academicJournals

Vol. 9(7), pp. 411-416, July, 2015
DOI: 10.5897/AJFS2015.1319
Article Number: 22C955F54264
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

Full Length Research Paper

Composition of sorghum-millet flour, *Aframomum danielli* essential oil and their effect on mycotoxins in kunu zaki

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Received 21 April 2015, Accepted 2 July 2015

This paper determines the composition of sorghum-millet flour, *Aframomum danielli* essential oil and its effect on mycotoxins in kunu zaki. Sorghum and millet grains in ratio 1:2 were cleaned, soaked for 12 h, washed, drained and dried in the hot air oven. The dried sorghum-millet grains were milled and packed for kunu zaki production. Kunu zaki was produced and different concentrations of *Aframomum danielli* essential oil (0.25, 0.50, 1.0, 2.5, 5.0 and 10%) were added. The bulk density, swelling index, water absorption capacity, solubility and oil absorption capacity of the flour were 0.75 g/cm^3, 1.16, 2.49 ml H_2O/g, 2.56% and 0.79 ml oil/g, respectively. The flour had low phenols, oxalate, phytate and saponins contents. Peak viscosity of the flour was 1234 Cp while the holding viscosity was 811 Cp. Breakdown and final viscosities were 423 and 1824.5 Cp, respectively. The components detected in the essential oil of *A. danielli* seed were dominated by 1, 8-Cineole (56.16%). There were gradual reductions in the mycotoxins contents of kunu zaki with addition of *Aframomum danielli* essential oil. Kunun zaki with 10 % *A. danielli* essential oil resulted in 76% reduction in fumonisin B_1. *A. danielli* exhibited ability to reduce FB_1, FB_2 and Ochratoxin A in kunu zaki.

Key words: *Aframomum danielli,* anti-nutrients, essential oil, kunu zaki, millet, sorghum.

INTRODUCTION

Cereals belong to the grass family and constitute important crops which serve as industrial raw materials and staple for the world (Enwere, 1998). The geographical location and climatic conditions of each area determine the kind of cereals that can be grown and utilized (FAO, 1989). The three most important cereals in the world are wheat, rice and corn. Other important cereal grains include sorghum, oats, barley, millet and rye. In Nigeria, the cereals cultivated are sorghum, millet, maize, rice, wheat and hungry rice to a lesser extent (Okon, 1998). Cereals have high carbohydrate, low fat and fair protein content (Enwere, 1998). Various food products are obtained from grains based on the processing techniques and the type of cereals employed (Gaffa et al., 2002). Cereals apart from being eaten directly are use largely to make various other products

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like flour, gruel, porridge and drinks especially alcoholic and non-alcoholic beverage.

Sorghum [*Sorghum bicolour* (L) Moench] locally called guinea corn is the most extensively grown cereal grain in the country (Aba et al., 2004). It is considered as one of the most important food crops in the world, following wheat, rice, maize and barley (FAO, 2006). Sorghum is the most amenable cereals grain to different processing technologies including primary, secondary and tertiary methods (Obilana and Manyasa, 2005). Primary processing involves: fermentation, malting, wet and dry milling, boiling, roasting and popping. Secondary processing involves: brewing, beverages and drinks production, baking and confectionery making, steaming, extrusion (for paste and noodles) while tertiary processing involves composite flour, biofortification and chemical fortification with additives. Sorghum drinks are also high in minerals, vitamins and some essential amino acids which are further enhanced through biofortification thus making them superior to other cereal foods. They contribute more energy and digestible protein in the diets of the majority of the people in the sub-Saharan regions than those obtained from root and tuber crops (Aba et al., 2004). In addition, its polyphenol content are used as antioxidants just as the slow digestibility of sorghum starch and protein makes its food useful in diabetic treatments. However, millets also have high starch, fiber content and poor digestibility of nutrients which severely limits their values in nutrition and influence their consumers acceptability (FAO, 1995). This paper determined the composition of sorghum-millet flour, *A. danielli* essential oil and its effect on mycotoxins in kunu zaki.

MATERIALS AND METHODS

Sorghum and millet grains were purchased at Bodija market, Ibadan while the fresh pods of *A. danielli* spice was obtained at Ibode market in Ibadan, Nigeria.

Raw material preparation

Sorghum and millet grains in ratio 1:2 were cleaned and soaked for 12 h. The grains were washed, drained and dried in the hot air oven (50°C) for 18 h. The dried sorghum-millet grains were milled and packed for kunu zaki production.

Production process of kunu zaki

The traditional method described by Gaffa et al. (2002) was adopted for use in the production of kunu zaki with slight modification. Four hundred grams of the sorghum-millet flour was mixed with 600 ml of water to form paste. The slurry was then divided into two portions (¾ and ¼). The larger portion was gelatinized by addition of boiling water. The two portions (gelatinized and ungelatinized) were then mixed together and then left overnight at room temperature for chance fermentation. It was then filtered using a muslin cloth. Spices were not added to the mixture intentionally. This is to ensure that no other spices interfere with the study.

