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Table of Content: Volume 12 Number 10 October 2018

ARTICLES

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Full Length Research Paper

The effect of mechanical kneading and absit preparation on tef injera quality

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The aim of this study was to investigate the effect of mechanical kneading and 'absit' preparation difference on the quality of tef injera, the staple food of Ethiopians. Standard methods were adopted to determine the starch fraction, total phenol, flavonoid, phytate and tannins of injera. Sensory injera quality was assessed using 9-point-hedonic scale. Change in kneading conditions (time/speed) did not significantly affect the free sugar (FSG), slowly digestible starch (SDS), resistant starch (RS), total starch(TS) and starch digestion rate index (SDRI). On the other hand, significant variation was observed in rapidly available glucose and rapidly digestible starch (RAG and RDS). Flavonoids, total phenolics and phytate contents varied significantly at different kneading time- speed combinations. Injera sensory quality was also significantly affected due to change in kneading conditions. Kneading condition 5 (3 min at speed 6) has the highest injera overall acceptability while kneading condition 9 (7 min at speed 12) had the lowest. In addition to kneading conditions, absit preparation (water to fermented dough ratio) was also found to affect the quality of tefinjera. Absit # 3 made from 100 ml of fermented dough and 900 ml of water had the highest injera overall acceptability while, the lowest was observed on Absit # 4 made from 300 ml of fermented dough and 100 ml of water. In conclusion, both kneading and absit preparation significantly influenced starch hydrolysis, flavonoids, total phenolics and phytate contents as well as sensory quality of injera.

Key words: Kneading, sensory quality, absit preparation, starch fractions.

INTRODUCTION

More than 70% of Ethiopian populations rely on injera for their diet, which is a traditional Ethiopian sourdough flatbread (Dijkstra et al., 2008). It is mostly made from flour obtained from the tef grain (*Eragrostis tef* [Zucc.] Trotter). In addition, injera can be made from different cereals such as wheat, sorghum, and maize having different quality (Yetneberk et al., 2004). Quality characteristics of injera are directly related to its

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Table 1. Kneading and absit variables.

Kneading variables			Absit variables		
Kneading treatment	**Time (min)**	**Speed (speed)**	**Absit treatment**	**Fermented dough (ml)**	**Water (ml)**
1	1	1	1	100	100
2	1	6	2	100	300
3	1	12	3	100	900
4	3	1	4	300	100
5	3	6	5	300	300
6	3	12	6	300	900
7	7	1	7	0	100
8	7	6	8	0	300
9	7	12	9	0	900

appearance, texture and taste. According to Assefa et al. (2018), a normal and typical injera is round, soft, spongy and resilient, about 6 mm thick with uniformly spaced honeycomb-like ‘‘eyes’’ on the top.

The fermentation of injera starts by adding water to tef flour and mixing or kneading it with a starter (back-slopped culture) called ersho. This process commences the ‘primary fermentation’ (Attuquayefio, 2014). According to Dobraszczyk and Morgenstern (2003), even though it is obvious that mixing in the development of rheology and texture in wheat dough is important, there is very little information on these changes at the different stages in the mixing process. There is little information on mixing and its effect on the texture of injera. In the traditional preparation of injera, tef flour, water and Ersho are kneaded into a thick paste or dough (Ashagrie and Abate, 2012; Girma et al., 2013).

Kneading in bread making is known to aerate the dough and according to Maloney and Foy (2003), gas retention depends on the development of the proper dough structure which requires adequate enough mixing. According to Keiffer (2006), during kneading, the wheat dough will wind up the hook when optimum kneading approaches. He described this as the ‘so-called Weissenberg effect’ and stated that it is a sign of elasticity. It is not known whether the Weissenberg effect (rod-climbing phenomenon) occurs in gluten-free dough or whether kneading enhances this phenomenon and hence has a significant effect on the quality of the final baked injera.

Dough processing is also an important factor determining the quality of baked goods (Amjid et al., 2013). The most important mechanical steps in industrial dough processing are kneading, extrusion, and molding. In all of these processing steps, considerable changes in the structure and properties of the dough can occur (Amjid et al., 2013). Moreover, Li et al. (2015) also described as food processing including: mixing, kneading, and heating affect the antioxidant properties of foods for which polyphenols are responsible.

Once fermentation takes place, part of the fermented batter is gelatinized by cooking to form the absit which is then added back to the fermented batter. This step initiates the ‘secondary fermentation’ (Assefa et al., 2018). Zannini et al. (2012) stated that the functionality of absit in the injera can be described as that of hydrocolloids in gluten-free breads, providing the batter with a better gas-holding capacity because of increased viscosity. Ashenafi (2006) also reported that absit is a dough enhancer (improves the texture of the dough) and Girma et al. (2013) also mentioned that absit is a dough binder, but does not define these terms or suggest a mechanism for the effect. It is believed that the main function of a dough enhancer and binder is to enhance the viscosity of batters. Yetneberk et al. (2004) stated that the objective of gelatinization is primarily to bring about cohesiveness of the batter and secondly to provide easily fermentable carbohydrate to leaven the injera. Yetneberk et al. (2004) also reported that by cooking part of the fermented batter to gelatinize the starch, the carbon dioxide produced by the fermentation is trapped and leavens the injera on baking. However, this study is lacking on the influence of absit preparation on injera quality. Therefore, the present study was done to investigate the effect of mechanical kneading and ‘absit’ preparation difference on tef injera quality.

MATERIALS AND METHODS

Among the different varieties released by Debre Zeit Agricultural Research Center of the Ethiopian Institute of Agricultural Research (EIAR), farmers predominantly prefer the Qouncho tef variety (DZ-Cr-387). This variety was selected and obtained from Debre Zeit Agricultural Research Center. Tef sample was hermetically stored in cool and dry place using polyethylene bag. Before milling, tef grain was cleaned by sifting. The kneading conditions (time/speed) (Table 1) were used based on preliminary assessment of injera exporters, kneading machine capacity, and other works on bread dough kneading; similarly the ratio of water to fermented dough for absit preparation was based on previous work (Assefa et al., 2018) and traditional practices. Based on the sensory results, Kneading # 1, with shorter kneading time and slower kneading speed, had moderate overall injera acceptability; #5, with moderate kneading time and moderate speed, had the highest injera acceptability; and

#9, with longer kneading time and fastest kneading speed, had the lowest injera acceptability; they were selected for starch hydrolysis, flavonoids, total phenolics, phytate and tannins analysis.

Tef dough kneading and Injera preparation

Tef dough samples were prepared according to Parker et al. (1989) and Zegeye (1997) with little modification. Amount equal to 60 ml of starter (ersho) was initially added to each kg of flour. Accordingly, the tef flour (from stone-disc mill) was mixed at 2:3 (w/w) with potable water and kneaded by kitchen aid (Moulinex Masterchif 720, France). The dough was allowed to ferment for 60 h at room temperature 30 ±5°C. After this primary fermentation, different dough-water combinations (Table 1) were used to prepare absit that was heated for 15 min with continuous stirring. The hot cooked dough (absit) was then mixed back into the fermenting dough, and sufficient potable water was added to make a batter. The batter was left covered for 2 h for secondary fermentation. Additional water was added to thin and form the right consistency of batter. Finally, half a liter of batter was poured onto the hot clay griddle in a circular form. After 2 to 3 min of cooking using electric injera baking equipment, injera was removed and placed in a basket.

Starch fractions analysis

Englyst et al. (1992) methods were used to measure *in vitro* starch digestibility of tef injera with modifications by Englyst et al. (1999 and 2000). The hydrolyzed glucose at 20 min (G20) and 120 min (G120) and the total glucose (TG) were measured by the glucose oxidase colorimetric method (Englyst et al., 2000). The free sugar glucose (FGS) content was measured by a separate test according to Englyst et al. (2000). Rapidly digested starch (RDS) = 0.9 * (G20 - FGS), slowly digestible starch (SDS) = 0.9 *(G120 - G20), resistant starch (RS) = 0.9 * (TG - G120), for total starch, (TS) = 0.9 * (TG -FGS) and rapidly available glucose of the sample (RAG) = G20 were calculated. As used by Abebe et al. (2015), starch digestibility rate index (SDRI) was computed from the percentage of RDS in TS in the flours.

Sample extraction for further analysis

Samples were extracted based on the procedures outlined by Barros et al. (2007) and Ferreira et al. (2007). Five gram of injera sample was extracted by stirring with100 ml of methanol at 25˚C and 150 rmp for 24 h using temperature shaker incubator (ZHWY-103B, China) and then filtered through Whatman No. 4. The residue was then extracted with two additional 100 ml portions of methanol as described above. The combined methanolic extracts were evaporated at 40^0C to dryness using rotary evaporator (Stuart R3300, UK) and re-dissolved in methanol at the concentration of 50 mg/ml and stored at 4^0C for further use.

Determination of total phenolic content

Phenolic compounds concentration in the injera methanolic extracts was estimated based on procedures described by Ferreira et al. (2007). One milliliter of sample (2000 µg) was mixed with 1 ml of Folin-Ciocalteus reagent. After 3 min, 1 ml of saturated sodium carbonate (20%) solution was added to the mixture and adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm. Gallic acid was used to construct the standard curve (0.5–100 µg/ml). Total content of phenolic in injera extracts and gallic acid equivalent (GAE) was calculated by the following formula:

$$C = \frac{c \times V}{m}$$

Where, C is the total content of phenolic compounds (mg/g fresh material in GAE) and c the concentration of gallic acid established from the calibration curve.

Determination of total flavonoids

Total flavonoid was determined by a colorimetric method as described by Xu and Chang (2007). Briefly, 0.25 ml of sample (50 mg) was mixed with 1.25 ml of deionized water and 75 µl of a 5% $NaNO_2$ solution. After 6 min, 150 µl of a 10% $AlCl_{3}.6H_2O$ solution was added to the mixture. The mixture was incubated at room temperature for 5 min, after which 0.5 ml of 1 M NaOH and 2.5 ml of deionized water were added. The mixture was then thoroughly vortexed and the absorbance of the pink color was measured at 510 nm against the blank. For the calibration curve, (+)- catechin was used with a concentration range of 10–1000 µg/ml. Results were expressed as mg (+)- catechin equivalent (CE)/g of extract.

Determination of phytate

The phytate content in the sample was determined according to Oyaizu, (1986). About 0.1 g of fresh samples was extracted with 10 ml 2.4% HCl in a mechanical shaker for 1 h at a room temperature. The extract was centrifuged at 3000 rpm for 30 min. The clear supernatant was used for phytate estimation. One ml of Wade reagent (containing 0.03% solution of $FeCl_3.6H_2O$ and 0.3% of sulfosalcilic acid in water) was added to 3 ml of the sample solution (supernatant) and the mixture was mixed on a vortex for 5 s. Absorption readings at 500 nm were taken against a blank sample consisting of 3 ml extract solution with 2 ml of 2.4% HCl without Wade reagent. Sodium salt of phytic acid (4.5-36 mg/ml) was used as standard for construction of calibration curve.

Determination of condensed tannins

Tannin was determined by Burns (1971) as modified by Maxson and Rooney (1972). One gram of sample was weighed and mixed with 10 ml 1% HCl in methanol in a screw cap test tube. Then, the tube was shaken for 24 h at room temperature on a mechanical shaker (ZHWY-103B, China). The solution was centrifuged at 1000 rpm for 5 min. One ml of supernatant was transferred to another test tube and mixed with 5 ml of vanillin-HCl reagent (prepared by combining equal volume of 8% concentrated HCl in methanol and 4% vanillin in methanol). After 20 min, the absorbance of the solutions and the standard solution were measured at 500 nm. Blank sample consisted of 1 ml of extract solution with 5 ml of 1% HCl without vanillin-HCl reagent. (+) catechin (0.5-12 mg /100 ml) was used as standard for construction of calibration curve.

Sensory evaluation

The sensory evaluation was carried out by a panel trained according to Einstein (1991). The selected panelists were tested for their ability to detect basic tastes (Jellinek, 1985). The panel comprised 10 people as recommended by Stone and Sidel (1985). They were female and male, who were students in Addis Ababa University, Ethiopia. Nine injera quality descriptors were used for evaluation: color, taste, odor, texture (degree of softness), injera number of eyes, eye size, eye distribution (eye uniformity), top and bottom surface (degree of being powdery and sticky); overall

Table 2. Effect of kneading conditions on starch fraction of tef injera.

Kneading	FSG	RAG	RDS	SDS	RS	TS	SDRI
1	0.13[a]±0.0	69.3[b]±0.0	62.3[b]±0.0	5.4[a]±1.3	13.0[a]±4.5	77.1[a]±0.8	80.8[a]±0.9
5	0.13[a]±0.0	70.6[c]±0.0	63.4[c]±0.0	9.23[a]±5.5	6.4[a]±0.0	76.9[a]±0.9	82.3[a]±1.0
9	0.13[a]±0.0	68.0[a]±0.1	61.0[a]±0.1	10.0[a]±5.0	4.3[a]±5.9	74.8[a]±4.1	81.8[a]±4.6

Data are expressed as mean ± standard deviations (SD); n=10; FSG= free sugar; RAG = rapidly available glucose; RDS = rapidly digestible starch; SDS = slowly digestible starch; RS = resistant starch; TS = total starch; and SDRI = starch digestion rate index. Different superscripts in the same column indicate statistically significant differences (P < 0.05).

Table 3. Effect of kneading conditions on total phenolics, flavonoids, phytate and tannins contents (mg /100 g).

Kneading	Flavonoids	Total Phenols	Phytate	Tannins
1	0.12[b]±0.00	0.13[c]±0.00	94.1[a]±0.16	198.5[a]±6.8
5	0.15[a]±0.00	0.32[b]±0.00	70.0[b]±0.31	177.6[a]±0.00
9	0.15[a]±0.02	0.38[a]±0.00	46.5[c]±0.31	172.4[a]±7.4

Data are expressed as mean ±SD, n=10 ; means with different superscripts in the same column are statistically different (α <0.05).

acceptability was also evaluated. A score sheet was prepared using the selected descriptors. Each one of attribute was evaluated using a 9-point numerical scale (0–9) anchored on both sides with verbal descriptions (that is 0 = much too dark, 9 = much too light) to allow the panel to score the intensity on a framed common scale. Good sensory practices were followed according to Lawless and Heymann (1999). Injera samples were presented to the panelists on a tray at ambient temperature (≈25°C) within 3-4 h after baking. A glass of drinking water was used for rinsing between samples.

Statistical analysis

Analysis of variance was performed on the data to establish significant (p <0.05) differences between the samples. The descriptive categories were converted to numerical scores. The scores were then subjected to analysis of variance using SPSS statistical software (Version 20) (SPSS Inc., USA) and means of duplicate results were compared by Tukey's Honestly Significant Difference Test.

RESULTS AND DISCUSSION

Effect of kneading (time/speed) on injera quality

Starch fractions at different kneading conditions

Table 2 presents the effect of kneading at different time/speed combinations on starch fractions. The changes of kneading conditions (time/speed) did not significantly affect the FSG, SDS, RS, TS and SDRI. On the other hand, significant variation was observed on RAG and RDS which were kneaded at different time/ speed combinations. The digestion of starch is an important process with respect to dietary requirements (Sujka and Jamroz, 2013). Factors which influence the digestibility of starch are the compositional and morphological properties and the physical access of enzymes to the starch (Singh et al., 2010). Though insignificant difference was observed with increasing time and speed of kneading, the SDS value increased with increasing time and speed. Alonso et al. (2000) and Altan et al. (2009) mentioned the effect of processing on starch digestibility. According to their finding, starch loses structural integrity due to shearing and kneading, making it more susceptible to enzymatic attacks; increased hydrolysis; faster digestion. Kneading #5 (3 min at speed 6) had higher RAG (70.6) and RDS (63.4) while the lower RAG (68.0) and RDS (61.0) were observed in Kneading #9 (7 min at speed 12). According to Canja et al. (2014), the formation of dough and its rheological properties may be affected by some factors like flour quality, the quantity of water, electrolytes (NaCl) and the kneading conditions (intensity of kneading, the amount of energy transmitted to the dough and time of kneading). The kneading conditions influence the properties of the dough and they can lead to an optimal growth, an incomplete development or to extra-kneaded dough. The end of kneading is appreciated through sensorial analyses. Well-kneaded dough should be homogeneous, tight, consistent, may be elastic and easy to come down from the mixer's arm and from the walls of the kneading container. The dough must become a thin strip, transparent and flexible without breaking (Rus et al., 2008).

Effect of kneading conditions on total phenolics, flavonoids, phytate and tannin contents

Table 3 presents the effect of kneading conditions on flavonoids, total phenolics, phytate and tannins contents. The finding showed that changing kneading conditions

Table 4. Effect of kneading conditions on the sensory quality of injera.

