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Microbiological evaluation and shelf life of seed flour mixes used for infant feeding in rural northern Nigeria

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This study investigates microbial load and shelf life of locally processed cereal-legume-oil seed flour mixes used for infant feeding in rural northern Nigeria. Free fatty acid (FFA), water activity (a_w), pH, total viable count (TVC) and presence of yeast, coliform bacteria and mold were the parameters determined. Hungry rice or *Digitaria exilis*, benne seed or *Sesamum indicum* and soybean or *Glycine max* seeds flours were produced using traditional processing methods, including washing, boiling, fermenting and roasting. The test samples were milled into flour that could pass through a 70 mm mesh sieve and blended on protein basis at 70:30 cereal-legume/oilseed ratios to produce D₇₀S₃₀, D₇₀G₃₀, D₇₀S₁₅G₁₅, D₇₀S₂₀G₁₀ and D₇₀S₁₀G₂₀ and stored at 30 to 32°C for 60 days. The parameters were determined at day 0 and intervals of 14, 28, 42 and 60 days. At the end of the storage period, all the parameters increased. FFA increased from 0.15 - 0.16% to 0.47 - 0.58%; a_w from 0.46 - 0.48 to 0.72 - 0.80; pH from 3.9 to 4.7 - 4.9 and TVC from $<1.0 \times 10^2$ to 2.2×10^3 to 3.6×10^4 cfu/g for 0 and 60 days, respectively. The differences between the values of the parameters of the formulations were significant ($p < 0.05$) especially from day 14. Yeast and coliform were not detected in all the samples but mold was detected from days 14 to 60. The formulation D₇₀S₃₀ had best shelf life; however, the samples should be consumed within 14 days of production.

Key words: Seed flour, multi-mixes, children, microbial load, shelf life.

INTRODUCTION

Poor child-feeding practices and high rate of infection result in malnutrition and poor health of children (WHO, 2010; UNICEF, 2011). The period from birth to two years is a 'critical window' for the promotion of exclusive breastfeeding (0 to 6 months) and quality/adequate complementary feeding (6 to 24 months) to optimize

growth, health, and development in children. In rural northern Nigeria, cereal, legume/oilseed based complementary porridges are local diets fed to children 6 to 24 months of age with continued breast feeding. Rural mothers combine different plant foods because they understand that dietary diversity (multi-mixes) is an

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Abbreviations: FFA, Free fatty acid; a_w , water activity; TVC, total viable count.

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effective approach to improve the quality of young children's plant-based diets especially without access to fortified food products (WHO/UNICEF, 2008). Combining cereals and legumes at the ratio of 70:30 have been shown to have nutrient potentials that complement one another in general (Fernandez et al., 2002) and have amino acid pattern that can compare with that of animal protein. Cereal and legume commonly blended for infant feeding are maize or millet and soybean. Other cereals and legumes/oilseed which are also cheap sources of plant protein but are under exploited and not commonly used in infant feeding include hungry rice (cereal) and benne seed (oilseed). They are under exploited in infant feeding general in Nigeria and in the south in particular; they are however used in rural north.

Hungry rice is one of the nutritious grains in the world (Thiam, 2008). It is particularly rich in many essential amino acids (National Academy of Science, 2006). Benne seed is an oil legume that is rich in micronutrients and phytochemicals (Bedigian, 2003). In the north of Nigeria where hungry rice and benne seed are combined in infant porridge, mothers frequently process these foods to produce complementary porridges. This is a gruesome task and in most cases facilities such as refrigerators to store the processed flour or paste are not available, they therefore store processed flour and pastes for less than seven days. The potentials of storing processed flour in standard household pantry beyond seven days could be exploited to reduce the work load and time for mothers and caregivers.

Shelf life is the length of time that a commodity such as food may be stored without becoming unfit for use or consumption. It is the recommended maximum time for which products can be stored during which the defined quality of a specified proportion of the goods remain acceptable under expected conditions of distribution, storage and display (Gyesley, 1991). A food product within its shelf life should be safe to eat, keep its appearance, colour, texture and flavour and meet any nutritional claims provided on the label (New Zealand Food Safety Authority, 2005). All foods spoil with time but the rate differs from food to food. Factors that affect food quality include microbial (bacteria, yeast and mold) growth, non-microbial spoilage (rancidity, browning and loss of nutrients) and product related spoilage such as water activity, pH and oxygen availability (Fontana, 2008; Sivasankar, 2010).

