel

In this study, removal of fumonisin B_1 appeared to be time dependent. Our results show that more fumonisin B_1 was removed when longer fermentation time (24 hours) was allowed (Table 1 & Figure 1); and that the use of starter LAB cultures was superior at removing fumonisin B1 within the first 4 hours of fermentation compared to natural fermentation or back-slopping methods (Table 1). Therefore, fermenting maize flour into porridge using LAB cultures at 30°C can significantly reduce levels of fumonisin B_1 by about 10.0 – 17.0% within 4 hours, compared to natural fermentation method, which only showed about 7.0% reduction. However, reduction of fumonisin B_1 in maize based porridges was more substantial after 24 hours (table 1 & figure 1), in the presence of LAB (14.0 – 27.0% reduction), indicating that longer fermentation time is essential in achieving greater elimination of mycotoxins in fermented foods.

At the end of fermentation period, we also observed that back-slopping method was superior to natural fermentation process in the elimination of fumonisins from maize based fermented porridges. Back-slopping produced a 30.0% reduction after 24 hours (Table 1 & figure 1), while natural fermentation had a 20.0% reduction of fumonisin B_1 over the same time period, in maize based porridge samples fermented at 30°C. This might be due to the fact that seeding with previously fermented porridge samples (back-slopping) may facilitate selection of strains which are best adapted to the food substrate. Other authors (Fandohan *et al.,* 2005) also reported a 13.0% reduction of fumonisin in ogi (a fermented suspension of wet milled maize in water) when fermented for 24 hours. It seems therefore, from our results, that back-slopping is a process with great potential to decrease bioavailability and toxic effects of fumonisins on weaning foods such as porridges fed to the children.

Table 1: Effect of lactic acid fermentation on reduction of fumonisin B_1 in maize based porridge

Culture treatments	Levels of Fumonisin B1 in porridge sample [1]		
	0 hrs	4 hrs	24 hrs
Natural fermentation (NF)	1.00 ± 0.00^{f}	0.93 ± 0.03^{def}	0.80 ± 0.03^{abcd}
Back Sloping (BS) [3]	1.00 ± 0.00^{f}	0.93 ± 0.03^{def}	0.70 ± 0.03^{a}
Pediococcus pentosaceus (PP)	1.00 ± 0.00^{f}	0.80 ± 0.00^{abcd}	0.76 ± 0.03^{abc}
Lactobacillus plantarum (LP)	0.96 ± 0.03^{ef}	0.86 ± 0.03^{bcdef}	0.76 ± 0.03^{abc}
Lactobacillus casei (LC)	0.93 ± 0.03^{def}	0.90 ± 0.03^{cdef}	0.83 ± 0.03^{abcd}
Lactobacillus fermentum (LF)	1.00 ± 0.00^{f}	0.86 ± 0.03^{bcdef}	0.83 ± 0.03^{abcd}

***** *Fermentation was performed at 30°C*

[1] *Values are means of replicate measurements*

[2] *Change is expressed as %, relative to readings recorded at 0 hour*

[3] *Back-sloped with 10% (v/v) previously fermented porridge, (BS)*

Means with the same letter within a column are not significantly different ($p<0.05$).

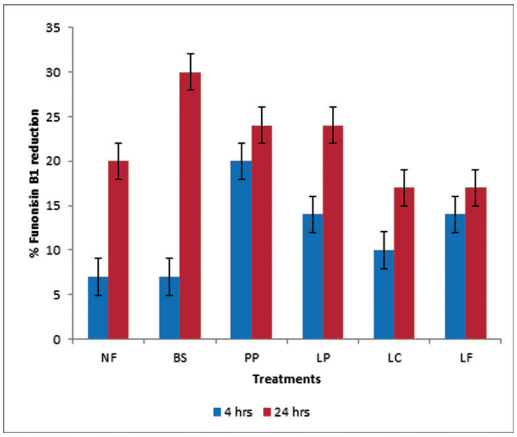
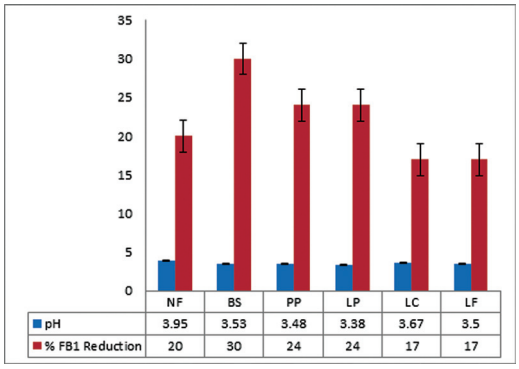