Kneading	Color	Taste	Texture	Number of eyes	Eye size	Eye distribution	Top and bottom surfaces	Odor	Overall acceptability
1	$5.3^{a}\pm1.3$	$5.5^{a}\pm0.1$	$4.1^{ab}\pm0.7$	$6.7^{a}\pm1.4$	$1.4^{a}\pm0.4$	$5.6^{ab}\pm1.1$	$5.6^{b}\pm0.9$	$5.5^{a}\pm0.4$	$5.1^{bc}\pm0.3$
2	$6.0^{a}\pm0.0$	$5.4^{a}\pm0.6$	$6.5^{bc}\pm1.0$	$6.1^{a}\pm0.1$	$3.6^{b}\pm0.1$	$3.7^{ab}\pm0.1$	$6.2^{b}\pm0.2$	$6.3^{a}\pm0.3$	$7.3^{cde}\pm0.3$
3	$7.4^{a}\pm0.1$	$6.2^{a}\pm0.3$	$6.3^{bc}\pm0.4$	$5.2^{a}\pm0.2$	$6.3^{c}\pm0.4$	$4.4^{ab}\pm0.8$	$5.8^{b}\pm0.6$	$5.4^{a}\pm2.1$	$8.2^{de}\pm0.0$
4	$6.0^{a}\pm0.5$	$5.1^{a}\pm1.5$	$2.8^{a}\pm1.8$	$4.2^{a}\pm1.1$	$4.7^{bc}\pm0.9$	$3.1^{ab}\pm1.8$	$3.9^{ab}\pm0.1$	$4.2^{a}\pm2.1$	$3.1^{ab}\pm2.2$
5	$6.3^{a}\pm1.6$	$5.3^{a}\pm1.8$	$6.7^{bc}\pm0.1$	$5.3^{a}\pm0.8$	$5.2^{bc}\pm0.1$	$6.9^{b}\pm0.4$	$6.7^{b}\pm1.4$	$6.1^{a}\pm1.1$	$8.6^{e}\pm0.1$
6	$4.8^{a}\pm0.3$	$4.9^{a}\pm0.8$	$3.1^{a}\pm0.4$	$5.5^{a}\pm0.4$	$5.9^{c}\pm0.3$	$5.9^{ab}\pm0.3$	$6.2^{b}\pm0.7$	$5.1^{a}\pm0.5$	$3.5^{ab}\pm0.6$
7	$5.4^{a}\pm0.8$	$5.2^{a}\pm0.8$	$6.8^{bc}\pm0.6$	$6.0^{a}\pm0.4$	$6.0^{c}\pm1.0$	$3.3^{ab}\pm0.7$	$5.2^{b}\pm1.1$	$6.2^{a}\pm0.1$	$5.3^{bcd}\pm0.2$
8	$4.6^{a}\pm1.2$	$4.5^{a}\pm0.2$	$4.3^{ab}\pm0.1$	$6.0^{a}\pm0.1$	$3.5^{ab}\pm0.6$	$4.8^{ab}\pm1.3$	$1.8^{a}\pm0.2$	$6.1^{a}\pm0.4$	$5.1^{bc}\pm0.2$
9	$6.3^{a}\pm0.1$	$6.3^{a}\pm0.1$	$8.7^{c}\pm0.2$	$5.4^{a}\pm0.6$	$4.5^{bc}\pm0.3$	$2.0^{a}\pm0.7$	$1.3^{a}\pm0.2$	$6.4^{a}\pm0.5$	$1.9^{a}\pm0.1$

Data are expressed as mean ±SD, n-10; means with different superscripts in the same column are statistically different ($\alpha < 0.05$).

have non- significant effect on the tannins content of the final product. Unlike tannins, flavonoids, total phenolics and phytate contents showed significant variation due to change in kneading conditions. Chlopicka et al. (2012) observed losses of antioxidants during dough mixing and kneading. According to their explanation, antioxidant activity of breads could be modified by active oxidative enzymes presented in ingredients of compounds used in breads production, or oxidized by ambient oxygen. The addition of water will initiate enzyme activities, while a substantial incorporation of oxygen occurred during the initial dough mixing and the remolding into smaller pieces. Contrary to the observation of Chlopicka et al. (2012), the total phenolic content increased with increasing time and speed of kneading. The bound phenolics may be released with elongated kneading time and speed of kneading as a result of heat induced due to friction. This might be the other possible explanation for the increment in flavonoids and total phenol contents of injera. Phytate content degraded significantly as kneading time and speed increased. It decreased in the order: Kneading #1 (94.1 mg/100 g)> Kneading #5 (70.0 mg/100 g)> Kneading #9 (46.5 mg/100 g). According to Baye (2014), phytate can be degraded by endogenous phytases which can be activated by food processing techniques. According to Hurrell and Egli (2010), high values in phytate are likely to impair the absorption of iron and zinc. Moreover, phytates can form complexes with minerals which are secreted endogenously such as calcium (Morris and Ellis, 1985) and zinc (Manary et al., 2002) and, making these minerals unavailable for re-absorption into the body. Increasing the kneading time and speed can degrade phytate and minimize these effects. On the other hand, according to Curhan et al. (2004), phytate can prevent kidney stones by serving as crystallization inhibitor of calcium salts. They also have anti-cancer properties (Singh et al., 2003) and glucose lowering effects (Lee et al., 2005, 2006).

Effect of kneading conditions on sensory quality of injera

The effect of kneading conditions on the sensory quality of injera is presented in Table 4. There was non-significant difference in the color, number of eyes, taste and odor of injera made from dough obtained from different kneading conditions. On the other hand, the remaining sensory attributes like texture, eye size, distribution, top and bottom surfaces and over all acceptability were significantly affected due to changing of kneading conditions. This might be explained based on the relationship between kneading and formation of gas. Kneading or remixing of the dough favors the release of large gas bubbles, resulting in a more even distribution of the bubbles within the dough which finally contribute to the quality of the product (Rosell, 2011). The sensory qualities texture, eye size and distribution, top and bottom surfaces and overall acceptability rated more with kneading time of 3 min and speed 6 (Kneading #5). On the other hand, Kneading #9 (7 min at speed 12) had the lower injera overall acceptability. Banu (2000) described that kneading is one of the most important operations in the manufacturing of bread. The main purpose of the kneading operations is to obtain a homogeneous mixture of the raw and auxiliary materials and at

Table 5. Effect of different water to fermented dough proportions on the sensory quality of injera (mean ± SD).

Absit	Color	Taste	Texture	Number of eyes	Eye size	Eye distribution	Top and bottom surfaces	Odor	Overall acceptability
1	5.4^{a}±0.0	4.1^{a}±0.9	4.9^{ab}±0.8	5.8^{a}±0.4	3.9^{bc}±1.3	4.8^{ab}±0.8	5.3^{b}±0.6	5.7^{a}±0.2	4.4^{bc}±0.3
2	6.0^{a}±0.2	4.3^{a}±0.1	6.0^{ab}±0.2	6.0^{a}±0.2	3.2^{bc}±1.3	6.1^{ab}±1.9	5.4^{b}±0.0	6.8^{ab}±0.1	5.0^{c}±0.1
3	6.6^{a}±0.7	4.8^{a}±0.0	7.5^{b}±0.4	6.2^{a}±0.0	5.2^{b}±0.4	6.9^{ab}±0.0	6.3^{b}±0.9	7.3^{b}±0.2	7.95^{d}±0.1
4	5.9^{a}±0.4	5.1^{a}±0.1	3.9^{a}±1.1	4.8^{a}±0.4	2.9^{bc}±0.6	2.4^{a}±0.4	5.5^{b}±0.5	5.7^{a}±0.1	2.2^{a}±0.0
5	6.9^{a}±0.7	4.3^{a}±0.2	4.1^{a}±0.5	5.2^{a}±1.5	1.9^{a}±1.0	4.5^{ab}±2.1	5.7^{b}±0.0	6.3^{ab}±0.8	3.2^{ab}±0.8
6	6.9^{a}±0.8	4.1^{a}±0.0	7.5^{b}±0.3	5.5^{a}±0.2	8.7^{c}±0.1	8.4^{b}±0.3	6.3^{b}±0.2	6.6^{ab}±0.2	4.4^{ab}±0.0
7	5.5^{a}±0.1	4.8^{a}±1.3	5.1^{ab}±1.4	6.0^{a}±0.6	1.5^{a}±0.0	5.1^{ab}±0.4	5.1^{b}±0.3	6.3^{ab}±0.4	3.1^{ab}±0.4
8	5.7^{a}±0.4	4.1^{a}±0.2	4.4^{a}±0.1	5.9^{a}±0.5	1.2^{a}±0.1	5.3^{ab}±1	5.3^{b}±0.6	5.8^{a}±0.2	3.5^{ab}±0.4
9	6.3^{a}±0.3	4.3^{a}±0.3	4.9^{ab}±0.6	4.7^{a}±0.3	2.2^{a}±0.3	3.0^{a}±1.5	3.2^{a}±0.5	5.7^{a}±0.1	2.3^{a}±0.2

Data are expressed as mean ±SD, n=10, Means with different superscripts in the same column are statistically different (α <0.05).

the same time obtain dough with viscous-elastic structure and properties. In addition, while kneading, in dough it is included a quantity of air, which is very important for rheological properties of the dough, and for the quality of the final product. During kneading, frictional heat makes the dough temperature to rise. To control the desired dough temperature, the water temperature has to be adjusted. The formation of the dough with its specific structure and rheological properties occurs because of several processes such as physical, colloidal, biochemical, and the main role is being held by the physical and colloidal processes (Bordei, 2004).

Gómez et al. (2013) concluded that dough mixing parameters will always need to be optimized for each formulation and system, taking into account the speed and duration of mixing and the type of stand mixer. The size, distribution, growth, and failure of the gas bubbles released during proofing and baking have a major impact on the final quality of the bread in terms of both appearance(texture) and final volume (Cauvain, 2003).

Effects of water to fermented dough ratio during absit preparation on the quality of injera

The impacts of water to fermented dough ratio during absit preparation on the sensory quality of injera are presented in Table 5. The ratio of water to fermented dough did significantly affect the quality of injera. Texture, number of eyes, top and bottom surface, odor and overall acceptability of injera obtained from absit #3 prepared from a ratio of 900:100 ml of water to fermented dough with higher sensory score. Injera overall acceptability is the result of other quality attributes like texture, number of eyes, top and bottom surface, odor and others. Absit #6 had less overall acceptability than that of Absit # 3, but the individual sensory attribute gained higher value except injera texture and eye size. The possible reason for the difference in the sensory score of the different injera from different absit preparation might be due to the amount of gelatinized absit used primarily to bring about cohesiveness of the dough and secondly to provide easily fermentable carbohydrate to leaven the injera (Yetneberk et al., 2004). Abiyu et al. (2013) stated that absit used to activate yeasts is responsible for CO_2 production and the development of eyes during baking of injera. Ashenafi (2006) mentioned that injera baked without absit or with less absit than required will have a lesser amount of eyes on the upper surface. Also, according to Stewart and Getachew (1962), injera made from batter lacking absit has a powdery look and lacks the air spaces or the so-called eyes of the injera which give it an “inviting look”. Different findings reported the percentage of fermented dough needed to be used for preparation of absit. According to Ashenafi (2006) and Girma et al. (2013), 10% of the weight of the fermented batter is commonly used to make absit. However, other amounts such as 5, 15 and 20% (Zanniniet al., 2012) of the fermented batter are sometimes used. On the other hand, it was understood from the traditional injera making process that the amount of absit and the ratio of water to fermented dough significantly depend on tef varieties.

Conclusion

Kneading conditions considerably affect the starch fractions (RAG and RDS) and sensory quality of injera. Kneading time of 3 min and speed of 6 rpm had the highest injera overall acceptability. Flavonoid, total phenolics and phytate contents of injera were also affected by kneading conditions. On the other hand, absit making method (the ratio of water to fermented dough) also affects the quality of injera. Absit which is made from 100 ml of fermented dough and 900 ml of water had the highest injera overall acceptability and further studies are required on different tef varieties.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

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Full Length Research Paper

Weaning food fortification and improvement of fermented cereal and legume by metabolic activities of probiotics *Lactobacillus plantarum*

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The provision of cheap, balanced and adequate nutritional composition for growing infants is a major challenge in Africa. Most weaning foods are usually cereal-based gruels fermented by lactic acid bacteria (LAB). The major nutrient they supply being carbohydrate, uncontrolled or inappropriate intake might cause growth retardation and malnutrition in growing children. The nutritional requirement of weaning infants could be supplied by supplementation with protein rich diets. Soybean is a cheap and available source of protein in Nigeria; but it also contain some complex sugars (raffinose) which is associated with abdominal discomfort in children of weaning age. This study is designed to use the probiotics LAB to improve the nutritional composition of weaning food blends. Lactic acid bacteria were isolated from Ogi (a fermented cereal gruel) using standard morphological and biochemical tests; their identities were confirmed with molecular methods. Nutritional and organoleptic attributes of the food blend were determined following Association of Official Analytical Chemist procedures. The data were subjected to statistical analysis at 5% level of significance. Fortification of the gruel with pre-treated soybeans improved the nutritional quality (Protein: 8.4 to 17.8 %; Fat: 3.6 to 12.9 %; Ash: 2.0 to 3.8%; Fe: 6.4 to 10.7mg/100g and Ca: 156.7 to 211.0mg/100g) during fermentation. Utilization of raffinose by probiotic Lactobacillus plantarum from local food sources reduced the complex sugars in soybeans. Nutritional qualities and organoleptic attributes of cereal gruels were improved by fortification with soybeans and fermentation.

Key words: Probiotics, complex sugars, Lactobacillus plantarum, fermentation, nutritional improvement, soybeans.

INTRODUCTION

Lactic acid bacteria (LAB) are a group of bacteria characterized by their ability to synthesize lactic acid. Typical LAB are gram-positive, non-sporing, catalase-negative, lacking cytochromes. They are anaerobic but

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tolerate little amount of oxygen. They can be rod or cocci and they produce lactic acid as the major end product of fermentative metabolism (Axelsson, 2004; Wall et al., 2007; Arimah and Ogunlowo, 2014). Proteinaceou bacteriocins are also produced by several LAB strains and provide an additional hurdle for spoilage and pathogenic microorganisms. Furthermore, lactic acid and other metabolic products contribute to organoleptic and textural profile of the food item (Makarova et al., 2006; Khalid, 2011; Kaškonienė et al., 2017).

The industrial importance of the LAB is further evidenced by their reputation as generally regarded to as safe (GRAS) status, due to their ubiquitous appearance in food and their contribution to humanhealth (Wall et al., 2007). LAB are characterized by an increased tolerance to a lower pH range which partially enables LAB to outcompete other bacteria in a natural fermentation, as they can withstand the increased acidity from organic acid production, for example, lactic acid and acetic acid (Wall et al., 2007; Adeyemo, 2012; Magala et al., 2017).

In many West African countries, exclusive breastfeeding is usually adequate up to three to four months of age, but after this period, it may become increasingly inadequate to support the nutritional demand of the growing infant. Thus, in a systematic weaning process, there is always the need to introduce soft, easily swallowed foods to supplement the infant's feeding early in life (Mariam, 2002; Ozogul and Hammed, 2017). In Nigeria, the usual first weaning food is called pap, *akamu*, *ogi* or *koko*, made from fermented cereal such as maize (*Zea mays*), millet (*Pennisetum americanum*), or guinea corn (*Sorghum* spp.). Such weaning foods are prepared traditionally by first hand-picking the dirt or stones in the cereal and washing with water. This is then followed by soaking in water, wet milling and fermentation (Oguntona and Akinyele, 1995; Adeyemo, 2012).

In Nigerian *Ogi*, maize, millet or sorghum grains are washed and steeped for 24 to 72 h during which they undergo lactic acid fermentation. They are then drained, wet-milled and finally sieved to yield fine smooth slurry with about 8% solid content and high water content (Oguntona and Akinyele, 1995). Such weaning foods are usually high in carbohydrate content and are of low nutritive value. They are characterized by low protein, low energy density and high bulk (Mariam, 2002; Yelnett et al., 2014; Carevic et al., 2016). Cereal-based diets have been found to be of lower nutritional value than animal-based ones and this forms the primary basis for most of the traditional weaning foods in West Africa.

The protein content of maize and guinea-corn is of poor quality, low in lysine and tryptophan. These two amino acids are indispensable to the growth of the young child. The problems inherent in the traditional weaning foods and feeding practices in West Africa predispose the infant to malnutrition, growth retardation, infection and high mortality (Nagai et al., 2012; Magala et al., 2017).

Legumes are rarely used for weaning because of their associated problems of indigestibility, flatulence, diarrhoea and discomfort in children (Hou et al., 2009). They may, however, be introduced later in life (after 6months) or be used as food blend to supplement or fortify the fermented cereal nutritionally.

Food fortification or supplementation is the public health policy of adding micronutrients (essential trace elements and vitamins) to foodstuff to ensure that minimum dietary requirements are met (Mariam, 2002; Nagai et al., 2012). Addition of micronutrients to staples and condiments can prevent large-scale deficiency disease and prevent the attended cases of malnutrition that is associated with using cereal based diet as weaning food .Several types of food supplements have been recognized including additives which repair a deficit to "normal" levels or additives which appear to enhance a food and supplements taken in addition to the normal diet (Mariam, 2002; Narwade et al., 2015).

A dietary supplement, also known as food supplement or nutritional supplement, is a preparation intended to provide nutrients, such as vitamins, minerals, fiber, fatty acids, proteins or amino acids that are missing or are not consumed in sufficient quantity in a person's diet. Some countries define dietary supplements as foods, while in others they are defined as drugs.

Soybean is one of such legumes that are used to supply additional nutrients and vitamins to cereal-based weaning food. It is rich in high level of dietary protein and it is easily accessible to the local populace (Wakil and Onilude, 2009). It has been used by several authors to formulate food blend that are rich in protein and used as weaning food in different part of Nigeria.It is thus able to supply the other nutrients that are lacking in such food. Also, formulated infant weaning foods from fermented blends of cereal and soybeans using *Lactobacillus plantarum* and other lactic acid bacteria have been formulated by different authors (Wakil and Onilude, 2009; Nagai et al., 2012; Magala et al., 2017).