Free fatty acid in stored foods is used to measure rancidity. Enzymes inherent in foods hydrolyze fat in the food into free fatty acid and glycerol during storage (Morrison, 2006). Light, atmospheric oxygen and moisture also precipitate rancidity which changes the flavour and taste of food (Modi et al., 2004). Water activity of a food is the ratio between the vapour pressure of the food itself, when in a completely undisturbed balance with the surrounding air media and the vapour pressure of distilled water under identical condition (U.S.

Food and Drug Administration, 1984). A a_w of 0.80 means that the vapour pressure is 80% of that of pure water. It is the amount of water in a food that is available for microbial use and it helps to predict microbial growth (Fontana, 2001). Water activity predicts food stability with respect to microbial growth rate of deteriorative reaction and physical properties of foods (texture and shelf life) (Ukegbu and Anyika, 2012). High values support microbial growth, therefore, moist food spoils faster than dry food. A food or solution's pH is a measure of acidity or alkalinity using a numerical scale between 1 and 14. Low pH value shows acidity and it limits the growth of many microorganisms and vice versa (International Commission on Microbiological Standards for Foods, 1996). The pH limit for growth of any microorganism is 0.60 (Fontana, 2008)

Microbial load of a food is determined by the levels of microorganisms (measured in colony forming units per gram) in the food during production, packaging, storage and other handling as well as the type of food in question. The end of shelf life can be based on the quantity of microorganism present (NZFSA, 2005). The objective of this study was to evaluate at intervals (0, 14, 28, 42 and 60 days) microbial load and shelf life of cereal-legume/oilseed multi-mixes used for infant feeding in rural northern Nigeria.

MATERIALS AND METHODS

Digitaria exilis, *Sesamum indicum* and *Glycine max* used for the study were purchased from a local market in Kaduna state in northern Nigeria and processed using traditional methods. *D. exilis* was washed, soaked in water (1:3 ratio W/V), fermented for 48 h (with the water changed every 24 h) and sundried. *S. indicum* seeds were washed, sundried and roasted at 80°C for 10 min. *Glycine max* seeds were also washed and boiled at 100°C for 60 min dehulled manually, soaked in water (1:3 ratio W/V), fermented for 24 h (with the water changed every 24 h) and sundried. The dried *Glycine max* seeds were roasted at 80°C for 10 min. The residual moisture after the processing of the food items was approximately 3%. All the processed seeds were milled (to pass through a 70 mm mesh sieve) separately into flour and analyzed for crude nitrogen (N) before being blended on the basis of their crude nitrogen (N) concentration. Each blend derived 70% of its dietary nitrogen from cereal (hungry rice) and 30% from legume (soybean) and or oilseed (benne seed) and furnished 20 g of protein per day. Five multi-mix formulations were *D. exilis*₇₀ and *S. indicum*₃₀ (*D*₇₀*S*₃₀); *D. exilis*₇₀ and *G. max*₃₀ (*D*₇₀*G*₃₀); *D. exilis*₇₀ *S. indicum*₁₅ *G. max*₁₅ (*D*₇₀*S*₁₅*G*₁₅); *D. exilis*₇₀, *S. indicum*₂₀ and *G. max*₁₀ (*D*₇₀*S*₂₀*G*₁₀) and *D. exilis*₇₀, *G. max*₂₀ and *S. indicum*₁₀ (*D*₇₀*G*₂₀*S*₁₀). 10 g of each mix was packaged in self-sealed polyethylene food bags and stored in a standard household pantry at 30 to 32°C for 60 days.

Free fatty acid (FFA), water activity (a_w), pH and microbiological load of each mix were analyzed in triplicate using standard procedures at days 0, 14, 28, 42 and 60. Free fatty acid was determined by the method of Association of Official Analytical Chemists (AOAC) (2005). The pH was determined using standard electrode pH meter as described by AOAC (1990). Water activity value analyzer model 5803, Germany was used to assess the water activity of the samples. The microbiological load was determined by serial dilution of each sample in ten-fold with sterile 0.1% media by

Table 1. Percentage free fatty acids of seed flour mixes made from *Digitaria exilis*, *Sesamum indicum* and *Glycine max* seed flours.