Extraction of the essential oil

The essential oil extraction was done by hydro-distillation method using Clevenger's apparatus. The flask with weighed samples, condenser and other gadgets were connected to complete the hydro-distillation arrangement using Clevenger-type apparatus. The crushed sample in the flask was entirely covered with de-ionized water as suspension and placed on the heating mantle. The water was allowed to boil in the flask and the essential oil is carried over to the condenser along with the steam. The essential oil and the steam are separated below the condenser through a separator. The procedure was repeated until a sufficient amount of oil was obtained for the analysis. The essential oil was dried over anhydrous sodium sulphate and stored in a 2 ml sealed Agilent vial protected from light at 4°C before analysis.

Functional, pasting and anti-nutritional properties sorghum-millet flour

Bulk density was done using Udensi and Okaka (2000) method, water absorption and swelling index using method of Iwuoha (2004). Oil absorption capacity was done using the method of Balogun and Olatidoye (2010) method. The pasting property of the flour was determined by RVA (RVA-4, Newport Scientific, Australia) according to Vongsawasdi et al. (2009). Method of Dairo (2008) was used for phytate determination while oxalate content was determined using the method of Nwinuka et al. (2005). Total phenol, flavonoid, saponin and alkaloid determination were done using method of Obadoni and Ochuko (2001) and Sahoré and Amani (2012), respectively.

Determination of the composition of essential oil

Gas chromatograph HP 6890 Powered with HP ChemStation Rev. A 09.01 [1206] software with the following condition was used for the determination of the essential oil component. The Injection method was split injection, the split ratio of 20.1, Carrier Gas is Hydrogen, Flow Rate: 1.0 ml/min. The inlet temperature of 150°C, Column Type of HP 5MS. Column Dimensions were: 30 m x 0.25 mm x 0.25 µm. Oven Program was: Initial at 40°C; ramped was from 5°C/min to 200°C, run at 200°C for 2 min. Detector was FID; detector temperature was 300°C; hydrogen pressure was 22 psi and Compressed air was 28 psi.

Mycotoxin detection and quantification

All analytical procedure for mycotoxin includes three steps. These include: extraction, purification and quantification. Gas chromatograph used for the mycotoxin analysis was HP 6890 powered with HP Chem Station Rev. A 09.01 [1206] software. The Gas chromatograph conditions were as follow: Carrier gas was hydrogen gas; detector used was PFPD. The simultaneous analysis of the fumonisins and ochratoxins in the sample with gas chromatograph was of good sensitivity. The reagent grade solvents and salts were used for sample extraction, cleanup and derivations; Deionised water was used where applicable. Standards were obtained from Sigma Aldrich. The modified AOAC method originally developed for corn and its products for mycotoxin analysis was followed for the extraction of the fumonisin in the sample. The pH was adjusted to 6.0 before filtration into the clean borosilicate beaker. 50 ml of the sample was applied to the anion exchange SPE column previously conditioned with 10 ml methanol followed by 10 ml of the methanol and water ratio 3 to 1 and followed by another 6 ml of ethanol. The fumonisins was eluted with 20 ml of the methanol and acetic acid ratio 95 to 5.

The eluents was concentrated using a stream of the nitrogen gas in a 60°C water bath. The modified method from R-Biopharm as follows, the sample was free of gas by mixing with a magnetic stirrer at 100rpm for 6ominutes. The pH was adjusted to 7.2. The column was washed with 20 ml of the deionised water at the flow rate of 5 ml per minute. An aliquot of 30 ml was applied to the column (Ochraprep, R-Biopharm). The sample was eluted into the vial with 3 ml of the methanol. The eluents was concentrated using a stream of the nitrogen gas in a 60°C water bath. The concentrated extract was combined and derivatised for the injection into the gas chromatography. 1.0 µl of the derivatised extract was injected into the chromatograph.

Statistical analysis

The mean and standard deviation of the triplicate data were calculated and subjected to analysis of variance (ANOVA) and a difference was considered to be significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Properties of sorghum-milet flour

Table 1 shows the physical and functional properties of sorghum-millet flour, the major raw materials in the production of kunu zaki beverage. The bulk density, swelling index, water absorption capacity, solubility and oil absorption capacity were 0.75 g/cm^3, 1.16, 2.49 ml H_2O/g, 2.56% and 0.79 ml oil/g, respectively. Bulk density of flour is important in determining the packaging requirement and material handling (Ezeocha et al., 2011). Swelling index is an indication of the water absorption index of the granules and reflects the extent of the associative forces within the granules (Moorthy and Ramanuhan, 1986). Water absorption capacity is the ability of flow properties to entrap large amount of water and also refers to total amount of water held by starch gel under a defined state of condition (Pinnavaia and Pizzirani, 1998; Chen and Lin, 2002). The physico-chemical properties of flour affects the textural characteristics of the food preparations made from grain. The behavior of starch in water is temperature and concentration dependent. Grain starches shows low uptake of water and swelling power at room temperature.