Soybean utilization is, however, limited because it contains some anti-nutritional factors.There is also the presence of some oligosaccharides such as raffinose, stachyose and verbascose which possess flatulence-inducing properties in children (Hou et al., 2009; Adeyemo and Onilude, 2014; Carevic et al., 2016). This problem has been solved by various authors in the past by dehulling, cooking, wet or dry milling, addition of sugar and oil, use of chemicals, germination and fermentation.

Some of these processes, however, may alter the composition of the weaning food, some are time and energy consuming, while others may be expensive or difficult to preserve. Thus, there has been agitation over the years to look for simple fermentation technology approach in order to arrive at nutritionally adequate ready-to-feed weaning foods that can be preserved safely without getting contaminated (Nagai et al., 2012; Carevic et al., 2016).

Fermented foods are associated with ‘good bacteria’ referred to as probiotics. *L. plantarum*has however been named as one of those good, useful and safe bacteria. Probiotics, as defined in a FAO/WHO (2002) report, are ‘live microorganisms which when administered in adequate amounts confer a health benefit on the host’. Robiotics are beneficial bacteria in that they favourably alter the intestinal microflora balance such as reconstruction of normal intestinal microflora after disorders caused by diarrhoea, antibiotic therapy and radiotherapy. It inhibits the growth of harmful bacteria, promote good digestion, boost immune function and increase resistance to infection (Waugh et al., 2009; Parvez et al., 2006; Makarova et al., 2006,Yelnett et al., 2014; Kaškonienė et al., 2017; Ozogul and Hammed, 2017).

Other physiological benefits of probiotics include removal of carcinogens, lowering of cholesterol, immune-stimulating and allergy lowering effect, synthesis and enhancing the bioavailability of nutrients. People with flourishing intestinal colonies of beneficial bacteria are better equipped to fight the growth of disease causing bacteria (Body Ecology., 2012; Ozogul and Hammed, 2017).

*L. plantarum*as a potential probiotics that shows antimicrobial activity by producing organic acids and bacteriocin. Some assayed strains of the organism showed safety with regard to haemolytic and gelatinase activity. They were able to produce exopolysaccharidesand some were able to utilise Fructooligosaccharide, all of which are characteristics attributed to probiotics. *L. plantarum* ferments raffinose and lactose efficiently and possesses alpha-galactosidase, beta-galactosidase and beta-phosphogalactosidase activities (Makarova et al., 2006; Bulhões et al., 2007; Yelnett et al., 2014; Kaškonienė et al., 2017; Ozogul and Hammed, 2017).

Problems usually arise from the wayweaning food is introduced to the child. While some mothers believe that additives such as milk should be added as supplement, others, especially from the low-income group, do not see a need for this at all. They do not make special effort to adequately prepare the child’s weaning diet. And so, such are usually monotonous, boring, unhygienically prepared and lacking sufficient nutrient for the growth of the child (Mariam, 2002; Magala et al., 2017).

Another problem that is associated with weaning foods in West Africa is the problem of diarrhoea and diarrhoea-related diseases that are highest at this level, especially in children that are less than one year. At this stage, these foods are gradually introduced to supplement and succeed breast feeding. This also plays a major role in child’s mortality and morbidity. This is because due to unhygienic and inappropriate preparation of weaning foods, episodes of diarrhoeal diseases are highest with the combination of nutrient malabsorption and malnutrition (Mariam, 2002; Ozogul and Hammed, 2017).

This approach would require knowledge about the nutritive values of a variety of local food commodities, indigenous to the affected communities. A number of cereals and legumes that are readily available in Nigeria have been found to have nutrient potentials that could complement one another if properly processed and blended (Mariam, 2002; Magala et al., 2017). Therefore, it is imperative that efforts to formulate composite blends and scientific studies are carried out to ascertain the nutritive adequacy of these locally available blends (cereal and legumes) for possible use as complementary foods with health benefits, especially by the rural and poor urban mothers during weaning period.

MATERIALS AND METHODS

Preparation of processed samples of soybeans and sterilization of samples

All the samples of soybeans used for this analysis were first surface-sterilized by cleaning in 1% sodium chloride solution for 5 min. It was rinsed several times with distilled water and allowed to dry in an oven at 50°C before further processing.

Preparation of samples

Raw

1 kg of soybeans was used at each time for the processing. The samples were picked manually to remove the dirts.It was milled into powder, sieved with a fine sieve to pass through 0.5 mm diameter sieve and stored in air tight plastic container for further use.

Cooked

One kilogram soybeans was added to distilled water (1:5,w/v) and cooked at 80°C on a hotplate (Hanna instruments 7010, UK). In all the cooking process, the level of cooking water was kept constant by the addition of boiling distilled water. It was dehulled and washed several times with distilled water to remove the seed coat; drained and later dried in the oven at 50°C. This was milled into powder and sieved with a fine sieve and later kept in airtight plastic container for further use.

Roasted

One kilogram soybeans was roasted in an oven (Hearson Willow model,UK) at 100°C, dehulled to remove the seed coat and milled into powder. It was sieved with a fine-sieve into fine powder and stored in airtight container until further use.

Proximate analysis

Moisture content, ash content, crude fat, protein, crude fibre, carbohydrate and total sugar were determined by the method of Horwitz and Latimer (2010).

Table 1. Analysis of *Ogi* samples only.

Parameter	*Ogi* only		
	White maize	**Yellow maize**	**Brown(Sorghum)**
Moisture content (%)	*62.3±0.02	61.5±0.01	62.6±0.14
Protein (%)	3.2±0.10	3.5±0.10	3.9±0.50
Etherextract(fat) (%)	1.6±0.10	1.8±0.020	1.8±0.02
Ash (%)	1.3±0.05	1.3±0.02	1.5±0.01
Crude fibre (%)	1.3±0.05	1.3±0.02	1.7±0.02
Carbohydrates (by difference) (%)	30.3±0.02	30.6±0.02	28.5±0.02
Ascorbic acid (mg/100g)	3.5±0.03	3.8±0.05	4.2±0.05
Reducing sugar (%)	0.7±0.30	0.8±0.50	0.5±0.01
Total sugar (%)	1.2±0.15	1.1±0.20	1.1±0.01
Thiamine (mg/100 g)	0.4±0.50	0.7±0.02	0.3±0.02
Riboflavin (mg/100 g)	0.05±0.50	0.08±0.13	0.10±0.12
Niacin (mg/100 g)	0.5±0.05	0.6±0.10	0.5±0.20
Ca^{2+} (mg/100 g)	24±0.30	27±0.01	28±±0.01
Fe^{2+} (mg/100 g)	2.2±0.02	2.6±0.02	4.2±0.02
PO_4^{3-} (mg/100 g)	140±0.30	142±0.01	145±0.05

*All value recorded are means of replicatedetermination±SE

Determination of vitamins and minerals in food

Ascorbic acid, vitamins B (riboflavinand thiamine), niacin and the determination of iron, calcium and phosphate in food samples were determined by the method of AACC (2008).

Preparation of the cereal blend

The three samples of the fermented gruels *ogi* were used for the formulation-white, yellow and brown (sorghum) *ogi*. It was prepared in the ratio 1:3 with the various soybeans prepared in different ways, namely:-raw, cooked and roasted. It was allowed to ferment with *L. plantarum* and the proximate analysis, antinutritional and residual oligosaccharides (and their profiles)and reducing sugars (and their profiles) were determined as described earlier. The food blend produced was also served as samples to nursing mothers and the organoleptic attributes of the various food blends such as taste, colour, aroma, texture, flavour, odour, shelf life, acceptability and appearance were assessed with questionnaires.

Packaging of the weaning food blend

The method of Oguntona and Akinyele (1995) as modified by Adeyemo (2012) was used in the preparation of *ogi*. *Ogi* was made using clean fermented cereals.The maize cereal was fermented for five days, milled and sieved thereafter. It was allowed to settle and srored in a clean platic container. The water was changed regularly to make it as fresh as desired and retain the desired taste, aroma and flavour.

Soybean was prepared by hand picking stones and dirt. It was roasted at 100°C on a hotplate. It was broken into small pieces with a waring blender (Kenwood model, UK) to remove the seed coat , milled into powder and sieved through a 0.5 mm sieve. It was stored in air tight plastic container. It can also be sealed in nylon containers and stored at room temperature. *Ogi* can be stored by refrigeration or at room temperature. The fermenting water may be changed regularly. This allows further fermentation to take place.

The blend or formulationwas done when the meal was to be consumed. Three parts *ogi* sample was mixed with one part roasted soybeans and left forone hour before cooking.

RESULTS

The nutritional analysis of different varieties of *Ogi*is presented in Table 1 while the Analysis of Ogis samples fortified with raw and processed soybeans in ratio 3:1 is shown in Table 2. There was significant diference between the three samples. The protein content was 3.9% in *Ogi* from sorghum while the one from white maize was 1.6% and the one from yellow maize 1.8%. The crude fibre was 1.7% in sorghum and 1.3 in white and yellow maize respectively. The riboflavin content (mg/100 g) was 0.10 in sorghum and 0.05 and 0.05 in white and yellow maize respectively. Fe^{2+} content in mg/100 g was 4.2 in sorghum and 2.2 and 2.6 in white and yellow maize respectively. The phosphate content also follows the same trend. Analysis of Ogis samples fortified with raw and processed soybeans in ratio 3:1. This is shown in Table 2.

The nutritional composition of the weaning food blend was presented in Table 3. There was an increase in the nutritional composition when compared with the *ogi* samples only. There was an increase in the protein content (13.8%), fat content (6.8%) and ash content (2.2%) while there was a reduction in the carbohydrate

Table 2. Analysis of *Ogi*samples fortified with raw and processed soybeans in ratio 3:1.

Parameter	Raw soy			Roasted soy			Cooked soy		
	White maize	Yellow maize	Brown (sorghum)	White maize	Yellow maize	Brown (sorghum)	White maize	Yellow maize	Brown (sorghum)
Moisture content (%)	*63.9±0.02	64.9±0.25	64.6±0.05	64.2±0.50	64.6±0.15	63.7±0.03	65.1±0.03	64.2±0.03	65.5±0.02
Protein (%)	11.2±0.10	11.5±0.25	13.6±0.50	11.5±0.02±0.02	11.6±0.20	13.8±0.02	10.9±0.02	11.0±0.04	11.4±0.01
Ether extract (fat) (%)	5.9±0.10	6.1±0.13	6.0±0.25	6.3±0.25	6.5±0.04	6.8±0.04	5.4±0.12	5.5±0.05	5.6±0.05
Ash (%)	1.8±0.14	1.8±0.12	1.7±0.05	2.0±0.12	2.1±0.20	2.2±0.02	1.4±0.02	1.5±0.03	1.5±0.05
Crude fibre(%)	2.1±0.05	2.1±0.33	2.4±0.13	2.0±0.04	1.9±0.02	2.2±0.03	2.0±0.04	1.9±0.03	2.0±0.04
Carbohydrates (by difference) (%)	15.1±0.02	13.6±0.30	11.7±0.14	14.0±0.03	13.3±0.03	11.3±0.04	15.2±0.13	15.9±0.13	13.0±0.20
Ascorbic acid (mg/100g)	7.0±0.02	7.0±0.50	7.4±0.05	4.0±0.03	3.9±0.03	4.3±0.02	3.2±0.03	3.5±0.02	3.8±0.10
Reducing sugar(%)	0.7±0.13	0.8±0.05	0.8±0.02	0.3±0.10	0.3±0.30	0.6±0.25	0.3±0.02	0.2±0.13	0.3±0.03
Total sugar(%)	0.9±0.33	0.9±0.20	0.8±0.04	0.7±0.04	0.7±0.02	0.8±0.04	0.3±0.05	0.3±0.05	0.4±0.02
Thiamine (mg/100g)	0.8±0.02	0.9±0.02	0.7±0.12	0.4±0.04	0.4±0.13	0.7±0.50	0.6±0.04	0.7±0.02	0.5±0.02
Riboflavin (mg/100g)	0.1±0.020	0.10±0.20	0.11±0.02	0.03±0.0.12	0.03±0.03	0.15±0.30	0.09±0.02	0.09±0.13	0.10±0.05
Niacin (mg/100g)	1.5±0.30	1.6±0.01	1.9±0.12	1.3±0.20	1.3±0.13	1.5±0.04	1.1±0.03	1.1±0.02	1.3±0.20
Ca^{2+} (mg/100g)	83±0.25	82±0.01	76±0.01	88±0.01	88±0.01	92±0.05	86±0.50	87±0.55	80±0.03
Fe^{2+} (mg/100g)	3.5±0.50	3.6±0.14	4.4±0.10	3.2±0.55	3.2±0.13	3.8±0.04	3.1±0.50	3.2±0.02	3.2±0.03
PO_4^{3-} (mg/100g)	205±0.05	208±0.20	216±0.10	226±0.55	225±0.50	228±0.04	210±0.02	212±0.55	210±0.05

*All value recorded are means of replicate determination±SE

content. There was also an increase in the vitamin content thiamine, riboflavin and niacin (0.7, 0.2 and 1.5 mg/100 g) respectively. Analysis of variance shows a significant difference (p=0.05) in the sample before it was fortified with the roasted soybeans sample. The mineral content also increased significantly (p=0.05). Also, when the ingredients were fermented together, there was a further increase in the nutritionalcomposition.The protein content increased (17.8%), fat (12.%), ash (3.8%), iron (10.7 mg/100 g), calcium (211.0 mg/100 g) and phosphate (288 mg/100 g).

The vitamin content also increased significantly, thiamine, riboflavin, and niacin (0.7, 0.2 and 1.5 mg/100 g respectively). In a trial experiment for the choice of the best weaning food composition, recipe A which consisted of roasted soybean and sorghum *ogi* was preferred by the nursing mothers. This is shown in Tables 2 and 4. Few people prefer recipes B and C. Therefore, further work was done with sample A alone.This was also used as a criteria for selection of the best weaning food for infants. The mothers were taught to prepare the weaning food themselves through a community development programme and their feed back were assessed with a questionnaire.

The weaning food blend were evaluated for their taste, colour, aroma, texture, flavour, odour, shelf life, acceptability and appearance based on a 5-point hedonic scale representing ‘well acceptable, acceptable, fairly acceptable, moderately acceptable and rejected; this is shown in Table 4. The Organoleptic attributes of the fermented cereal blend is shown in Table 4 and Figure 1. Sample A compared favourably with sample D, a commercially sold weaning food set up as control

The organoleptic attributes of the food blend is shown in Figure 1. Samples fermented with *L. plantarum* have pleasant odour, taste and aroma longer shelf life. The colour was creamy and was accepted by mothers. The market surveywas done among nursing mothers who were earlier

Table 3. Nutritional Analysis of the weaning food blend (Sorghum *Ogi*-fortified with roasted soybeans and fermented with *L.plantarum*).

Parameter	Sorghum *ogi* only	Sorghum *ogi* with roasted soybeans	Sorghum *ogi* with roasted soybeans and fermented with *L.plantarum*
Moisture content (%)	62.6±0.14	63.7±0.02	52.3±0.30
Protein (%)	3.9±0.50	13.8±0.15	17.8±0.30
Ether extract(Fat) (%)	1.8±0.02	6.8±0.20	12.9±0.02
Ash (%)	1.5±0.01	2.2±0.05	3.8±0.10
Crude fibre (%)	1.7±0.02	2.2±0.10	1.7±0.01
Carbohydrates (by difference) (%)	28.5±0.02	11.3±0.20	11.5±0.01
Ascorbic acid (mg/100g)	4.2±0.05	4.3±0.03	18.3±0.03
Reducing sugar (%)	0.5±0.01	0.6±0.10	1.6±0.20
Total sugar (%)	1.1±0.01	0.8±0.01	3.2±0.12
Thiamine (mg/100g)	0.3±0.02	0.7±0.02	1.8±0.13
Riboflavin (mg/100g)	0.10±0.12	0.2±0.03	1.0±0.50
Niacin (mg/100g)	0.5±0.20	1.5±0.50	2.7±0.03
Ca^{++} (mg/100g)	28±±0.01	92.0±0.05	211.0±0.02
Fe^{++} (mg/100g)	4.2±0.02	3.8±0.10	10.7±0.13
PO_4^{---} (mg/100g)	145±0.05	228.0±0.01	288.0±0.01

*All value recorded are means of replicate determinations± SE

Table 4. Organoleptic attributes of the fermented cereal blend.

Organoleptic attributes	Roasted Soybeans +sorghum *Ogi* (A)	Roasted soybeans + dried sorghum *Ogi* (B)	Roasted soybeans + sorghum*Ogi* fermented with L. *plantarum* (C)	Commercially sold weaning food(D)
Taste	Sweet	Sweet	Sweet	Sweet
Colour	Creamy	Creamy	Creamy	Creamy
Aroma	Acceptable	Fairly acceptable	Moderately acceptable	Acceptable
Texture	Acceptable	Fairly acceptable	Moderately acceptable	Acceptable
Flavour	Acceptable	Fairly acceptable	Moderately acceptable	Acceptable
Odour	Acceptable	Fairly acceptable	Moderately acceptable	Acceptable
Shelf life	2 months	2½ months	2 months	6months
Acceptability	Well accepted	Fairly acceptable	Moderately acceptable	Well Accepted
Appearance	Well accepted	Fairly acceptable	Moderately acceptable	Well Accepted

taught the recipe. It showed that mothers had different erroneous believes about factors responsible for the presence of gas production, bloating and flatulence in young infants. Their responses were favourable when the sample was compared with commercially sold weaning food set up as control.