Sample code	0 day	14 days	28 days	42 days	60 days
D ₇₀ S ₃₀	0.15±0.01 ^b	0.26±0.01 ^b	0.27±0.01 ^c	0.37±0.01 ^b	0.47±0.02 ^c
D ₇₀ G ₃₀	0.15±0.01 ^b	0.36±0.01 ^a	0.46±0.01 ^a	0.47±0.02 ^a	0.58±0.04 ^a
D ₇₀ S ₁₅ G ₁₅	0.15±0.02 ^b	0.36±0.02 ^a	0.46±0.02 ^a	0.47±0.01 ^a	0.58±0.05 ^a
D ₇₀ S ₂₀ G ₁₀	0.15±0.01 ^b	0.25±0.02 ^c	0.36±0.01 ^b	0.47±0.03 ^a	0.57±0.01 ^b
D ₇₀ S ₁₀ G ₂₀	0.16±0.01 ^a	0.36±0.01 ^a	0.46±0.02 ^a	0.47±0.05 ^a	0.58±0.01 ^a

Means ± SD of 3 determinations; means with different superscript on a column are significantly different. D₇₀S₃₀ = *Digitaria exilis*₇₀/*Sesamum indicum*₃₀; D₇₀G₃₀ = *Digitaria exilis*₇₀/*Glycine max*₃₀; D₇₀S₁₅G₁₅ = *Digitaria exilis*₇₀/*Sesamum indicum*₁₅/*Glycine max*₁₅; D₇₀S₂₀G₁₀ = *Digitaria exilis*₇₀/*Sesamum indicum*₂₀/*Glycine max*₁₀; D₇₀S₁₀G₂₀ = *Digitaria exilis*₇₀/*Sesamum indicum*₁₀/*Glycine max*₂₀.

Table 2. Water activity (a_w) of seed flour mixes made from *Digitaria exilis*, *Sesamum indicum* and *Glycine max* seed flours.

Sample code	0 day	14 days	28 days	42 days	60 days
D ₇₀ S ₃₀	0.48±0.01 ^a	0.53±0.01 ^e	0.55±0.01 ^d	0.61±0.01 ^c	0.72±0.01 ^e
D ₇₀ G ₃₀	0.47±0.00 ^a	0.55±0.01 ^c	0.60±0.01 ^a	0.61±0.01 ^c	0.80±0.01 ^a
D ₇₀ S ₁₅ G ₁₅	0.47±0.01 ^a	0.58±0.01 ^a	0.58±0.01 ^b	0.60±0.03 ^d	0.78±0.02 ^b
D ₇₀ S ₂₀ G ₁₀	0.48±0.01 ^a	0.54±0.02 ^d	0.57±0.02 ^c	0.65±0.01 ^a	0.75±0.01 ^d
D ₇₀ S ₁₀ G ₂₀	0.46±0.01 ^b	0.57±0.01 ^b	0.58±0.01 ^b	0.62±0.01 ^b	0.77±0.01 ^c

Means ± SD of 3 determinations; means with different superscript on a column are significantly different. D₇₀S₃₀ = *Digitaria exilis*₇₀/*Sesamum indicum*₃₀; D₇₀G₃₀ = *Digitaria exilis*₇₀/*Glycine max*₃₀; D₇₀S₁₅G₁₅ = *Digitaria exilis*₇₀/*Sesamum indicum*₁₅/*Glycine max*₁₅; D₇₀S₂₀G₁₀ = *Digitaria exilis*₇₀/*Sesamum indicum*₂₀/*Glycine max*₁₀; D₇₀S₁₀G₂₀ = *Digitaria exilis*₇₀/*Sesamum indicum*₁₀/*Glycine max*₂₀.

pour plate technique as described by AOAC (1990). Coliform count was determined using nutrient agar with 24 h incubation at 37°C; sabour and dextrose agar was used for yeast/mold with 48 h incubation at room temperature while for TVC it was 24 h incubation at 37°C. One way analysis of variance (ANOVA) and Duncan's multiple range tests were used to separate/compare means obtained between groups. Differences were considered significant at p<0.05.