The pasting properties of sorghum millet flour are as shown in the Table 2. Peak viscosity of the flour was 1234 Cp while the holding viscosity was 811 Cp.

Breakdown and final viscosities were 423 and 1824.5 Cp respectively. Final viscosity showed the ability of the flour to form viscous gel after cooking and cooling. Setback value was 1013.5 Cp. Setback viscosity is a process that occurs during cooling in which the starch molecules start to re-order and subsequently form a gel structure. The higher setback value is indicative of higher rate of starch retrogradation. The viscosities observed by Liu et al. (2012) for sorghum flours were higher than the viscosities value observed for sorghum-millet flour. Peak time was 4.97 mm while peak temperature was 79.53 Cp.

Table 1. Functional properties of sorghum-millet flour.

Functional properties	Value
Bulk density (g/cm^3)	0.75±0.11
Swelling index (%)	1.16±0.09
Water absorption capacity (ml H_2O/g)	2.49±0.11
Solubility (%)	2.56±0.23
Oil absorption (ml oil/g)	0.79±0.17

Table 2. The Pasting properties of sorghum millet flour.

Pasting properties	Value
Peak (Cp)	1234±0.32
Holding strength (Cp)	811±0.53
Breakdown (Cp)	423±0.19
Final viscosity (Cp)	1824.5±0.20
Setback (Cp)	1013.5±0.23
Peak time (min)	4.97±0.017
Pasting temp (°C)	79.53±0.37

Table 3. Anti-nutritional composition of sorghum-millet flour.

Anti-nutritional factor	Concentration
Phenols %	0.11
Oxalate %	0.01
Phytate (mg/100 g)	0.05
Saponins (mg/100 g)	0.83
Flavonoid (mg/100g)	2.31
Alkaloid (mg/100 g)	2.22
Tannin (mg/100 g)	1.69

Peak time is the time required to achieve peak viscosity (Liu et al., 2003). The anti-nutritional compositions of the flour are shown in Table 3. The flour had low phenols (0.11%), oxalate (0.01%), phytate (0.05 mg/100 g) and saponins (0.83 mg/100 g). The values for flavonoid, alkaloid and tannin were 2.31, 2.22 and 1.69%, respectively.

Composition of *A. danielli* essential oil

Essential oil compositions of *A. daniellii* seed are shown in Table 4. The essential oil of *A. daniellii* contained 56 components. The components detected in the essential oil of *A. danielli* seed were dominated by 1, 8-Cineole (56.16 %), and five other main component being, β – Pinene (14.77 %), Alpha Terpineol (11.46 %), Alpha

Table 4. Composition of the major content of the essential oil of *Aframomum danielli*.

Component	Retention time (min)	%
Alpha pinene	9.70	4.28
β - Pinene	11.38	14.77
Cis-ocimene	12.26	1.59
Pinene-2-oL	13.84	1.16
Gama terpinene	14.89	0.05
Geranial (Neral)	15.40	0.01
1,8-Cineole	17.71	56.16
Citronellal	18.21	0.04
Alpha Terpineol	18.69	11.46
Terpinen-4-ol	18.79	3.05
Citronellol	19.27	0.04
Alpha terpinenyl acetate	21.10	4.24
Neryl Acetate	21.72	0.02
Humulene (alpha Caryophylene)	27.78	0.04

Table 5. Concentration of mycotoxins in grains and kunun zaki.

Mycotoxin	Concentration (µg/kg)	
	Millet	Sorghum
Fumonisin B_1	7.87	12.55
Fumonisin B_2	0.92	1.27
Ochratoxin A	12.13	21.27

Pinene (4.28 %), Alpha Terpinenyl Acetate (4.24 %) and Terpinen-4-ol (3.05 %). The result is in agreement with the findings of Adegoke and Krishna (1998). Though, Adegoke and Krishna (1998) isolated 41 essential oil components but 1,8-cineole (59.8%), Alpha pinene (4.3%) and Alpha terpinyl acetate (3.2%) were also among the major compounds detected in the essential oil. The composition of essential oil vary depending on genetic variability inside plant species, the geographical region of plant cultivation, the occurrence of biotic and abiotic stresses, the phonological stage of the plant and its chemotype, the method of drying and the method of extraction of the oils (Cosentino et al., 1999; Jerkovic et al., 2001; Labra et al., 2004; Rota et al., 2008; Zheljazkov et al., 2008). It had been reported that spices owe their antimicrobial properties mostly to the presence of alkaloids, phenols, glycosides, steroids, essential oils, coumarins and tannins (Ebana et al., 1991).