Statistical analysis

The statistical analysis carried out include mean, standard deviation and standard error, anova and Duncan multiple range of variables using SAS package and bar chart.

DISCUSSION

Provision of healty, nutritionally adequate, cheap, easy-to- prepare weaning food with prolonged shelf life is major challenge in West Africa. Weaning food is usually required when breast milk alone cannot meet the nutritional requirement of a young child. Commercial weaning foods are available in the market butthey are usually very expensive and not within the reach of low income earners. Some of these weaning foods however

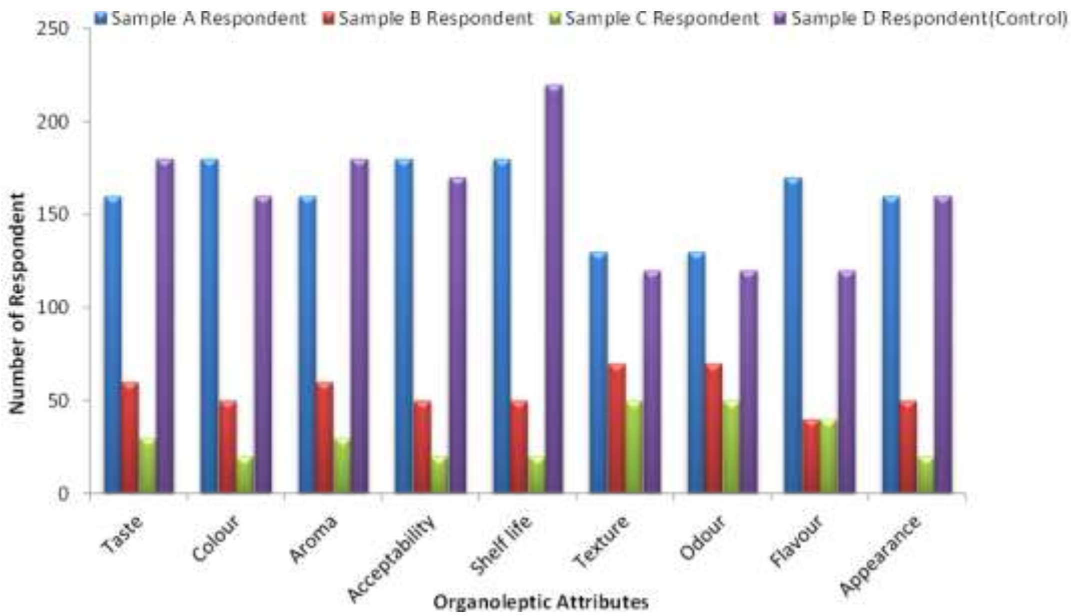

Figure 1. Survey carried out among nursing mothers on the organoleptic attributes of the weaning food blend. Sample A = Roasted soybeans+sorghum *Ogi;* Sample B = Roasted soybeans + dried sorghum*Ogi;* Sample C = Roasted soybeans + sorghum *Ogi* and Fermented *L. plantarum;* Sample D = Commercially sold weaning food (Control).

have their various challenges. Domestic preparation of some results in a combination of raffinose family of oligosaccharides that causes abdominal pain, bloating, gas formation and flatulence in young children, while some also contain some anti nutritional factors as a result of poor method of preparation. Others may not be prepared prepared hygienically which may result in series of diarrhoeal episodes in children. Some of these problems were addressed in this research with the metabolic and probiotics activities of *L. plantarum* through fermentation. This is in accordance with the work of Mariam (2002) and Macdonald et al. (2012) on problems preparation of a good weaning food and its associated.

The presence of raffinose oligosaccharide and some antinutritional factors in soybeans has limited its use as a good source of protein in Nigeria despite its several advantages (Oguntona and Akinyele, 1995; Nagai et al., 2012). Soyabeans can be consumed by adults and infants when the anti nutritional factors and Raffinose (RFO) have been removed successfully. This has been reported earlier by the authors Adeyemo and Onilude (2013, 2014).

Furthermore, the nutritional composition of soybeans was enhanced by fermentation using *L. plantarum.* Also, soybeans is not usually consumed raw except with some form of pre-treatment methods such as soaking, dehulling, milling, cooking and roasting (Onilude et al., 2004; Nagai et al., 2012). Fortification of sorghum *ogi* with roasted soybean improved the nutritional composition of the cereals. Cereals have been known to be deficient in lysine, but are rich in cystein and methionine. Legumes on the other hand are rich in lysine but deficient in sulphur containing amino acids. Thus by blending cereals with legumes, the overall protein quality was improved in the blend (Nagai et al., 2012; MacDonald et al., 2012).

On nutritional composition, the increase in the quality of the food blend by the addition of roasted soybeans flour was significant ($p<0.05$). The result was consistent with other report on the improvement in quality of corn protein by protein complementation or supplementation as determined by more traditional evaluation methods and as observed by other researchers like Onilude et al. (2004); Bulhões et al. (2007) and Wakil and Onilude (2009). There was an increase in protein, ash content, ascorbic acid, thiamine, riboflavin, niacin and mineral content. This is similar to the work of Nagai et al. (2012) who reported that when *ogi* was fortified with a high quality vegetable protein such as soybean flour, the fermented meal could serve as a major source of protein, especially in weaning foods for infant. They also opined that fortifying corn meal with defatted soybean caused a further improvement in the vitamin content of the food blend.

The formulation made in this research could easily serve as a good weaning formula with probiotics properties. The traditional fermentation of *ogi* which consist of metabolic activities of *L. plantarum* and inclusion of roasted soybeans which caused an increase in the protein content is however, a desirable quality. Even thou the development of weaning formulation in developing countries is a great challenge,this can be addressed by the use of such weaning food composition. In a similar observation, Macdonald et al. (2012) opined that the traditional fermentation which contribute an important role in food supply especially fermented cereal from indigenous raw materials play an important role on daily nutrition in developing countries.

The formulated blend meets the daily nutritional requirements of growing infants. This agrees with the nutritional composition and specification for home-prepared and commercially-processed food blends as laid down by PAG (2009) and recommended by FDA, FAO, WHO and UNICEF (Reports 1997-2012; 2007; 2000-2016). It is also able to supply the nutritional and energy requirement of a growing child and also confer health benefits on them as stated in the FDA report (2000-2016) while meeting the daily nutrient composition of the recommended standard for weaning when compared with commercially available ones.

This is also corroborated by the work of Wakil and Onilude (2009) who reported a significant increase in nutritional composition of fermented cereals when the fermentation time increases. Furthermore, the probiotics activities of LAB was able to increases the acidity of the fermenting cereal during the process by lowering of the pH and preventing the growth of spoilage microorganisms which is a characteristic of a good probiotics (Bulhões et al., 2007; Arimah and Ogunlowo, 2014; Ozogul and Hammed, 2017).

This corroborates the work of Adeyemo and Onilude (2014) which reported a reduction of galacto-oligosaccharide content of soybeans during fermentation with enzymatic activities.The reduction of RFO and other unwanted /toxic substances during fermentation and improvement of the nutritional composition of the food by *L. plantarum* is one of the attributes of a good probiotic that can be used as starter culture (Amankwah et al., 2012; Nagai et al., 2012).

In conclusion, cereals and legumes are abundant in West Africa Sub-region; thus these potentials can be harnessed to prepare adequate weaning foods for children. Preparation of weaning foods through fermentation should be as natural as much as possibleand carried out under strict hygienic condition. This will reduce diarrhoea, infection, kwashiorkor and high infant mortality rate. Also, preparation of adequate and well balanced weaning food is cheap, easy to prepare and has so many probiotic properties and health benefits. The Sustainable Development Goals (SDG) of the UN will be achieved as this will reduce the problem of poverty, malnutrition and infant mortality rate. The food blend can compete adequately with the commercially available infant weaning formula.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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Determinants of traders' nutrition knowledge and intake of traditional African vegetables in Tanzania

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Indigenous and traditional African vegetables are receiving increasing attention because of their potential to contribute to food and nutritional security as well as enhance livelihoods in sub-Saharan Africa. Traditional African vegetables are important sources of nutrients like iron and calcium and also vitamins A, B complex, C, and E. The consumption of vegetables in sub-Saharan Africa, however, falls way below the recommended levels by the World Health Organization and Food and Agriculture Organization. This situation has led to micronutrient deficiencies and widespread nutritional disorders among the low-income and food insecure populations. This study explored the factors that determine the nutritional knowledge, frequency of intake, and attitudes of traders towards consumption of traditional African vegetables. Data were collected from 65 purposively selected households of traders in Manyire, Embaseny, and Bangata markets in Arumeru District of Tanzania from July to November 2015. Generalized Poisson and factor analysis were used in the analysis of data. The results showed that education, age, and annual income influenced traders' nutritional knowledge. The consumption of traditional African vegetables was influenced by education, household size, occupation, nutritional value, and preparation time. Factor analysis results indicated that knowledge of health benefits, taste, preparation time, and perception influenced consumption of traditional African vegetables. These findings imply that the consumption of traditional African vegetables can be enhanced by educating traders about the health benefits of these commodities as well as the taste-preserving preparation techniques. The study recommends inclusion of the health attributes of traditional African vegetables in promotional campaigns.

Key words: Diet, health, education, nutrients, vitamins.

INTRODUCTION

Indigenous and traditional African vegetables (TAVs) are receiving increasing attention because of their potential to contribute to food and nutritional security as well as enhance the livelihoods of smallholder farming households in sub-Saharan Africa (Afari-Sefa et al., 2012). Compared with exotic vegetables, the TAVs contain high levels of nutrients such as vitamins A, B complex, C, and E as well as iron and calcium. For example, amaranth greens have

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been shown to contain 57 times more vitamin A precursor, 13 times more iron, and 8 times more calcium compared with cabbage (Yang and Keding, 2012). The TAVs are also marketed and traded at the local, regional, and international levels, thus providing a revenue stream (Humphry et al., 1993; Smith et al., 1995; Smith et al., 1996).

The consumption patterns and preferences for traditional African vegetables vary from household to household within different countries (Uusiku et al., 2010). In Tanzania, 17.2% of the TAVs consumed were naturally occurring in the wild, while 56.9% were cultivated (Weinberger and Msuya, 2004). Keding et al. (2007) also found that about 78% of the participants in a study in Tanzania were cultivating TAVs with 75% of these households having consumed these vegetables on the previous day. Furthermore, 50% of all cultivated TAVs was sold in the markets while the remaining half was either consumed at home or given out as gifts (Keding *et al*., 2007).

Kobe (2004) points out that the level of consumption of vegetables and fruits (27 to 114 kg/capita per year) in sub-Saharan Africa is much less than the WHO/FAO recommendation (146 kg/capita per year). Low vegetable consumption is a major factor causing micronutrient deficiencies and widespread nutritional disorders including birth defects, weakened immune systems, and mental and physical retardation in Sub-Saharan Africa (FAO, 2003). In Tanzania, for instance vegetable consumption per capita is below the minimum recommended intake of 200g per person per day (FAO, 2009). Vegetable consumption in the country only grew from 107 to 113 g per capita per day between 1993 and 2000 (FAO, 1994). By 2012, vegetable consumption had reached 317 g per capita per day for the highest quintile consumers (Keding et al., 2012). For most medium and low-income consumers, however, vegetable consumption is still below the recommended levels. Furthermore, little is known about the consumption and nutritional importance of TAVs in Tanzania since indigenous knowledge about them is no longer passed on from one generation to the next (Weinberger and Msuya, 2004).

According to the World Bank (2010), malnutrition in Tanzania is a problem with stunting affecting 42% of children under five years old while anemia affects 53% of pregnant women, 60% of children under five years old, and 81% of infants aged 9-11 months. In addition, vitamin A deficiency affects 33% of children and 37% of women. Given that TAVs provide dietary fiber and other important components of nutritional value, their consumption could significantly contribute to the prevention of chronic and lifestyle diseases (Uusiku et al., 2010). However, these vegetables are neglected and despised even though they are rich in nutrients like proteins, carbohydrates, vitamins, and dietary fiber which are essential for good health (Nnamani et al., 2009). The consumption of TAVs is threatened by competition from global vegetables (Chweya and Eyzaguirre, 1999).

This study focused on traders who consume and also trade in TAVs. Traders have a wealth of information unknown to researchers about supply, demand and markets emanating from their trading activities, extensive handling, and consumption of TAVs. Thus, establishing the factors that determine traders' household nutritional knowledge about TAVs as well as frequency of intake and attitude towards consumption of TAVs is essential for the promotion, marketing, and trading of these commodities to consumers. An understanding of these relationships could provide policy-makers with information to design policies and programs that can contribute to measures needed to expand the consumption of TAVs.

MATERIALS AND METHODS

Theoretical framework

Economic policies are often directed towards measurable outcomes. Models based on count data allow for regression-type analyses when the dependent variable of interest is a numerical count. Jörgen (2002) observes that count data modeling techniques have become important tools in empirical studies of economic behavior. Economic studies usually begin with the specification of a theoretical model, which attempts to explain an agent's (for example household, firm, individual) behavior or choice as depending on other variables (Jörgen, 2002). The Poisson regression model is the main building block in this kind of modeling framework. However, researchers routinely employ more general specifications, usually the negative binomial model. This approach constitutes the standard choice for a basic count data model in an attempt to avoid implicit restrictions on the distribution of observed counts in the Poisson model. From the theoretical model, an empirically feasible regression model is formulated. This study thus used the generalized Poisson model to determine the factors influencing nutritional knowledge and frequency of intake of TAVs.

Consul and Famoye (1992) have presented an excellent overview of the basic generalized Poisson regression model and its derivation. The probability distribution function of a generalized Poisson distribution is defined as:

$$f(y;\lambda,\theta)=\theta\left(\theta+\lambda_i^{y_i}\right)^{y_i}\frac{exp\left(-\theta-\lambda_i^{y_i}\right)}{y_i} \quad (1)$$

Consul and Famoye (1992) and Hilbe (2011) show that the log-likelihood (LL) transformation for the above generalized Poisson probability distribution is given by:

$$\mathcal{L}(\mu,\alpha;y)=\sum_{i=1}^{n}\left\{y_i ln\left(\frac{\mu_i}{1+\alpha\mu_i}\right)+(y_i-1)ln(1+\alpha y_i)-\left[\frac{-\mu_i(1+\alpha y_i)}{1+\alpha\mu_i}\right]-ln\Gamma(y_i+1)\right\} \quad (2)$$

In terms of $x\beta$ this distribution can be given as:

$$\mathcal{L}(\beta,\alpha;y)=\sum_{i=1}^{n}\left\{y_i ln\left(\frac{exp\left(x_i'\beta\right)}{1+\alpha exp\left(x_i'\right)}\right)+(y_i-1)ln(1+\alpha y_i)-\left[\frac{exp\left(x_i'\beta\right)(1+\alpha y_i)}{1+\alpha exp\left(x_i'\beta\right)}\right]-ln\Gamma(y_1+1)\right\} \quad (3)$$

Where, y_i= random response variable corresponding to the level of nutritional knowledge possessed by respondent (i); x =covariate vectors of explanatory variables and β= linear predictor of random response variable.

Model specification: factors affecting TAV nutritional knowledge

Parmenter and Wardle (1999) designed a reliable and valid instrument which provides a comprehensive measure of nutritional knowledge possessed by adult consumers. This instrument helps to identify areas of weakness in peoples' understanding of healthy eating and also provides useful data for examining the relationship between nutritional knowledge possessed by an individual and dietary behavior. The instrument has five scale levels which are administered to determine the nutrition knowledge possessed by consumers. The scale/levels used are: (i) understanding of nutrition terms; (ii) awareness of dietary recommendations; (iii) knowledge of foods as sources of nutrients; (iv) ability to apply the information in each scale chosen by the respondent, and v) awareness of diet-disease associations.

This study adopted this design to assess the amount of nutritional knowledge possessed by traders in relation to TAVs. Traders of traditional African vegetables were asked to indicate their knowledge at each level of the nutrition scale and the implications. The level of nutritional knowledge possessed was tallied for each trader case interviewed. For example, if a trader possessed knowledge and application of scale item number 1 on understanding of nutrition terms and scale item number 2 on awareness of dietary recommendations out of the five, then the total number of nutrition knowledge possessed by this trader would be two (2). The dependent variable was, therefore, the number of items on the nutritional knowledge scale known to the trader, including an understanding of their implications. The expected responses ranged from zero to five. Some respondents indicated lack of awareness of all items in the nutritional knowledge scale, and hence their response implied zero knowledge. Few traders indicated having knowledge of all the five items identified in the nutritional knowledge scale. Ultimately, the implicit functional form of the generalized Poisson regression model estimated was:

Scale/level of nutritional knowledge possessed (y) = f (lnage, gender, education, lnincome, type of occupation, social capital) + e (4)

Model Specification: factors influencing frequency of intake of TAVs

The demand for TAVs was modeled using the frequency of consumption. In order to assess the factors influencing the frequency of intake of nutrient-dense vegetables, this study specified the dependent variable as the average number of times that TAVs were consumed by the trader's household per week. Hence, the dependent variable for the frequency of intake was a number such as zero times a week, three times a week, and so on. This study used the Standard Poisson and Generalized Poisson Regression models to isolate the determinants of the frequency of intake because the dependent variable was a count data variable. The model was specified as follows:

Frequency of TAV intake (Z) = f (lnage, gender of household head, household size, lnincome, occupation, education) + e (5)

Traders' attitudes towards traditional African vegetables (TAVs)

Descriptive and inferential statistics were used to examine the attributes of TAVs that were most preferred by traders' households. The descriptive statistics used were percentages and mean scores. Factor analysis was used to identify the latent dimensions underlying the variables used to measure the preferred attributes. The responses, which were given on a five-point Likert scale, were subjected to principal component factor analysis (PCA) with Varimax rotation. The component factors were subjected to the Kaiser-Meyer-Olkin and Bartlett's test (KMO and Bartlett's test) to determine the sampling adequacy. According to Leech et al. (2012), a KMO measure greater than 0.7 is preferable, while one that is less than 0.5 is inadequate. The KMO test is used to determine whether enough items are predicted by each factor. The above procedures were adopted for this study and used to analyze the preferred attributes leading to a higher frequency of intake of nutrient-dense vegetables by traders' households.

