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Short Communication

Control of ochratoxin A (OTA) in *kunu zaki* (a non-alcoholic beverage) using Daniellin^{TM}

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***Kunu-zaki*, a non-alcoholic beverage, was produced using modified traditional method incorporating Daniellin^{TM}. Treated samples kept for 5 days at ambient condition (26^{+}-2 C) while untreated samples kept for only 1 day. Protein contents and calorific values of *kunu-zaki* treated with Daniellin^{TM} (0.5 to 5.0%, w/v) were between 5.76 to 5.93% and 1606.47 to 1626.8 KJ/100g while values for untreated samples were 5.72% and 1547 KJ/100g respectively. Ochratoxin A (OTA) in raw materials used for *kunu-zaki* production was reduced from 50 mg/kg to <1.5 mg/kg with the incorporation of Daniellin^{TM} at 1.5, 2.0 and 2.5%.**

Key words: Ochratoxin A, *kunu-zaki* beverage, Daniellin^{TM}, food safety.

INTRODUCTION

Kunu-zaki is a cereal-based, non-alcoholic fermented beverage. The nutritional and medical importance of *kunu-zaki* have been reported (Gaffa and Ayo, 2002). *Kunu-zaki* has poor keeping qualities (Osuntogun and Aboaba, 2002) owing to faulty processing and storage methods and it is known to be prone to microbial contamination. Microbial contamination of plant products has been reported elsewhere (Beuchat and Brackett, 1990). Bacterial species associated with the contamina-tion of *kunu-zaki* have been reported (Ayo et al., 2004; Umoh et al., 2004). Fungi notably *Aspergillus* and *Penicillium* species have also been isolated from *kunu-zaki* (Osuntogun and Aboaba, 2004). To date, there are no data on mycotoxin contamination of *kunu-zaki* (a cereal-based beverage). In cereals and oil seeds, mycotoxins of public health significance include aflatoxins, citrinin and ochratoxin A (Pillet, 1998; Janardhane et al., 1999). The adverse effects of mycotoxins in man include genotoxicity, carcinogenicity, mutagenicity, teratogenicity and immunotoxicity (IARC, 1993).

Ochratoxin A (OTA) is a moderately stable molecule that can withstand most food processing techniques like malting and brewing (Boudia and Lebars, 1995; Baxter, 2001). As OTA may appear in finished cereal products like beverages (Scott, 1996), this present work was carried out in order to extend the shelf life of *kunu-zaki* and also control the level of OTA contamination of the beverage using Daniellin^{TM} (Adegoke, 2005; 2006). The multifunctional profiles of *Aframomum danielli* from where Daniellin^{TM} was obtained have been reported (Adegoke and Gopalakrishna, 1998; Ashaye et al., 2006; Adegoke et al., 2006).

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MATERIALS AND METHODS

Preparation of *kunu-zaki*

The method described by Gaffa and Ayo (2002) was modified as follows: about 300 g of millet *(Pennisetum typhodeum*) cleaned and washed thrice with potable water was steeped together with 300 g of peeled sweet potato (*Ipomoea batata*) for about 3 h at 62 ± 1°C. The soaked grains were removed, washed again with potable water and wet-milled into a paste which was divided into two parts in a ratio 1:3 (v/v). To the larger part (3 parts), boiling hot water was added to gelatinize the milled grains to which the smaller part (1 part) was added with vigorous and constant stirring. The suspension was left to ferment at room temperature (26 ± 2°C) for 8 - 10 h after which it was filtered using a sterile muslin cloth. Chemical additives or spice like ginger, pepper were not added to the filtrate (*kunu-zaki).*

Treatment of *kunu-zaki* with DANIELLIN^{TM}

To 100 ml of freshly-prepared *kunu-zaki* was added in duplicate, 100 ml of cold, aqueous solution of Daniellin^{TM} to give final concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5% ($^{w}/_{v}$) respectively (Table 1 were used for analytical works. Daniellin^{TM} ^{was} not added to the control samples.

Table 1. Percentage reduction of OTA in *Kunu-zaki* using Daniellin^{TM}

Sample	Ota level (µg/kg)	% Reduction
Untreated *Kunu-zaki* *	10	-
Kunu + 0.5% Daniellin^{TM}	5	50
Kunu + 1.0% Daniellin^{TM}	< 2.5	75
Kunu + 1.5% Daniellin^{TM}	< 1.5	100
Kunu + 2.0% Daniellin^{TM}	< 1.5	100
Kunu + 2.5% Daniellin^{TM}	< 1.5	100