RESULTS

Table 1 presents the percentage of FFA in the seed flour mixes. The FFA values of all the blends increased with storage (p<0.05). At day 0, D₇₀S₁₀G₂₀ had the highest FFA (0.16%) while the D₇₀G₃₀, D₇₀S₃₀, D₇₀S₁₅G₁₅, and D₇₀S₂₀G₁₀ had 0.15%. At day 60, the D₇₀S₃₀ had the least FFA value (0.47%) while D₇₀G₃₀, D₇₀S₁₅G₁₅ and D₇₀S₁₀ had the highest value (0.58%). There were differences (p<0.05) among the FFA values of the samples especially on days 14 and 28. Table 2 shows water activity (a_w) values of the seed flour mixes at different intervals. The values ranged from 0.46 in D₇₀S₁₀G₂₀ to 0.48 in D₇₀S₃₀ and D₇₀S₂₀G₁₀ at day 0. At day 14, D₇₀S₁₅G₁₅ had the highest water activity of 0.58, followed by D₇₀S₁₀G₂₀ (0.57 a_w), D₇₀G₃₀ (0.55 a_w) and D₇₀S₂₀G₁₀ (0.54 a_w). D₇₀S₃₀ had

the least value (0.53 a_w). At day 28 of storage, D₇₀G₃₀ had the highest value (0.60 a_w) and the D₇₀S₃₀ had the least value (0.55 a_w). The water activity of all the samples were similar at days 28 and 42, but showed significant differences (p<0.05) between days 0 and 60. There was a progressive increase in the pH values of the samples with storage (Table 3). At day 0, all the samples had the same pH value (3.9). The pH values at day 14 ranged from 4.0 in D₇₀S₁₀G₂₀ to 4.2 in D₇₀S₁₅G₁₅ while at day 42, it ranged from 4.5 in D₇₀S₃₀ and D₇₀S₁₅G₁₅ to 4.8 in D₇₀G₃₀ and D₇₀S₁₀G₂₀ and 4.9 in D₇₀G₃₀. The decrease in acidity was significant (p<0.05) from day 0 to day 60. However, there were no significant differences (P>0.05) in the pH values of the samples at a particular interval except in days 28, 42 and 60. At day 0, all the samples had the same TVC value of <1.0x10² cfu/g except for D₇₀G₃₀ (Table 4). At day 14, D₇₀S₃₀ and D₇₀S₂₀G₁₀ had the least (1.0x10²) TVC value and D₇₀G₃₀ had the highest (1.4x10² cfu/g). At the end of 60 days of storage, D₇₀G₃₀ had the highest (3.6x10⁴cfu/g) and D₇₀S₃₀ the least (2.2x10³ cfu/g) TVC value. Only mold (< 100 cfu/g) was detected from the 14 days. At day 60, the D₇₀G₃₀ had the highest mold value (2.4x10⁴ cfu/g), followed by D₇₀S₁₅G₁₅ (2.6x10³ cfu/g), D₇₀S₁₀G₂₀ (1.7x10³ cfu/g), D₇₀S₂₀G₁₀ (2.7x10²

Table 3. pH of seed flour mixes made from *Digitaria exilis*, *Sesamum indicum* and *Glycine max* seed flours measured at intervals.

Sample code	0 day	14 days	28 days	42 days	60 days
D ₇₀ S ₃₀	3.9±0.01 ^a	4.1±0.00 ^a	4.4±0.03 ^b	4.5±0.00 ^c	4.7±0.03 ^c
D ₇₀ G ₃₀	3.9±0.02 ^a	4.1±0.01 ^a	4.5±0.05 ^a	4.8±0.01 ^a	4.9±0.04 ^a
D ₇₀ S ₁₅ G ₁₅	3.9±0.01 ^a	4.2±0.00 ^a	4.4±0.01 ^b	4.5±0.00 ^c	4.7±0.02 ^c
D ₇₀ S ₂₀ G ₁₀	3.9±0.02 ^a	4.1±0.04 ^a	4.4±0.02 ^b	4.7±0.02 ^b	4.8±0.01 ^b
D ₇₀ S ₁₀ G ₂₀	3.9±0.03 ^a	4.0±0.00 ^a	4.3±0.02 ^c	4.8±0.03 ^a	4.9±0.03 ^a

Means ± SD of 3 determinations; means with different superscript on a column are significantly different. D₇₀S₃₀ = *Digitaria exilis*₇₀/*Sesamum indicum*₃₀; D₇₀G₃₀ = *Digitaria exilis*₇₀/*Glycine max*₃₀; D₇₀S₁₅G₁₅ = *Digitaria exilis*₇₀/*Sesamum indicum*₁₅/*Glycine max*₁₅; D₇₀S₂₀G₁₀ = *Digitaria exilis*₇₀/*Sesamum indicum*₂₀/*Glycine max*₁₀; D₇₀S₁₀G₂₀ = *Digitaria exilis*₇₀/*Sesamum indicum*₁₀/*Glycine max*₂₀.