Data and variables

This study used data collected from trading households in Arumeru District located in Arusha Region in Tanzania. This is one of the districts where a project called "Improving Income and Nutrition in Eastern and Southern Africa by Enhancing Vegetable-based Farming and Food Systems in Peri-urban Corridors (VINESA)"[1] [VINESA is led by World Vegetable Center with financial support from Australian Center for International Agricultural Research (ACIAR)] is being implemented. The district is composed of three major ethnic groups, namely Wameru, Waarusha, and Maasai. A cross-sectional survey of TAVs traders was conducted from July to November 2015. A representative sample of the TAV traders was obtained through purposive sampling involving the selection of villages where the VINESA project was being implemented in the Arumeru District. A total of 65 small-scale TAVs traders from Manyire, Embaseny, and Bangata local markets were selected. A structured questionnaire was administered to the traders through face-to-face interviews. The variables used in the study were extracted from the household survey data and are shown in Table 1.

RESULTS AND DISCUSSION

Descriptive statistics results

Table 2 shows the descriptive statistics of the socio-economic characteristics of traders in the study. Majority of the traders interviewed (97%) were females. The results imply that the majority of those who trade in traditional African vegetables are women. The traders were also consumers of TAVs as shown by the ratio variables in Table 2. The results below therefore reflect traders' consumption of TAVs.

The results indicate that 89.23% of those surveyed had attained primary school education, 3.08% had no formal education, 7.69% had secondary school education, and none had attained middle-level college education. These results imply that the TAV trader group consists majorly of those with a low level of education. The mean age of traders consuming TAVs was 38 years, while the mean period of involvement in TAVs business was eight years, implying that the youth were relatively few among the traders. This situation raises the concern that non-consumption of TAVS by the youth (age group 15 to 35 years) and other individuals with higher levels of education is likely to promote poor diets and the incidence of nutritional deficiency disorders and diseases

Table 1. Description and expected sign of variables included in the nutritional knowledge and frequency intake models.

Variable name	Variable description	Variable coding	Expectations: nutritional knowledge, frequency intake models	Nutrition knowledge sign	Frequency intake sign
Gender	Gender of household head	1 if male, 0 if female	Men are generally expected to be more knowledgeable about their environment than women	+	+
Years of schooling	Number of years spent in school	Natural logarithm of number of years of schooling	The more the number of years of schooling the more the nutritional knowledge and frequency of intake of TAVs	+	+
Age	Age of the household head	Natural logarithm of age in years	One is expected to accrue more nutritional knowledge with age and also increase intake of TAVs	+	+
Occupation	Consumer's regular work or profession	1 if nutritionist, exposed to agricultural training, or teacher; 0 otherwise	Consumers that are nutritionists or those exposed to agricultural training are expected to have more nutritional knowledge and higher intake of TAVs	+	
Income	Annual household income	Natural logarithm of income	The higher the income, the more the nutritional knowledge and higher intake of TAVs	+	+
Group membership	Consumer is a member of a group	1 if yes; 0 otherwise	Consumers with membership in groups are expected to have more nutritional knowledge and higher intake of TAVs	+	+
Household size	Number of members in a household	Natural logarithm of the size of household	The higher the number of members in a household, the more the nutritional knowledge and higher intake of TAVs	+	+
Medicinal value	Medicinal value influences intake	1 if yes; 0 otherwise	The more the attachment to medicinal properties of TAVs, the higher the intake of these commodities		+
Nutritional value	Nutritional value influences intake	1 if yes; 0 otherwise	The more the attachment to nutritional properties of TAVs, the higher the intake		+
Time to prepare TAVs	Less time to prepare TAVs influence intake	1 if yes, 0 otherwise	The shorter the time required to prepare TAVs, the higher the intake		+
TAVs weekly expenditure	Amount spent weekly to purchase TAVs	Natural logarithm of expenditure	The higher the weekly expenditure on TAVs, the more the intake of TAVs		+

in the township areas. There is, therefore, need to promote the consumption of TAVs among the youth as well as educated traders.

The ethnic composition of the traders consisted of 41.54% Meru, 13.85% Arusha, and 21.54 Chagga. Business activity was the main occupation for 91% of the traders. The mean yearly income of the traders was 1,634,692 Tanzania shillings (Tshs) or (US$ 730), which was majorly derived from the sale of TAVs, other global vegetables, and fruits. The results further showed that traders spent approximately Tshs 9,143.10 (US$ 4.1) per week in purchasing TAVs for household consumption. The average frequency of household intake of TAVs was 1.4 times per week (that is basically once a week).

Factors affecting TAV nutritional knowledge

The respondents were asked five questions to assess their nutritional knowledge and scores from each case were added together. Knowledge about the nutritional value of TAVs ranged from zero to five. The mean nutritional knowledge of the traders was 2.27 out of 5 (that is 45.4%), implying that traders had moderate nutritional knowledge about TAVs. The mean deviance and the Pearson chi-square ratio (the Pearson chi-square value divided by its degree of freedom) were used to assess the goodness of fit of the standard Poisson model. The estimated deviance and Pearson ratios were as shown below:

Table 2. Socio-economic characteristics of the sample.

Demographic properties	Frequency	
Gender (%)		
Female	96.92	
Male	3.08	
Marital status of respondent (%)		
Married	85.16	
Single	9.23	
Separated	3.08	
Divorced	1.54	
Ethnicity group (%)		
Meru	41.54	
Arusha	13.85	
Chagga	21.54	
Others (Sukuma, Nyakyusa, Iraqw, Pare)	23.07	
Main occupation (%)		
Agriculture	9.23	
Business	90.77	
Level of education (%)		
None	3.08	
Primary	89.23	
Secondary	7.69	
Socio-economic characteristics of the sample-Ratio variable scales	**Mean**	**Standard deviation**
Age of respondent (mean)	38.15	9.497
Number of years of schooling (mean)	7.14	1.424
Household size (count) mean	4.338	1.123
Distance to nearest market (minutes) mean	51	57.637
Amount spent to purchase TAVs per week (Tshs) (mean)	9,143.10	6585.37
Household income (Tshs) (mean)	1,634,692	1,408,851
Nutritional Knowledge (count) mean	2.769	1.272
Frequency intake (count) mean	1.415	0.6096184

Source: Author's survey of TAVs consumers in Arumeru District, July to November 2015.

Deviance/df = 40.79479437/53 = 0.76
Pearson/df = 30.81113652/53 = 0.58

The two ratios are significantly smaller than 1, which is evidence of under-dispersion and the fact that the standard Poisson model was not a good fit for the data. However, the generalized Poisson model was a better fit for the data and hence the results derived from this model (Table 3) form the basis of the discussion that follows. The model results show that nutritional knowledge of traders was influenced positively by age, number of years of schooling, annual income, household size, and interaction terms (that is age and annual income; years of schooling and annual income; and age and household size). Table 4 shows the results of Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) tests of goodness of fit for the two models. Lower values obtained for either one of the two tests indicate a better fit.

Age of the respondent significantly influenced (P-value <0.05) the level of nutrition knowledge possessed. Each additional year was expected to increase the level of nutritional knowledge by a factor of 1.60e-06, with all other variables in the model being constant. Thus, an individual acquires more nutritional knowledge with increasing age, which means that younger traders would be expected to possess relatively less nutritional knowledge compared with their older counterparts. The results also show that the number of years of schooling significantly (P-value<0.01) influenced the level of nutritional knowledge, which suggests that education plays an important role in enhancing the level of

Table 3. Factors which influence traders' awareness of nutrition knowledge.

Dependent variable = Scale/level of nutrition knowledge possessed by respondent	Standard Poisson		Generalized Poisson	
	IRR	P-values	IRR	P-values
Gender	1.051	0.708	1.134	0.423
Ln of Years of schooling	0.0000134	0.018***	3.67e-06	0.005***
Ln of Age	4.54e-06	0.058**	1.60e-06	0.020**
Occupation	1.012	0.818	1.027	0.578
Ln of Income	0.064	0.020**	0.046	0.005***
Group membership	1.056	0.662	1.090	0.398
Ln of Household size	0.059	0.141	0.065	0.071*
Ln Age &ln Income	2.113	0.082*	2.305	0.027**
Ln Yrs. in school & ln Income	2.271	0.016*	2.477	0.005***
Ln Age & Ln HH size	5.223	0.204	5.024	0.109
Constant	1.89e+18	0.011	1.15e+20	0.003
Number of observations	64		64	
Wald chi2(10)	18.86		19.07	
Prob>chi2	0.0421		0.0394	
Pseudo R2	0.0186		0.0398	

*, ** and *** denote significance level at 10, 5 and 1 percent respectively.

Table 4. Akaike's and Bayesian Information Criterion.

Model	Obs.	ll(null)	ll(model)	df	AIC	BIC
Standard Poisson	64	-110.4389	-108.3874	11	238.7749	262.5226
Generalized Poisson	64	-104.1484	-100.0033	12	224.0065	249.9131

nutritional knowledge. In this case, respondents with more years of schooling had a higher likelihood of being more nutritionally aware with respect to TAVS compared with those who had spent fewer years in school.

Annual income of the respondent significantly (P-value<0.01) influenced nutritional knowledge of the traders. For each additional 1.00 Tsh, the level of nutritional knowledge changed by a factor of 0.046. Thus, more than their lower income counterparts, higher income traders are able to obtain more nutritional information because they have the capacity to move around and attend meetings and other fora that may facilitate the acquisition of nutritional knowledge. Household size also significantly (P-value<0.1) influenced awareness of nutrition knowledge. This implies that with increase in household size, the level of nutritional knowledge would be expected to change by a factor of 0.065, while holding all other variables in the model constant.

The interaction term between age and annual income significantly (P-value<0.05) influenced awareness of nutrition knowledge. The older respondents who also had higher income were more likely to possess more nutritional knowledge. In addition, the interaction term between the number of years of schooling and annual income of the respondent significantly (P-value<0.001) influenced the traders' awareness of nutritional knowledge. Hence, more educated traders with higher incomes would be expected to possess more nutritional knowledge about TAVs.

Factors affecting the frequency of TAV Intake

This section presents the results of the factors that influenced the frequency of intake of traditional African vegetables among traders. The results of the regression models are shown in Table 5. The Prob-chi^2 test statistic showed that both the models fit the data well (p-value = 0.0000and 0.0000 respectively). The mean deviance and the Pearson chi-square ratio (the Pearson chi-square value divided by its degrees of freedom) were used to assess the degree of fit of the Standard Poisson Model. The estimated deviance and Pearson ratios are shown below:

Deviance/df = 13.83700644/51= 0.271
Pearson/df = 12.32726424 /51= 0.241

These results show that both ratios are significantly smaller than 1, thus indicating under-dispersion and the fact that the standard Poisson model does not fit the data

Table 5. Factors which influence traders' frequency intake.

Dependent variable = Intake frequency	Standard Poisson		Generalized Poisson	
	IRR	P-values	IRR	P-values
Gender	1.134	0.462	1.497	0.299
Education	0.797	0.043**	0.721	0.085*
Ln of Age	0.921	0.790	0.914	0.764
Occupation	0.941	0.300	0.873	0.048**
Ln of Income	1.038	0.597	1.048	0.598
Ln of household size	1.463	0.119	1.986	0.013**
Member of group/social	0.955	0.743	0.828	0.184
Medicinal value	1.219	0.286	1.288	0.220
Ln of TAVs Weekly spent	1.092	0.183	1.100	0.187
Nutrition value	1.405	0.101	1.619	0.044**
Time to prepare	1.216	0.174	1.387	0.042**
Constant	0.191	0.202	0.052	0.060
Number of observations	65		65	
Wald chi2(13)	138.01		114.05	
Prob>chi2	0.0000		0.0000	
Pseudo R2	0.0293		0.0283	

* and ** denote significance level at 10 and 5 percent respectively.

Table 6. Akaike's and Bayesian Information Criterion.

Model	Obs.	ll(null)	ll(model)	df	AIC	BIC
Standard Poisson	65	-80.95128	-78.58167	14	185.1633	215.6048
Generalized Poisson	65	-67.94971	-69.87097	14	167.7419	198.1834

well. Table 6 shows the results of Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) tests of goodness of fit for the two models. Generalized Poisson model was a better fit for the data because it had lower values for both AIC and BIC. The discussion below is based on the results of generalized Poisson model.

Years of schooling significantly (P-value<0.1) influenced frequency of intake of TAVs. The longer the period spent in school, the higher the likelihood that traders would consume more TAVs. This implies that education can play an important role in sourcing for and accessing nutritional knowledge, which is likely to translate into improved consumption of TAVs. However, this occurrence is still at the lowest significance level (that is, 10%). Occupation of the respondent significantly (P-value<0.05) influenced frequency of TAVs intake. The incidence rate ratio for frequency of intake changed by a factor of 0.873, implying that traders without occupational training in nutrition, agriculture, or teaching had 13% less frequency of TAVs consumption compared with those that possessed these qualities.

Household size significantly (P-value<0.05) influenced frequency of intake of TAVs. Thus, if the size of the household increases by one individual, the incidence rate for frequency intake of TAVs would be expected to change by a factor of 1.986, if all other variables in the model are held constant. The bigger the household size, the higher the frequency of consuming TAVs.

The frequency of TAVs intake was significantly (P-value<0.05) influenced by traders' perception of their nutrition value. The incidence rate for frequency of TAVs intake changed by a factor of 1.619, or increased by 62%, when the respondent indicated consumption of TAVs for nutritional purposes. The time spent to prepare TAVs also significantly (P-value<0.05) influenced the frequency of TAVs consumption such that less time spent in the preparation of these vegetables increased the consumption incidence rate by a factor of 1.387 (that is, approximately 39%).

Traders' attitude towards consumption of traditional African vegetables

Factor analysis was used to identify latent dimensions underlying the different variables applied in measuring traders' attitudes. Responses to the 11 items on the five-point Likert-type scale were subjected to principal component factor analysis. Exploratory factor analysis with Varimax rotation was employed to create

Table 7. Results of exploratory factor analysis.

Factor and item description	Factor loading	% variance explained
Factor 1: Health benefits		32.38
Fresh TAVs contain more nutrients than dried ones	0.835	
Intake of variety of TAVs each day guarantees vitamins and minerals required	0.915	
It is important to choose diet accompanied with TAVs	0.721	
Consumption of TAVs improves eyesight and boosts body immunity	0.872	
TAVs are best consumed when fresh	0.838	
Factor 2: Personal taste		20.78
TAVs are inferior foods (poverty food)	0.664	
TAVs are tasteless and bitter	0.879	
Factor 3: Time factor		10.84
TAVs take more time to prepare	0.941	
Factor 4: Personal perception		10.48
TAVs are not good for me	0.956	

Source: Author survey of TAVs consumers in Arumeru District, July to November 2015.

measurement scales. The objective was to obtain fewer dimensions reflecting the relationships among these inter-related variables. An Eigen-value greater than one rule was applied in identifying the number of factors. The variables that had large loadings on the same factors were grouped together. A factor loading value of 0.50 and above is normally considered as good and significant (George and Mallery, 2003). The analysis produced a solution with five factors that accounted for 74.48% of the total explained variance as shown in Table 7. The Kaiser's overall measure of sampling adequacy obtained was 0.695, which borders on the recommended threshold of 0.7 (George and Mallery, 2003) suggesting that the data were appropriate for factor analysis.

Five attitude variables concerning importance of consuming TAVs varieties were loaded on factor 1 with the cross-correlation coefficients of 0.835, 0.915, 0.721, 0.872, and 0.838 (Table 7). This factor accounted for 32.38% of the total variance and was termed ‘health benefits’ because these variables focused mainly on the importance attached to the consumption of TAVs by local traders. Higher scores and positive responses on this factor implied a general understanding of the health benefits of TAVs among traders and the significance of consuming these commodities. Factor 2 had cross-correlation coefficients of 0.664 and 0.879. Since these variables focused mainly on attitude towards taste of TAVs varieties, factor 2 was labeled as ‘personal taste’ and accounted for 20.78% of the total variance. These scores and the positive responses on this factor emphasize an important general opinion about the taste of TAV varieties.