*Sorghum used for *kunu-zaki* production had 50 µg/kg of OTA

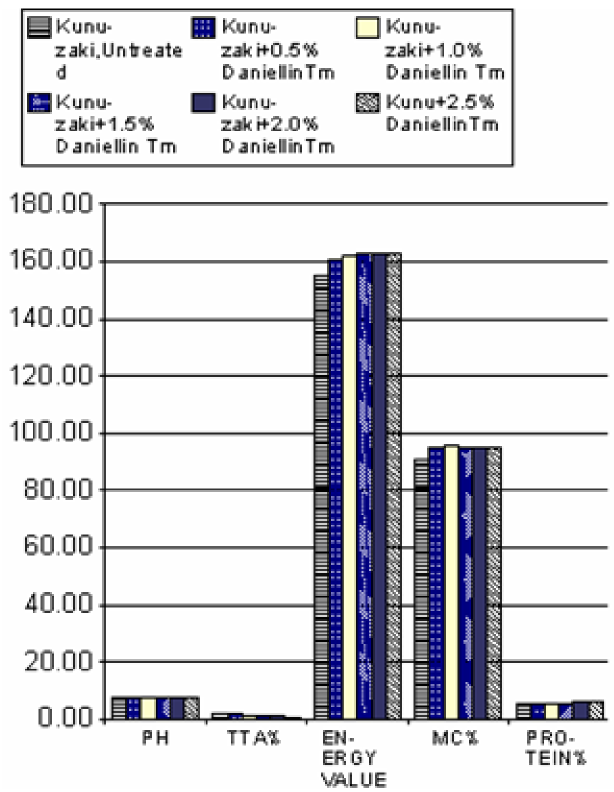

Figure 1. Proximate composition of *kunu-zaki* using Daniellin Tm

Chemical analysis

Crude protein and moisture contents were determined using standard methods (AOAC, 1984). pH measurements were done using pH meter (3305 Jenway) after initial standardization with pH 4 and 7 buffers solutions and titratable acidity (TA % lactic acid) was done by titrating 10 ml of *kunu-zaki* against O.1N NaOH to phenolphthalein end point. Mineral contents of *kunu-zaki* were determined using atomic absorption spectrophotometer (ashing process) as described by Cerwyn (1995) while caloric content of *kunu-zaki* was estimated using oxygen bomb calorimeter (Anon, 1961).

Determination of OTA level in *kunu-zaki*

The method for OTA qualification described by AOAC (2002), was modified as follows: 5 ml of aqueous solution of sodium bicarbonate was added to 50 g of each of control and treated samples of *kunu-zaki* followed by shaking for 5 min. The resulting mixture was extracted with 50 ml of chloroform over a bed of anhydrous sodium sulphate. Sodium bicarbonate (6.0 g) and celite mixture (25 ml of 5% aqueous $NaHC0_2$ solutions, celite and 50 g) were used for the clean-up process. Thereafter, 70 ml of n-hexane and 70 ml chloroform were used for washing the column. Elution was done using 700 ml of acetic acid: benzene solution (2:98). The eluate was evaporated to dryness and was later used for thin layer chromatography after dissolving each sample residue in 500 ml of benzene: acetic acid (98:1). Spotting was done using 5-50ml (5, 10, 20, 30, 40, 50 ml) of OTA standard (courtesy, IAEA/NAFDAC, Lagos) and 20 ml of each sample residue on TLC plates (20 x 20cm) in a developing solvent (benzene: methanol: acetic acid; 10:1:1). Spotted control and test samples were viewed under UV light after which quantification of OTA in each sample was calculated (AOAC, 2002).

RESULTS AND DISCUSSION

Within 1 day of storing *kunu-zaki*, microbial deterioration appeared while samples treated with Daniellin^{TM} (0.5 to 2.5% w/v) remained stable after 5 days of storage at 26 ± 2^{o}C. Our findings are thus in agreement with the report on the poor keeping qualities of *kunu-zaki* (Osuntogun and Aboaba, 2004). Upgrading the processing of *kunu-zaki* in line with HACCP principle will therefore involve controlling hazards associated with raw materials and processing methods. Hazards can arise from processing and handling operations of household foods (Abdulsalam and Kaferstein, 1993). Osuntogun and Aboaba, (2004) isolated some moulds from *kunu-zaki*. Moulds in addition to visible spoilage, can spoil foods through the formation of mycotoxins (Huis int' Veld, 1996).

In this present study, Daniellin^{TM} stabilized *kunu-zaki* for 5 days and also eliminated OTA with consistent lowering of pH of the beverage (Figure 1). The calorific and protein values of *kunu-zaki* treated with DaniellinTM are shown in figure 1. Millet used for *kunu-zaki* production was found to be contaminated with OTA (50 mg/kg). However, using Daniellin^{TM} at 1.5, 2.0, 2.5 (w/v) and a 100% reduction of OTA was found in treated *kunu-zaki* (Table 1). The presence of OTA in *kunu-zaki*, reported for the first time, is of public health significance. Nephrotoxicity and carcinogenity have been associated with OTA (IARC, 1993). While the control of growth and synthesis of some mycotoxins can be achieved using synthetic chemicals (Thompson, 1992; Nesci et al., 2003), the safety of some of these additives is of public health concern (Miller, 1989; Adegoke et al., 1998). The European Commission proposed 5 mg/kg of OTA in raw cereals and 3mg/kg in cereal-derived products (EC, 2002). The control of OTA to zero level (Table 1) and absence of aflatoxin B_1 (unpublished data) in *kunu-zaki* shows that Daniellin^{TM} can be used for controlling OTA in *kunu-zaki*. Our study has also confirmed the reports of Ashaye et al. (2006), Aroyeun and Adegoke (2007) that *Aframomum danielli* can be used in food processing /preservation.

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