Table 4. Total viable count (TVC), mold, yeast and coliform count (cfu/g) of seed flour mixes made from *Digitaria exilis*, *Sesamum indicum* and *Glycine max* seed flours.

Parameter	Intervals (days)	D ₇₀ S ₃₀	D ₇₀ G ₃₀	D ₇₀ S ₁₅ G ₁₅	D ₇₀ S ₂₀ G ₁₀	D ₇₀ S ₁₀ G ₂₀
TVC	0	<1.0×10 ^{2a}	1.0×10 ^{2a}	<1.0×10 ^{2a}	<1.0×10 ^{2a}	<1.0×10 ^{2a}
	14	1.0×10 ^{2d}	1.4×10 ^a	1.2×10 ^{2c}	1.0×10 ^{2d}	1.3×10 ^{2b}
	28	2.2×10 ^{2c}	2.0×10 ^{2c}	1.5×10 ^{2c}	2.0×10 ^{2c}	1.8×10 ^{2c}
	42	2.0×10 ^{3c}	2.2×10 ^{3b}	1.8×10 ^{3d}	2.3×10 ^{3a}	2.0×10 ^{3c}
	60	2.2×10 ^{3d}	3.6×10 ^{4a}	2.4×10 ^{3c}	2.6×10 ^{3b}	2.1×10 ^{4e}
Mold	0	N.D	N.D	N.D	N.D	N.D
	14	6.0×10 ^{1c}	5.0×10 ^a	8.0×10 ^a	6.0×10 ^{1c}	7.0×10 ^{1b}
	28	1.0×10 ^{2c}	1.1×10 ^{2b}	1.3×10 ^{2a}	1.0×10 ^{2c}	1.1×10 ^{2b}
	42	2.0×10 ^{2c}	2.2×10 ^{2c}	2.3×10 ^{2b}	2.0×10 ^{2c}	2.5×10 ^{2a}
	60	2.5×10 ^{2e}	2.4×10 ^{4a}	2.6×10 ^{3b}	2.7×10 ^{2d}	1.7×10 ^{3c}
Yeast	0 - 60	N.D	N.D	N.D	N.D	N.D
Coliform	0 - 60	N.D	N.D	N.D	N.D	N.D

Means ± SD of 3 determinations; means with different superscript on a column are significantly different. D₇₀S₃₀ = *Digitaria exilis*₇₀/*Sesamum indicum*₃₀; D₇₀G₃₀ = *Digitaria exilis*₇₀/*Glycine max*₃₀; D₇₀S₁₅G₁₅ = *Digitaria exilis*₇₀/*Sesamum indicum*₁₅/*Glycine max*₁₅; D₇₀S₂₀G₁₀ = *Digitaria exilis*₇₀/*Sesamum indicum*₂₀/*Glycine max*₁₀; D₇₀S₁₀G₂₀ = *Digitaria exilis*₇₀/*Sesamum indicum*₁₀/*Glycine max*₂₀. ND = Not detected.

cfu/g). D₇₀S₃₀ had the least value (2.5×10² cfu/g). The differences were significant (p<0.05) across the different intervals. The same trend was observed in mold. Yeast and coliform were not detected in all the samples throughout the storage period.