Only one attribute, that is, preparation time for TAVs, was loaded on factor 3 with cross-correlation coefficients of 0.941. This attribute focused on time taken to prepare TAVs. Therefore, factor 3 was termed ‘time factor’. Given that peri-urban consumers generally have very limited time to prepare TAVs, the traders usually sell already prepared packages of these commodities. On the other hand, this was not the case with rural consumers. Time factor accounted for 10.84% of the total variance. Factor 4 had a cross-correlation coefficient of 0.956, which was as a result of the attribute ‘TAVs are not good for me’. This variable was labeled as ’personal perception’ and it accounted for 10.48% of the total variance. This result suggested that negative perception towards TAVs still persisted among consumers. This perception has pervaded the communities for many years and is a hindrance to the consumption of these commodities. The cumulative percentage of variance for all the factors explained 74.47% of the total variance.

Conclusion and Recommendations

This study found that the factors which influenced traders' awareness of nutrition knowledge were level of education, age, annual income as well as the interaction of these factors. The factors which influenced traders' frequency of intake of TAVs were education, household size, occupation, nutrition value, and preparation time. The study, therefore, concluded that socio-economic factors have a significant effect on nutritional knowledge of TAVS and frequency of intake of these commodities. Traders had a positive attitude towards consumption of TAVs. The ‘health factor’ had the highest weight in the factor analysis, implying that traders strongly associate consumption of TAVs with health wellbeing. Moreover, they consider TAVs as normal food rather than inferior or food for the poor. There is need to improve the processing and cooking of TAVs so as to maintain the nutrient content and also preserve taste. The weight for ‘perception factor’ was relatively high, suggesting that there is need for change of attitude towards the consumption of TAVs because they are important to human health. The study recommends inclusion of the health attributes of TAVs in promotional campaigns to encourage consumption. Finally, promotional approaches such as advertisements, posters, road shows, and cooking demonstrations can be used to enhance the consumption of TAVs.

CONFLICT OF INTEREST

The authors have not declared any conflict of interest.

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Effect of pregelatinized corn and rice flour on specific volume of gluten-free traditional Algerian bread «*KhobzEddar*» using central composite design

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In the present study, the effect of addition of pregelatinized corn and rice flour on specific volume of gluten-free traditional Algerian bread *Khobz Eddar* was studied. The gluten-free breads were made from corn and rice/field bean formula in ratio 2/1 (w/w) and pregelatinized corn and rice flour were made by adding water to flour on the basis of 5/1 (w/w) and heated until 65°C. Response Surface Methodology (RSM) was used to optimize the gluten-free *Khobz Eddar* bread with the specific volume as the only response. The effect of hydration and pregelatinized corn and rice flour on specific volume of the two formula of gluten-free *Khobz Eddar* bread was studied. The optimum points were characterized by specific volume and image analysis as compared to a control bread based on durum wheat semolina. The optimum formulation for corn/field bean bread contained 115 g/100 g of water and 7.05 g of pregelatinzed corn (dry basic) with a specific volume of 2.39 cm^3/g and for rice/field bean bread the optimum was found by addition of 105 g/100 g of water and 6.3 g of pregelatinized rice (dry basic) with a specific volume of 2.24 cm^3/g. Specific volume of corn/field bean bread showed a higher value as compared to rice/field bean bread (*p<0.05*), for both breads, the specific volume was lower (*p<0.05*) than control wheat bread (3.64 cm^3/g). The image analysis by software Image J showed that the number of cells in both formula was greater than that of *Khobz Eddar* control bread.

Key words: Gluten-free *Khobz Eddar*, optimization, pregelatinized flour, rice, corn, specific volume.

INTRODUCTION

Khobz Eddar is a traditional leavened bread consumed in Algeria during major celebrations such as weddings and religious feasts. It is prepared from durum wheat semolina with added salt, oil, yeast, water, and whole egg.

Bread may be prepared by the use of gluten-free ingredients such as rice, corn, buckwheat, potato and sorghum flours (Arendt and Dal Bello, 2008). To present an acceptable gluten-free bread, the proposed loaves must have quality characteristics that are similar to those of wheat bread. Bread with rice or corn flour has a lower loaf volume than wheat flour bread; it is related to the

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absence of gluten, where the glutenin wheat dough is responsible for the dough's resistance to extension and it has the ability to retain gases produced during the yeast fermentation process (Hamada et al., 2013; Hoseney, 1994; Turabi et al., 2008). To solve this problem, different additives can be suggested to improve bread volume.

The pregelatinized flours and starches obtained by heating in the presence of water are widely used for their technological properties such as solubility in hot or cold water, high viscosity and smooth texture and they can be used in the treatment of foods whenever thickening is required (Lai, 2001). The addition of gelatinized rice flour or rice porridge to prepare gluten-free bread has been studied limitedly (Chang et al., 2014). The gelatinized rice can replace the gluten and affect the texture or physical properties of bread and give a higher dough expansion (Shibataa et al., 2011; Miyazaki et al., 2006).

Tang Zhong or pregelatinized flour is indeed a kind of 'flour paste,' a thick flour mixture was made by adding water to corn or rice flour on the basis of 5/1 (w/w) at 65°C (149°F) (Delcourt and Lefief, 2013; Herberth, 2013; Paquette, 2016; Yvonne, 2007). Pongjaruvat et al. (2014) reported that the starch has a potential to improve viscoelastic properties of the dough to efficiently trap and retain carbon dioxide gas bubbles produced during fermentation. The onset of viscosity of starch begins in excess water at about 65°C when amylopectin crystals begin to melt and proteins to hydrate (Fitzgerald and Reinke, 2006). On cooling, the dough becomes more viscous and amylose aggregates and forms a gel (Sindic, 2009).

The aim of this work was to investigate the effect of addition of pregelatinized corn flour or pregelatinized rice flour on the quality of gluten-free traditional Algerian *Khobz Eddar* bread and to optimize water and pregelatinized flour (corn or rice) addition for a better expansion by applying Response Surface Methodology (RSM).

MATERIALS AND METHODS

Raw materials

Sample preparation for gluten-free Khobz Eddar making

For control, durum wheat semolina was obtained from a local market in Benhamadi, Algeria (14.20± 0.00% moisture, 0.83±0.00% ash, 0.67±0.01% lipid, and 13.93±0.00% protein).

For gluten-free *Khobz Eddar*, rice (Basmati) was obtained from Thaïland (10.30±0.14% moisture, 1.66±0.23% ash, 0.06±0.04% lipid, and 9.42±0.10% protein), corn grain from Alicampo (Argentina) (6.66±0.00% moisture, 1.40±0.01% ash, 1.14±0.1% lipid, and 9.77±0.00% protein) and field bean seeds from Alamir company (Egypte) (10.00±0.8% moisture, 2.66±0.00% ash, 1.03±0.00% lipid, and 30.86±0.55% protein). They were milled using a laboratory mill and sifted. All semolina used in gluten-free *Khobz Eddar* had particle sizes between 200 and 500 µm.

Additional ingredients used were salt (ENAsel, Algeria), instant dry yeast (Saf-instant, France), commercial sunflower oil "Elio" from Cevital (Algeria) and fresh eggs. They were purchased from an Algerian local market.

For pregelatinized formulation

Rice flour (Basmati, Thaïland) had 10.66±0.47% moisture, 1.33±0.00% ash, 0.36±0.07% lipid and 6.04±0.38% protein and corn flour (Alicampo, Argentina) had 8.33±0.46% moisture, 1.40±0.01% ash, 3.97±0.23% lipid and 8.74±0.00% protein.

Hydration properties of corn and rice flours

Water absorption index (WAI), water solubility index (WSI) and swelling power (SP) of both flours (corn and rice) were determined.

The SP was determined using the method of Mccormock et al. (1991), as amended by Tang et al. (2002), while WAI and WSI were determined by the method of Li and Yeh (2001). A sample of 0.25 g of flour was weighed into a centrifuge tube with coated screw cap to which 5 ml of a 0.1% $AgNO_3$ solution was added. The suspensions obtained were mixed for 10 s using a Vortex mixer. The tubes were placed in shaking water bath at 70°C for 10 min and then transferred into a boiling water bath for 10 min. After the tubes were cooled in cold water (20°C) for 5 min and centrifuged at 1700 × g for 4 min. The supernatant was poured out from the tube and was dried in air oven at 105°C for 24 h and weighed (W1) (Tang et al., 2002). The sediment adhered to the wall of centrifuge tube was weighed (Ws). WAI, WSI and SP were calculated as follows (Anderson et al.,1969; Li and Yeh, 2001; Mccormick et al., 1991):

$$WSI=[W1/0.25] \times 100\%$$

$$SP=Ws / [0.25 (100\% - WSI)] (g/g)$$

$$WAI=Ws / 0.25$$

Pregelatinized flour process

Two types of pregelatinized flour were prepared with rice flour or corn flour. Pregelatinized rice flour was added to dough made with rice/field bean semolina and pregelatinizedcorn flour was added to dough made with corn/field bean semolina.

The pregelatinized flour was made by adding the water to flour on the basis of 5/1 (w/w). The mixture was then heated with stirring by using spatula until the temperature reaches 65°C (149°F). The pregelatinized flours were then cooled 1 h at room temperature and kept for 24 h at 4°C. They were mixed with other ingredients after maintaining it at room temperature for 1 h. The process flow diagram of pregelatinized flours is as shown in Figure 1.

Gluten-free *Khobz Eddar* formulation

The gluten-free formulations studied in this work were based on a mixture of cereal and leguminous in a ratio of 2:1 (w/w), aiming to offer a better nutritional balance in amino-acids (Benatallah et al., 2012; Micard et al., 2010). Two formulations were prepared based on corn/field bean semolina (CFBS) or rice/field bean semolina (RFBS). The hydration level was determined for each formula by preliminary experiments. Intervals of water and pregelatinized flour amount used were, respectively 51 to 115 ml of water and 0 to 14.10 g (dry basis) of pregelatinized flour corn for 100 g CFBS ingredients and 45 to 105 ml water and 0 to 12.60 g (dry basis) pregelatinized flour rice for 100 g of RFBS. The water contained in the eggs is not taken into account in the total water added. Preliminary tests on the control bread made with wheat semolina showed a specific volume (3.64±0.03 cm3/g) of Khobz Eddar with an optimal hydration rate of 55 g of water per 100 g of durum wheat semolina.

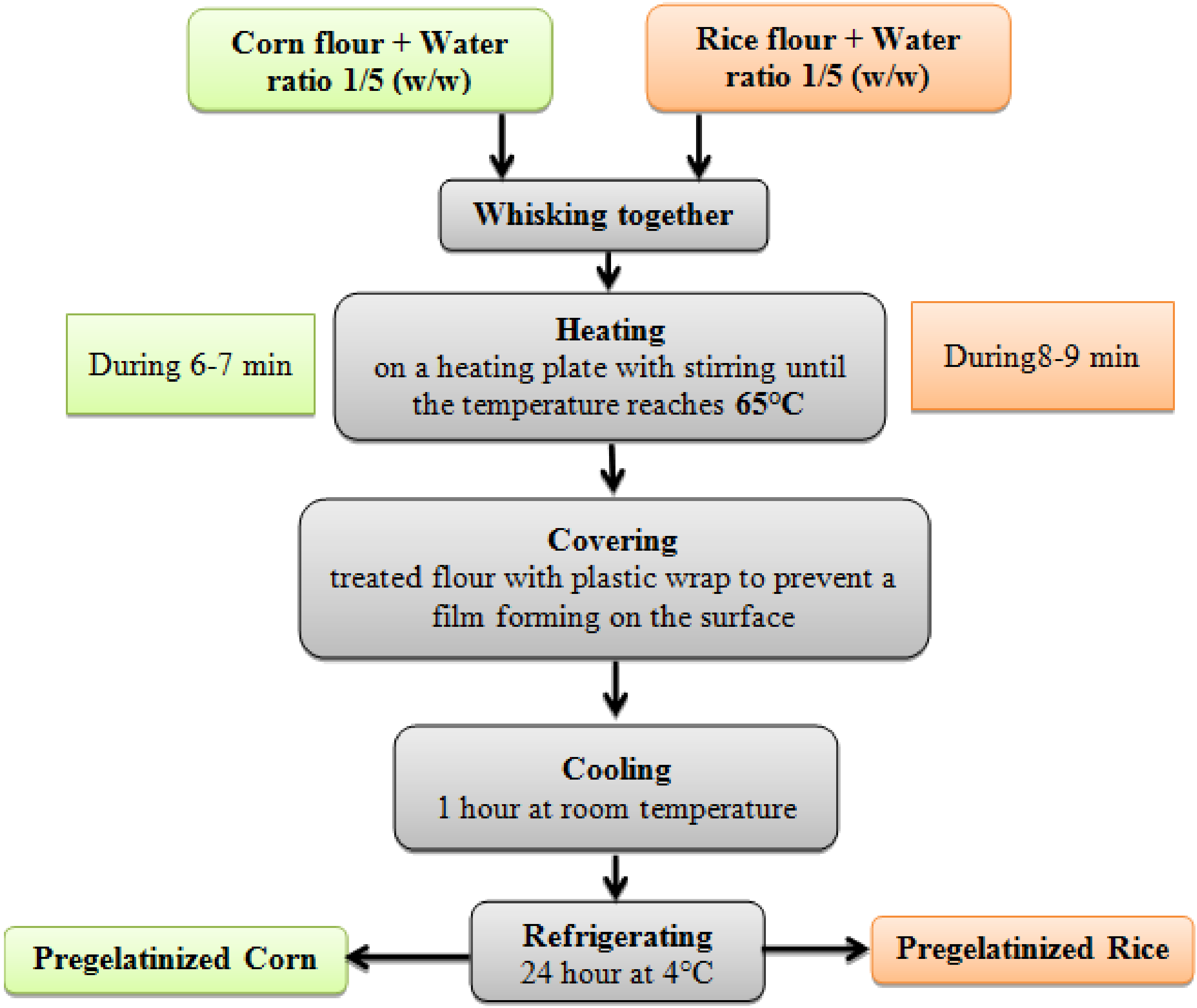

Figure 1. Flow diagram illustrating the formulation steps of the two type of pregelatinized flour.

Table 1. Coded and actual levels of the factors water and pregelatinized corn and rice in the central composite design..

Code value	Real values (g)			
	CFBS formula		RFBS formula	
	Water	Pregelatinized corn	Water	Pregelatinized rice
	X_1	X_2	X'_1	X'_2
-1.41421	51.00	0.00	45.00	0.00
-1	60.37	2.06	53.78	1.84
0	83.00	7.05	75.00	6.30
+1	105.62	12.03	96.21	10.75
+1.41421	115.00	14.10	105.00	12.60

CFBS: Corn/Field bean semolina; RFBS: rice/field bean semolina.

Experimental design

A central composite design of 2 factors was used to study the effects of water (X_1 for CFBS, X'_1 for RFBS) and pregelatinized flour level [X_2 for corn flour (CF), X'_2 for rice flour (RF)] on the specific volume of gluten-free bread (Y for CFBS, Y′ for RFBS). Each of the factors was tested at five levels (Table 1). The matrix of central composite design for CFBS and RFBS is shown in Table 1. The

Table 2. Central composite design arrangement, experimental data and the main resulting dough of corn/field bean semolina formula.

Run	Coded X_1	Coded X_2	Compositon of CFBS formula					
			Uncoded water (g)	Total water	Treated corn	Semolina	Water	
				Uncoded CFg in 100 g crude ingredients	CSg in 100 g crude ingredients	FBS g in 100g crude ingredients	g in mixed treated corn*	g added to 100 g of crude ingredients**
1	+1.41421	0	115.00	7.05	59.62	33.33	35.25	79.75
2	-1.41421	0	51.00	7.05	59.62	33.33	35.25	15.75
3	0	+1.41421	83.00	14.10	52.57	33.33	70.50	12.50
4	-1	+1	60.37	12.03	54.63	33.33	60.17	0.00
5	+1	-1	105.62	2.06	64.60	33.33	10.32	95.30
6	+1	+1	105.62	12.03	54.63	33.33	60.17	45.45
7	-1	-1	60.37	2.06	64.60	33.33	10.32	50.04
8	0	-1.41421	83.00	0.00	66.67	33.33	0.00	83.00
9	0	0	83.00	7.05	59.62	33.33	35.25	47.75
10	0	0	83.00	7.05	59.62	33.33	35.25	47.75
11	0	0	83.00	7.05	59.62	33.33	35.25	47.75
12	0	0	83.00	7.05	59.62	33.33	35.25	47.75
13	0	0	83.00	7.05	59.62	33.33	35.25	47.75

CFBS: Corn/field bean semolina formula, CS: corn semolina, FBS: field bean semolina, CF: corn flour. ***Water weight in mixed pregelatinizedcorn = CF weight × 5. **Water weight added to crude ingredients = Total water weight – (CFweight × 5).

factorial section is a 2^2 test; the star section includes four tests. Five replicates (runs 9, 10, 11, 12, and 13) at the center of the design were used to allow for estimation of the pure error at the sum of square, for a total of $2^2+2^2+5=13$ runs.

Bread making process

The formulations used in the preparation of experimental *Khobz Eddar* bread and the composition of CFBS and RFBS are shown in Tables 2 and 3, respectively.

The basic bread recipe consisted of rice or corn/field bean semolina, 2% of salt, 2% of instant dry yeast, 10 g powder egg, 10 mL of sunflower oil and the amount of water defined according to experimental design (Tables 2 and 3). The making procedure involved manual premixing of dry ingredients, with the exception of whole egg and then water was added. When pregelatinized rice and corn flour were added, the corresponding amount of rice or corn semolina and water were replaced. The mixture was left to rest for 10 min, then the fresh whole egg and the rest of water were added and mixed for 10 min and sunflower oil was added and mixed for additional 5 min. The resulting dough was divided in lumps (70 g) and put into mold and proofed for 45 min at 37°C in a fermentation cabinet. The baking tests were carried out at 230°C for 30 min into an electric oven. The process flow diagram is as shown in Figure 2.