Figure 1: Percentage fumonisin B_1 reduction

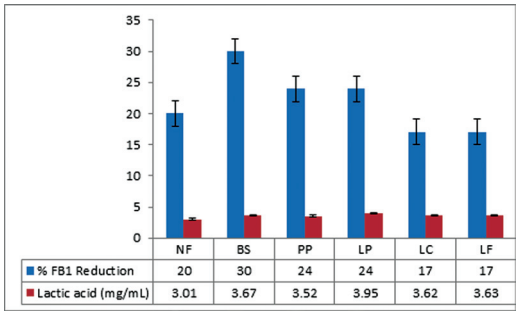
**NF: Naturally fermented, BS: Back slopped, PP: Pediococcus pentosaceus, LP: Lactobacillus plantarum LC: Lactobacillus casie LF: Lactobacillus fermentum*



	NF	BS	PP	LP	LC	LF
pH	3.95	3.53	3.48	3.38	3.67	3.5
% FB1 Reduction	20	30	24	24	17	17

Figure 2: Effect of pH on fumonisins reduction during lactic acid fermentation of maize based porridge

**NF: Naturally fermented, BS: Back slopped, PP: Pediococcus pentosaceus, LP: Lactobacillus plantarum LC: Lactobacillus casie LF: Lactobacillus fermentum*



	NF	BS	PP	LP	LC	LF
% FB1 Reduction	20	30	24	24	17	17
Lactic acid (mg/mL)	3.01	3.67	3.52	3.95	3.62	3.63

Figure 3: Effect of lactic acid on fumonisins reduction during lactic acid fermentation of maize based porridge

**NF: Naturally fermented, BS: Back slopped, PP: Pediococcus pentosaceus, LP: Lactobacillus plantarum LC: Lactobacillus casie LF: Lactobacillus fermentum*

Effect of pH and lactic acid on reduction of fumonisins B_1

Figure 2 and 3 shows the effect of pH and lactic acid reduction on fumonisin B_1. They show that, after 24 hours, pure LAB strains that had the ability to lower the pH of the fermented products to less than 3.50, i.e. *Pediococcus pentosaceus* and *Lactobacillus plantarum,* had greater ability to reduce greater amounts of fumonisin B_1 (24.0% reduction) than the rest commercial strains i.e. *Lactobacillus casei* and *Lactobacillus fermentum* (pH>3.5; 14.0% fumonisin B_1 reduction) (Figure 2). It follows therefore that production of organic acids by these organisms during fermentation is key to creation of such effects (Figure 3).

It was also observed that back-slopping method, due to its ability to promote faster bacterial growth and thus greater production of lactic acid in maize based porridges (and lower the pH of the porridge samples) than natural fermentation process, also seemed to have greater fumonisin reduction potential (Figures 2 & 3). When back-slopped method was used, the level of lactic acid produced after 24 hours of fermentation (Figure 3) was significant enough to create conditions that favoured greatest reduction of fumonisin B_1 (30.0% reduction) than natural fermentation (Figure 3).

Discussion

Lactic acid bacteria (LAB) strains have GRAS status (generally recognized as safe) and when used in food fermentation they contribute to the development of the desired sensory properties in the final product. Fermentation (a low energy preservation process) is an old but important food processing technology, it is simple and affordable. LAB fermentation may be an effective strategy to reduce fumonisins contamination in maize-based complementary

foods in Tanzania. The ability of lactic acid bacteria to detoxify mycotoxins has previously been reported (Shetty *et al.,* 2006). The use of this strategy in reducing mycotoxins from naturally contaminated maize is thus quite promising. The mechanisms of mycotoxin biosynthesis inhibition by lactic acid bacteria have been reported as strain dependent and could involve the binding of mycotoxin to the cell wall of bacteria and/or conversion of mycotoxin into less/non-toxic derivatives (Jard *et al.,* 2011).