Concentration of mycotoxins in cereal grains and beverages

The concentration of mycotoxin in cereal grains are shown in Table 5. Ochratoxin A and Fumonisin B_1 have higher values of 12.13 and 7.87 ug/Kg, respectively in millet. These values were increased in sorghum with 21.27 and 12.55 ug/Kg in Ochratoxin A and Fumonisin B_1 respectively. These results show that the cereal grains were contaminated during storage with sorghum being more susceptible to mycotoxin contamination. Likewise, Aroyeun et al. (2011) detected ochratoxin A in cocoa powder during storage. Therefore, susceptibility of cereal grains to mycotoxin contamination depend on storage conditions, climatic conditions and handling of the cereals after harvest. The level of FB_1 which was 7.09 µ/l is well above the provisional maximum tolerable daily intake of 2 µg/g set up by the Joint Food and Agricultural organisation and World Health Organisation (FAO/WHO) expert Committee on Food Additives (JECFA) (WHO, 2002). The presence of mycotoxins in cereal beverages like kunun zaki is expected due to poor handling of grains both in the field and during storage in developing countries like Nigeria. Amina et al. (2012) reported the occurrence of fumonisin and deoxynivalenol in stored maize used in industrial production in Zaria, Nigeria. This is a matter of concern because high cost of soft drinks and increased consumers awareness about synthetic products brought about high increase in the consumption of cereal beverages.

The initial concentration of FB_1 in kunu zaki is 7.09 µ/l, with the application of 0.25 % *A. danielli* essential oil. The value reduced to 6.56 signifying 8% reduction of FB_1 while the highest dose of 10 % resulted in 76% reduction (Table 6). Sumalan (2013) also recorded that reduction of fumonisin in wheat grain was dose dependent. The results revealed higher reductions in FB_1 than FB_2. *A. danielli* exhibited ability to reduce FB_1, FB_2 and Ochratoxin A, although in varying amount. Aroyeun et al. (2011) observed reduction in the level of ochratoxins in cocoa powder treated with different concentrations

Table 6. Effect of *Aframomum danielli* essential oil on the concentration of mycotoxin in kunun zaki.

Concentration of essential oil (%)	Concentration (µ/l)		
	FB1	FB2	Ochratoxin A
0	7.09±0.11^{b}	0.96±0.05^{c}	12.10±0.09^{a}
0.25	6.56 ±0.02^{f}	0.72 ± 0.01^{d}	10.66 ± 0.01^{f}
0.50	5.56 ±0.01^{e}	0.70 ± 0.01^{d}	9.93 ± 0.01^{e}
1.00	3.38 ± 0.01^{d}	0.42 ± 0.01^{c}	5.49 ± 0.01^{d}
2.5	2.67 ± 0.01^{c}	0.38 ± 0.01^{bc}	4.14 ± 0.01^{c}
5.0	2.15 ±0.01^{b}	0.36 ± 0.01^{d}	3.38 ± 0.01^{b}
10.0	1.67 ± 0.01^{a}	0.33 ± 0.01^{a}	2.81 ± 0.01^{a}

of *A. daniellii*.

It has been reported that *A. danielli* possesses broad-spectrum antimicrobial properties as it had been found to inhibit the growth of some microorganisms such as *Salmonella enteriditis, Streptococcus aureus*, *Aspergillus niger* and *Pseudomonas fragii* (Adegoke et al., 2002; Ashaye et al., 2006). The essential oil of *A. danielli* is able to penetrate into the amine group and commence the process of deamination. This may be responsible for the reduction in the toxins as the doses of essential oil increases. Yoon et al. (2000) describe that the essential oil is capable of depolarizing the mitochondrial membrane and decreasing the membrane potential, this affect the Ca^{2+} and other ion channels, resulting in the reduction of pH and also affecting the proto pump and ATP pool. The change of the fluidity of the membrane resulted into the leakage of radicals, cytochrome C, calcium ions and protein. Thus, permeabilization of outer and inner mitochondrial membrane leads to cell death by apoptosis and necrosis.

Conclusions

The combination of raw materials (sorghum and millet) for kunu zaki beverage showed high viscosities with high setback value which indicate its ability to retrogradate after cooling. Introduction of different doses of *A. danielli* essential oil into kunu zaki reduced mycotoxins in the beverage. Percentage reduction of mycotoxins in kunu zaki depends on the doses of *A. danielli* essential oil used.

Conflict of interests

The authors did not declare any conflict of interest.

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