Bread evaluation

The analyzed bread characteristics included specific volume (V*sp*) and image analysis for optimums proceded 1 h post-baking.

Specific volume

Specific volume (V*sp*) of the gluten-free *Khobz Eddar* bread was determined at room temperature, using the formula: Specific volume (cm^3/g) = Volume of bread (cm^3) / Bread weight (g). The volume of the samples was measured by the method of displacement of small particles of 0.67 cm^3/g in specific volume.

Image processing

Three samples *of Khobz Eddar* bread (control, CFBS optimum, and RFBB optimum) were sliced transversely (1 cm) and scanned using a flatbed scanner (Epson Stylus SX105). The scanned images were analyzed using the software Image J 1.48. The acquired color images were firstly saved in TIFF format. Using bars of known lengths, pixel values are converted into distance units. The center of each image was cropped to a square of 20×20 mm^2 and converted to gray scale (8 bits). A threshold method was used for differentiating gas cells and non-cells (conversion to a binaryimage). The black parts of the binary image

Table 3. Central composite design arrangement, experimental data and the main resulting dough of rice/field bean semolina formula.

Run	Coded X'_1	Coded X'_2	Compositon of RFBS formula					
			Uncoded Water (g)	Total water	Treated corn	Semolina	Water	
				Uncoded RFg in 100 g crude ingredients	RS g in 100 g crude ingredients	FBS g in 100 g crude ingredients	g in Mixed Treated rice*	g added to 100 g of crude ingredients**
1	+1.41421	0	105.00	6.30	60.37	33.33	31.50	73.50
2	-1.41421	0	45.00	6.30	60.37	33.33	31.50	13.50
3	0	+1.41421	75.00	12.60	54.07	33.33	63.00	12.00
4	-1	+1	53.78	10.75	55.91	33.33	53.77	0.00
5	+1	-1	96.21	1.84	64.82	33.33	9.22	86.98
6	+1	+1	96.21	10.75	55.91	33.33	53.77	42.43
7	-1	-1	53.78	1.84	64.82	33.33	9.22	44.56
8	0	-1.41421	75.00	0.00	66.67	33.33	0.00	75.00
9	0	0	75.00	6.30	60.37	33.33	31.50	43.50
10	0	0	75.00	6.30	60.37	33.33	31.50	43.50
11	0	0	75.00	6.30	60.37	33.33	31.50	43.50
12	0	0	75.00	6.30	60.37	33.33	31.50	43.50
13	0	0	75.00	6.30	60.37	33.33	31.50	43.50

RFBS: Rice/Field bean semolina formula, RS: rice semolina, FBS: field bean semolina, RF: rice flour. *****Water weight in mixed pregelatinized rice **=** RF weight × 5. ******Water weight added to crude ingredients = total water weight – (RF weight × 5).

were regarded as air bubbles (Rubel et al., 2015).

As a result, the surface plots and four air-bubble parameters of the bread samples were calculated: number of cells, average size, area fraction and circularity. A perfect circle has a shape factor of 1 (Rounds pores) and a line has a shape factor approaching 0.

Statistical analysis

The computational work, including the surface-contour graphical presentations of the response surface models, was performed using the statistical software JMP (Version 11.2, SAS Institute Inc., Cary, NC, USA). Multiple regression analysis was performed to fit second order model to the dependent variable by using Minitab Release 17 (Minitab Inc., State College PA, USA). The model was used to optimum conditions. These conditions were determined by using response optimizer in Minitab release 17 software. One way analysis of variance (ANOVA) was applied to compare the effects of water (X_1, $X`_1$) and pregelatinized flours ($X_2$,$X`_2$) on the dependent variable (Specific volume Y_1, Y_2). The significance level was set at 0.05. The model proposed for each response was:

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_{11} X_1^2 + b_{22} X_2^2 + b_{12} X_1 X_2$$

where b_0 is the value of the fitted response at the center point of the design, that is, point (0,0); b_1 and b_2 are the linear regression terms; b_{11} and b_{22} are the quadratic regression terms; and b_{12} is the cross-product regression term (Montgomery, 1991).

RESULTS AND DISCUSSION

Hydration properties of corn and rice flours

Table 4 shows WAI, WSI, and SP results of the corn and rice flours. WAI can be used to give an indication of cold paste viscosity, which correlates with the cooking degree and WSI can be used as an indication of the degree of molecular damage (Bryant et al., 2000; Yağci and Göğüş, 2008). Flour with high WSI and low WAI would be ideal for use in a sport drink because of the high solubility. Further, flour with high WAI and low WSI could be used in a product such as a low fat soup where the main concern is a high viscosity (Bryant et al., 2001).

Corn flour presented the highest values of SP (0.14±0.00 g/g) and WAI (12.48±0.28 g/g). Cornejo and Rosell (2015) have found that there is a positive correlation parameter between SP and WAI. According to Han et al. (2010), the SP and WAI increase when the size of the particles

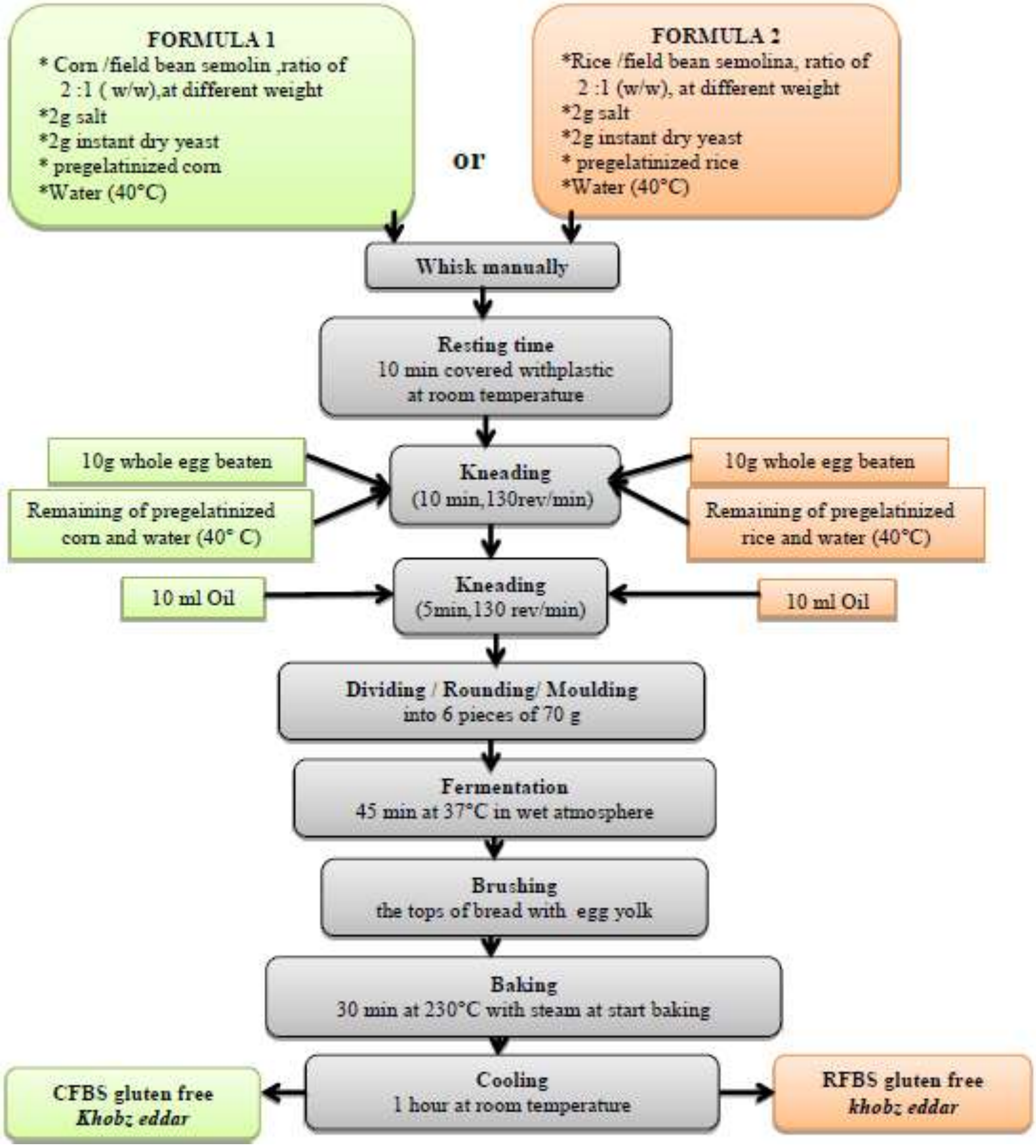

Figure 2. Flow diagram illustrating the formulation steps of two gluten-free breads.

Table 4. Hydration properties of corn and rice flour.

Parameter	WAI (g/g)	WSI (g/100 g)	SP (g/g)
Rice flour	8.60±0.62 [a]	26±2.82[a]	0.11±0.00 [a]
Corn flour	12.48±0.28 [b]	12±0.00 [b]	0.14±0.00 [a]

WAI: Water absorption index; WSI water solubility index; SP swelling power. [a,b]Indicated a significant difference ($p<0.05$).

increases.

WSI of rice flour (26±2.82 g/100 g) is higher than that of the corn flour, while the WAI indicates that the corn flour absorbs more water (12.48±0.28 g/g). So, the corn flour is more viscous than the rice flour because it has a high WAI and low WSI. These results are supported by Bryant

Table 5. Responses of gluten free corn/field bean semolina bread and rice/field bean semolina bread.

Run	Responses			
	Code values		V_{sp} CFBS	V_{sp} RFBS
	X_1 or X'_1	X_2 or X'_2	Y	Y′
1	+1.41421	0	2.39±0.02	2.24±0.01
2	-1.41421	0	1.87±0.07	1.74±0.03
3	0	+1.41421	2.12±0.02	1.85±0.02
4	-1	1	1.64±0.03	1.55±0.04
5	+1	-1	2.07±0.04	2.15±0.01
6	+1	1	2.12±0.06	1.78±0.02
7	-1	-1	1.67±0.01	1.61±0.07
8	0	-1.41421	1.79±0.03	1.60±0.05
9	0	0	2.16±0.03	1.96±0.02
10	0	0	2.13±0.01	1.99±0.02
11	0	0	2.05±0.02	2.02±0.03
12	0	0	2.12±0.02	2.04±0.02
13	0	0	2.10±0.01	1.93±0.01

CFBS: Corn/Field bean semolina bread; RFBS: rice/field bean semolina bread; Vsp: specific volume.

et al. (2000) and Yağci and Göğüş (2008).

Effects of pregelatinized flours on specific volume of breads (CFBS and RFBS) and optimization

The effect of a range of water and pregelatinized flours on specific volume of CFBS *Khobz Eddar* bread and RFBS *Khobz Eddar* bread is shown in Table 5.

CFBS bread had specific volume ranged from 1.64±0.03 to 2.39±0.02 cm^3/g. The highest specific volume (2.39±0.02 cm^3/g) was obtained at a level of 115 g of water and 7.05 g of pregelatinized corn. This specific volume was lower than that of durum wheat semolina (control) *Khobz Eddar* (3.64 cm^3/g) and higher than CFBS *Khobz Eddar* with no pregelatinized corn improver (1.79±0.03 cm^3/g) (Figure 3A, B, and C).

The plots in Figure 4 showed that the specific volume of samples has increased with an increase in the amount of water, as its linear effect was positive ($p<0.05$). However, the effect of the amount of pregelatinized corn showed a negative quadratic effect on the V*sp* of *Khobz Eddar*.

The specific volume of RFBS *Khobz Eddar* increases from 1.55±0.04 to 2.24±0.01 cm^3/g which is considerably lower than the value 3.64±0.03 cm^3/g reported in wheat durum semolina *Khobz Eddar.* A higher specific volume (2.24 ± 0.01 cm^3/g) was obtained at the level of 105 g of water and 6.3 g of pregelatinized rice. This specific volume was higher than RFBS *Khobz Eddar* with no pregelatinized rice improver (1.60±0.05 cm^3/g) (Figure 3A, D, and E). It can be observed (Figure 5) that the V*sp* depended on the amount of water added. The effect of water showed positive linear ($p<0.05$) effect and the effect of amount of pregelatinized rice showed a negative quadratic effect ($p<0.05$).

All gluten free *Khobz Eddar* containing pregelatinized corn had higher specific volume than that containing pregelatinized rice because the corn flour is more viscous than the rice flour.

Similar effects on specific volume of CFBS and RFBS *Khobz Eddar* have been reported with additions of pregelatinized flour. The specific volume for each gluten free bread formula gave a quadratic curve with a downward opening parabola, mainly due to the pregelatinize flour incorporation and not to the hydration which appears to increase linearly the V*sp*. Maxima of both surfaces, indicative of optimal gluten free bread, is near the axial point (+1.41421, 0) correspond to X_1=115 g and X_2=7.05 g of CFBS formula and to X'_1=105 g and X'_2=6.3 g. No great further improvement of a specific volume of CFBS or RFBS *Khobz Eddar* was observed at the highest pregelatinized flour addition.

The specific volume of the samples had increased as water addition increased; this may be related to the starchy nature of gluten-free bread (Schoenlechner et al., 2010). The viscosity of the starch-water mixture also changes during gelatinisation due to swelling of the granules. If the amount of water is insufficient to provide complete swelling and disruption of the starch granules, only part of the crystallinity of the starch granules is lost (Baks et al., 2007). The reason why the gluten free *Khobz Eddar* containing pregelatinized corn or rice had Vsp higher than that with no pregelatinized flour is that the high viscosity induced by pregelatinized rice and corn favor the entrapment of air bubbles in the dough structure, and it is even enough to hold the gas pressure during expansion at the early stage of baking (Shibata et

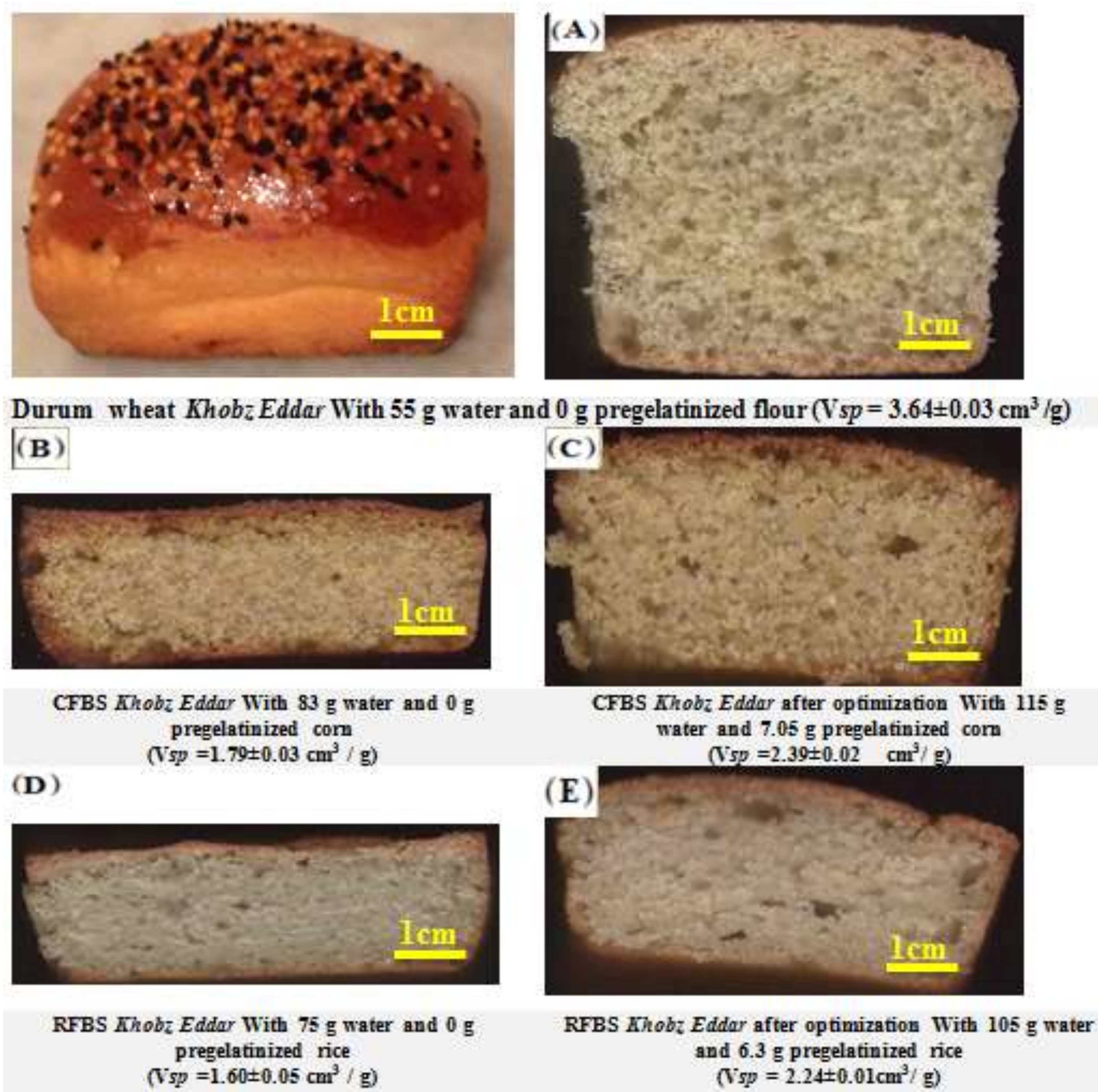

Figure 3. Crumb structure of gluten-free *Khobz Eddar* breads (with corn and with rice) as compared to wheat bread.

al., 2011).