In this study, the use of lactic acid bacteria showed promising potential as a food processing technique in greatly reducing levels of fumonisin B_1 in maize-based porridges. This indicates that reduction of fumonisin B_1 toxins in fermented porridges made with naturally contaminated maize is, to some degree, possible using lactic acid starter cultures. It was observed that processing maize into traditional products, such as fermented gruel, can reduce the levels of fumonisin in food. Fumonisin B_1 decreased when LAB strains were used to ferment maize-based gruel at 30°C.

Overall, reduction of fumonisin B_1 in maize based porridge samples was more substantial (30.0% reduction) using the back-slopping fermentation technique than the natural fermentation process (20.0% reduction). A 23.0% reduction of fumonisin was reported in South African non-fermented stiff porridge obtained from whole maize meal (Shephard., 2002). Additionally, a 13.0% fumonisin B_1 reduction was reported during the processing of ogi (fermented thick paste popular in West African countries) (Fandohan *et al.,* 2005), which has a method of processing similar to back-slopping method used in this study.

Greater levels of fumonisin toxin were reduced when samples were fermented for a longer period of time (24 hours). Lactic acid and pH levels in the porridge samples, which were also dependent on fermentation time, were both found as very effective in significantly reducing fumonisin B_1 contamination in the maize based porridge samples. Fumonisin B_1 were found to be more reduced at lower pH (after 24 hours) than at higher pH (after 4 hour) levels, suggesting that pH and time were intricately involved in elimination of the fumonisins.

It has been suggested that pH may contribute to reducing mycotoxins content by transforming mycotoxins to its less toxic compounds (Galvano *et al.,* 2001). In this study, however, we could not determine with 100% certainty that the observed fumonisins B_1 reduction was only influenced by pH reduction, since other factors such as higher bacteria populations after 24 hours of fermentation could also have been involved. Additionally, inhibition of fumonisins B_1 accumulation could also be related to production of low-molecular-weight metabolites produced by the lactic acid bacteria at the exponential growth phase (Shetty *et al.,* 2006). However, pH reduction (lactic acid production) may not be solely responsible for removal or inhibition of fumonisins B_1 in foods. More investigation is still needed on how the LAB strains used in this study and pH work together to reduce mycotoxin levels in maize-based substrates. This would also provide insight on how fermentation conditions could be optimized to achieve greater fumonisins reduction capacity of probiotic microorganisms.

Conclusion

Mycotoxin (especially FB1) contamination has become a global health issue especially in sub-Saharan African countries and the consumption of these toxins has been reported to cause acute and chronic effects in animals and human beings. The results of this study indicated that lactic acid fermentation could be a part of a comprehensive mycotoxicosis prevention strategy that can help detoxify the commonly consumed maize-based gruels in Tanzania. Consumers could benefit from enhanced food safety, through consumption of gruel less contaminated with mycotoxins and, might in addition, benefit from the probiotic effects of LAB. Infants, particularly those born in highly mycotoxin prone zones of the continent, may benefit when there maize-based weaning foods (i.e. porridges) are fermented with LAB. This may be achieved through reduction of pH during

fermentation and production of lactic acid by the LAB organisms, especially if fermentation process is extended for at least 24 hours.

For great impact on fumonisins B_1 reduction, careful selection of LAB strains is critical, However, for consumers who cannot afford LAB cultures from commercial sources, back-sloping technique seems to be the most economical, feasible and effective method to reduce fumonisin B_1. More investigation is still needed on the interactions between strains within the mixed LAB cultures and pH to provide insight on how fermentation conditions can be optimized to achieve greater mycotoxin reduction capacity of probiotic microorganisms. This study demonstrated the ability of some strains of lactic acid bacteria to reduce the initial concentration of fumonisin B_1 in maize-based complementary food. However, this intervention strategy should be rigorously tested and validated using clinical trials designed with biomarkers serving as object end points of efficacy. The examination can be conducted by determining the changes in blood and urinary levels of fumonisin B_1 (UFB1) as biomarkers of fumonisin B_1 exposure. The effects of detoxification on nutritive and sensory properties also need to be investigated.

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