DISCUSSION

At day 0, all the samples had FFA value of 0.15% except the D₇₀S₁₀G₂₀ which had 0.16%. These values gradually increased during the 60 days of storage probably due to hydrolysis of fat. This is in agreement with the report of Sewald and DeVries (2003) that hydrolysis of glycerides could account for increased values of free fatty acid in stored flours. The low FFA values observed in the D₇₀S₃₀

after 60 days of storage relative to the other mixes could be attributed to absence of benne seed in the blend. This implied that D₇₀S₃₀ would have a longer keeping quality because long shelf life is associated with low FFA of a food/product (Food Chain, 2001). However, the values of all the samples were below the range (0.5 to 1.5%) that Onwuika (2005) reported when rancidity began to be noticed in most oils and oil rich foods. Also, antioxidant (sesamol and sesamolina) content of benne seed could have contributed to the long shelf life of the mixes (Bedigian, 2003; Ologunde, 2011). The water activity (a_w) of each blend increased with storage. This could be due to concomitant increase in moisture content and as a function of time and temperature (Rong, 2007). The low water activity of the mixes on day 0 relative to the subsequent days could be associated with fermentation

of the staples. This is in agreement with an earlier report by Mba-Anyadioha (2008) that fermentation increased acidity which reduced the water activity and moisture level in which the fermenting organisms thrive successfully. In addition, sun drying and roasting might have contributed to the reduced values obtained. The water activity values of all the mixes were below the value (0.91) in which most food spoilage bacteria do not grow (Sivasankar, 2010). Spoilage bacteria and mold require $0.91a_w$ and $0.70a_w$, respectively for growth (Sivasankar, 2010). The lower limit for growth of mycotoxigenic mold is about $0.78 a_w$. Also, yeast was not detected in all the blends throughout the storage period because a_w values were below the value (0.88) that supports yeast growth (Fontana, 2008).

The pH of all the samples was 3.9 on production. This indicated acidity, which might be due to production of weak acid during fermentation of the grains (Mba-Anyadioha 2008). The pH of a food encourages or discourages growth and survival of microflora in that food (Fontana, 2008). Low pH value (acidity) did not favour the growth of pathogens such as *Enterobacteriaceae* and *Aspergillus* which are implicated in infantile diarrhoeal diseases (Rong, 2007). The optimum pH for growth of *Enterobacteriaceae* is in the range of 6.0 to 8.0 (ICMSF, 1996). As the storage progressed, the pH of the samples gradually increased to 4.9 in $D_{70}S_{15}G_{15}$ and $D_{70}S_{15}G_{15}$. Such increase according to Rong (2007) could be attributed to chemical changes. The final pH value (4.9) of the samples was below the value that encourages microbial growth. The low pH of samples at the end of storage period meant that microbial activities in food products were reduced and that helped to extend the shelf-life of the samples.

Yeasts and coliforms were not detected in the mixes throughout the storage period of 60 days probably because of the acidification of the medium by lactic acid fermenters (Mba-Anyadioha, 2008) and storage temperature. The low microbial load of the complementary mixes was due to low water activity and low pH caused by fermentation of the grains (Mensah et al., 1990). Fermentation induced an anti-microbial environment that reduced the risk of microbial contamination as observed by Ayoya et al. (2010) in home-made complementary foods. The low microbial load could also be due to roasting of the food samples. Roasting reduces micro-organisms and enzyme activity and destroys insects to improve keeping qualities (FAO/WHO, 2012). However, the total viable counts of formulated complementary mixes were within the acceptable limits of 10^7 cfu/g (ICMSF, 1996) for flours. The detection of mold at day 14 could be attributed to post production contamination from the environment (including the storage material), temperature, increased water activity and pH. The temperature and pH ranges that favour mold growth are 10 to 35°C and 2 to 9, respectively. However, the mold counts of all the samples were within ICMSF

(1996) specifications (10^5 cfu/g) for flours. Microbial proliferations in foods need certain conditions - namely available water (water activity), proper pH, right temperature and nutrients and time. By controlling these conditions one can prevent microbial growth and extend the shelf life of a food. At the end of the storage period, the samples had FFA values below the range that rancidity was observed in a previous study. Also, a_w values were below that which allows for proliferation of microorganisms. The pH was slightly above the value (4.6) at which a product is said to be acidic however, it was below the level which encourages microbial proliferation. Yeasts and coliforms were not detected in the mixes throughout the storage period of 60 days. The presence of mold from day 14 meant that the mixes should be consumed within 14 days of production. Sanitation is a factor in the control of microbial growth. The flours should be prepared under hygienic conditions and stored in air-tight container and in clean and dry pantry that is not dark to discourage growth of mold. Also, porridge made from the flour should be well cooked to further ensure safety.

Conflict of interests

The authors did not declare any conflict of interest.

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