During the bread baking, the starch granules absorb water, swell, and set to form the rigid bulk of the walls that surround the bubbles of carbon dioxide. At the same time, their swollen rigidity stops the expansion of the bubbles (Mcgee, 2007). So in the pregelatinized flour, the starches are already swollen before they were mixed with other ingredients. This means that they do not need to compete with the starch of semolina (corn or rice) to absorb water in the oven.

For samples containing pregelatinized corn or pregelatinized rice, the contour plots of response surfaces are shown in Figures 4 and 5, respectively. By analyzing the contour plots and evaluation of the

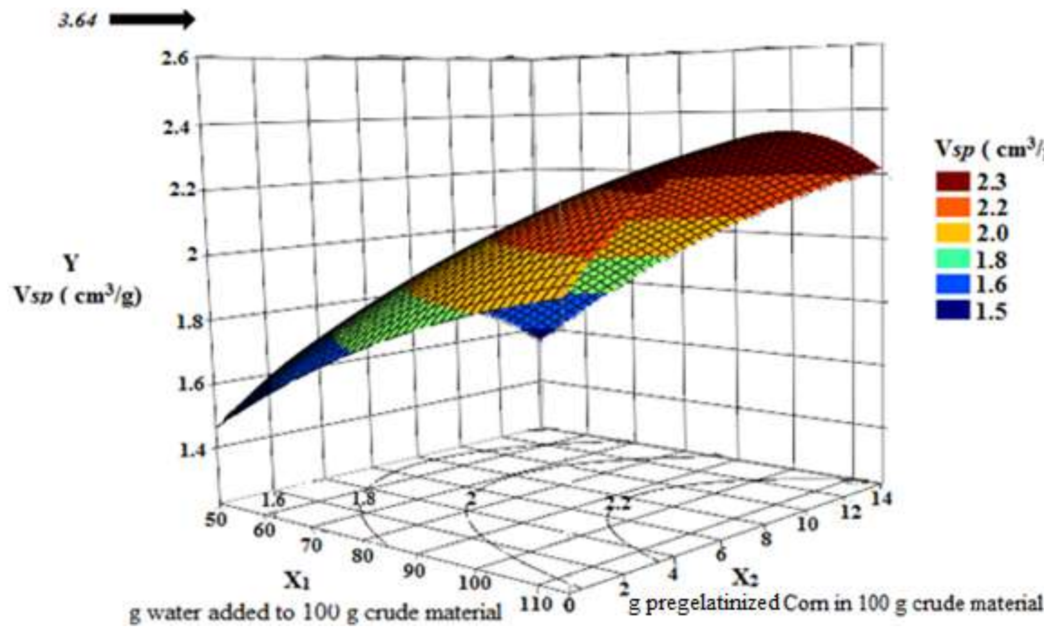

Figure 4. Effect of hydration and pregelatinized corn on V*sp* of CFBS gluten free *Khobz Eddar*. 3.64 cm^3/g: Specific volume (V*sp)* obtained from durum wheat semolina bread.

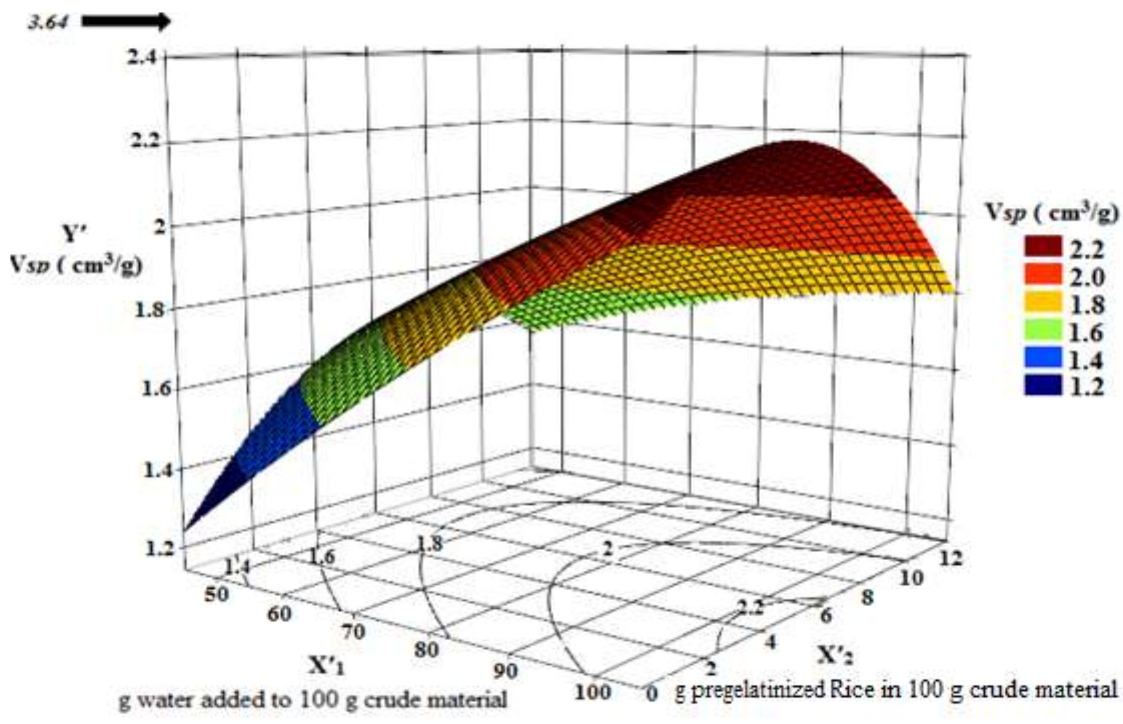

Figure 5. Effect of hydration and pregelatinized rice on specific volume (V*sp)* of RFBS gluten free *Khobz Eddar.*

relationships between response and variable, an optimum formulation of gluten-free traditional *Khobz Eddar* is presented as a bread having an acceptable specific volume. In the current study, it is suggested that the optimum formulation of CFBS bread was $X_{1=}$115 g of water and X_2=7.05 g of pregelatinized flours corn and the optimum formulation of RFBB was $X`_{1=}$105 g of water and $X`_2$=6.3 g of pregelatinized flours rice.

Table 6. Results of breads crumb images analysis (Control, CFBS, and RFBS).

Parameter	Number of cells	Average size	Area fraction (%)	Circularity
Control	112	0.92±2.71	25.95	0.72±0.25
CFBS optimal	278	0.13±0.31	11.30	0.83±0.21
RFBS optimal	241	0.18±0.45	9.63	0.81±0.24

CFBS: Corn/Field Bean Semolina Bread; RFBS: Rice/Field Bean Semolina Bread.

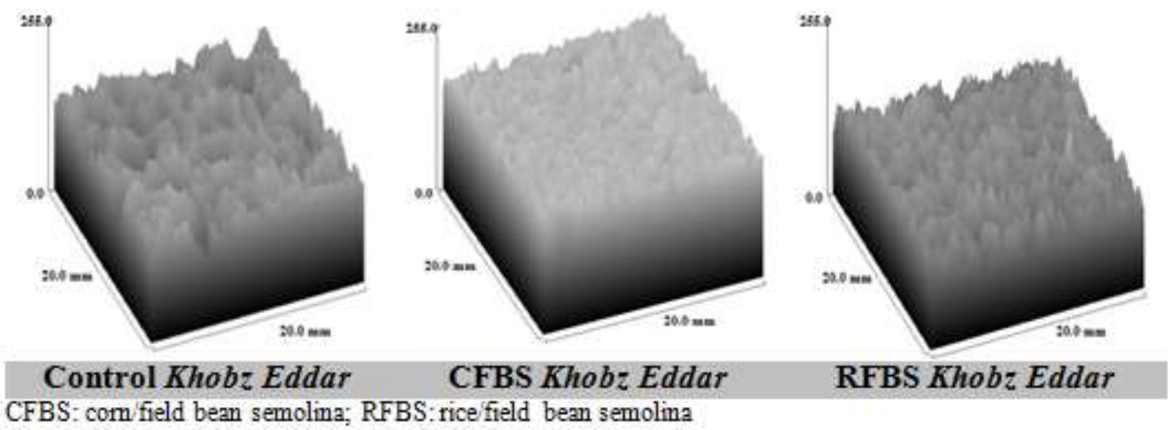

Figure 6. The surface plots of *Khobz Eddar* breads crumb (20 × 20 mm^2). CFBS: Corn/Field bean semolina bread; RFBS: rice/field bean semolina bread.

Image analysis

Specific volume as an important quality parameter was found to have good correlations with longitudinal average size and cross circularity (Pourfarzadv et al., 2012). The number and size of the cells have a remarkable effect on the rheological properties of the dough. The characteristics of air bubbles of *Khobz Eddar* crumbs (control, CFBS optimal and RFBS optimal) obtained from the analysis of scanned images are shown in Table 6 and the surface plots are as shown in Figure 6.

The total number of cells of control *Khobz Eddar* (112) was lower than that of gluten free *Khobz Eddar* CFBS optimal (278) and RFBS optimal (241), whereas the average size and the area fraction of cells were lower than the control bread (Figure 6). So the pregelatinized flours (corn or rice) have a similar effect on the gluten free bread *Khobz Eddar* (CFBS and RFBS).

The addition of pregelatinized flours (corn or rice) facilitated the formation of air bubbles during mixture, but inhibited the growth of these bubbles during fermentation. This might be due to the addition of pregelatinized flour which increased the viscosity of the dough and slow gas diffusion hence the growth of the smaller bubbes were difficult.

The roundness value of CFBS optimal and RFBS of optimal (0.83±0.21 and 0.81±0.24) *KhobzEddar* bread was considerably more than control *Khobz Eddar* (0.72±0.25).

Conclusion

This study confirmed that the pregelatinized flours can be used as natural improver to optimize the specific volume of gluten free *Khobz Eddar* bread, but this specific volume stay lower than *Khobz Eddar* durumone. The air bubbles of both formulas were more in number (278 for corn and 241 for rice) with smaller sizes and more roundness than the control *Khobz Eddar* bread with a number of cells of 112.

The recipe optimized may be given a higher specific volume by replacing the water mixed with pregelatinized flour by milk at the same weight or used the pregelatinized flour in powder form.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Fate of aflatoxins during traditional melon cake and sauce processing

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Melon cake is a non-fermented, traditionally processed food product obtained from melon seeds, and it is consumed as a snack or added to soup. Melon sauce is obtained during the final step of melon cake processing and it is used with melon cake during soup making. This study focused on evaluating the fate of aflatoxin during traditional melon seed processing into melon cake and sauce as a combined product. Shelled melon seeds were purchased from local markets and traditionally processed into melon cake and sauce. Samples were obtained at each processing step and analysed for aflatoxins by LC-MS/MS. Aflatoxin B_1 (AFB_1), AFB_2, AFG_1 and total aflatoxin (sum of AFB_1, AFB_2 and AFG_1) levels in the starting raw material (melon seeds: 39.5, 3.5, 1.97 and 44.9 µg/kg) were reduced by 95, 82, 85 and 94%, respectively, in the finished product (boiled melon cake and sauce) ready for direct consumption. The traditional process of making melon cake and sauce reduced aflatoxin levels below the regulated limits and may lower aflatoxin exposure among melon consumers.

Key words: Aflatoxin, consumer health, food processing, food safety, melon cake, melon sauce.

INTRODUCTION

Melon (C*olocynthis citrullus* L.) seed is a highly nutritious oil seed consumed widely in Nigeria and across West Africa. It contains more than 50% oil and is rich in essential amino acids, vitamins and micronutrients (Akobundu et al., 1982). Melon seeds and their products are very susceptible to infection by aflatoxigenic fungi due to high ambient temperature and relative humidity during storage and hence aflatoxin contamination (Bankole et al., 2004a, b, 2006, 2010; Ezekiel et al., 2016; Somorin et al., 2016), and may possibly increase

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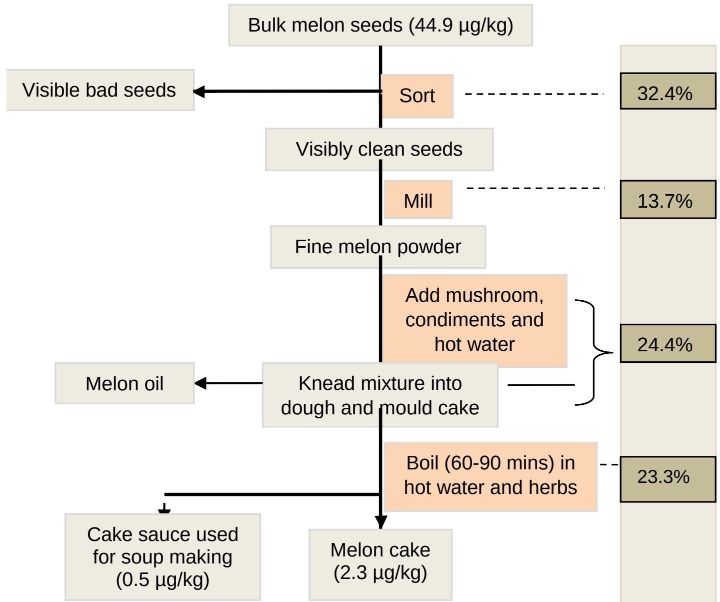

Figure 1. Flow chart for the production of melon cake and sauce with the resulting percentage total aflatoxin reduction. µg/kg = total aflatoxin concentrations in foodstuff: Inputs, processes and products Processing interventions Percentage reduction of total aflatoxins by intervention step.

exposure to aflatoxin among its consumers. Aflatoxin exposure has been associated with liver cancer, immune system suppression and growth retardation in humans (especially children) (Gong et al., 2002, 2012; Turner et al., 2003, 2007). In addition, aflatoxin contamination of melon seed restricts its trade, especially to the European Union where this foodstuff is highly sourced as an alternative oilseed to groundnut. This high demand of melon seeds both locally in Nigeria and internationally, and the aflatoxin menace it faces continues to arouse interest on possible exposure control measures among its consumers. While there are several strategies to control exposure to aflatoxins, traditional processing has been shown to be simple and effective for aflatoxin reduction in some foodstuffs (Karlovsky et al., 2016).

Melon seeds are traditionally processed into several products such as melon ball snack 'robo', 'egusi' soup and the fermented condiment 'ogiri' (Bankole et al., 2010). Melon cake is a non-fermented, traditionally processed product from melon seeds in Nigeria and some parts of West Africa. It is a common snack (Ajuru and Okoli, 2013) for some tribes during traditional meetings and is mainly used as substitute for animal protein sources (e.g. meat and fish) during the preparation of "egusi" soup. The processing scheme for melon cake from shelled melon seeds is shown in Figure 1. Melon sauce is a side product obtained during the final step of melon cake processing and is mainly used for soup making. Traditional food processing (e.g. hand sorting and fermentation) has been shown to significantly reduce mycotoxin (e.g. aflatoxin) levels in maize during processing by up to 96 to 99% and 61 to 83%, respectively (Matumba et al., 2015; Okeke et al., 2015). Roasting groundnut and fermentation of *Aspergillus flavus*-contaminated melon seeds to *ogiri* were shown to reduce aflatoxin levels by about 10 times and 100%, respectively (Afolabi et al., 2015; Ogunsanwo et al., 1989). In spite of the wide consumption of melon cake and the reports of high mycotoxin contamination of melon seeds, there is limited evidence on the effects of traditional processing on mycotoxin levels in melon seed-based foods. As such, this study aimed to investigate the fate of aflatoxins during the processing of melon cake so as to determine whether traditional processing of melon seeds to melon cake and sauce reduces aflatoxin levels.

MATERIALS AND METHODS

Samples

Shelled melon seeds were purchased from local urban markets where this crop is sold in large quantities in Lagos State, Nigeria in September 2014. The bulk melon sample weighed about 1 kg. The sample was traditionally processed into melon cake by a frequent producer and consumer of the product according to Figure 1. Samples (10 g each) for analysis were collected from each of the

Table 1. Aflatoxin levels from raw materials (melon seeds) to their products (melon cake and sauce) and their percentage reduction.

Materials	Concentrations (µg/kg)			
	Aflatoxin B_1	Aflatoxin B_2	Aflatoxin G_1	Total aflatoxin[a]
Melon input[b]	39.5	3.5	1.97	44.9
Mushroom input[c]	–	–	–	–
Cake sauce	0.125[f]	0.2[f]	0.15[f]	0.48
Melon cake	1.74	0.43	0.15[f]	2.32
Melon cake + sauce[d]	1.87	0.63	0.3	2.8
Aflatoxin reduction (%)[e]	95.3	82.0	84.8	93.8

[a]Total aflatoxins: Σaflatoxin B_1 (AFB_1), $AFB_2$and AFG_1; [b]Aflatoxin level in bulk melon/raw material; [c]Aflatoxin level in mushroom; no aflatoxin detected; [d]Sum of aflatoxin levels in melon cake and sauce because of their combined usage in soup preparation; [e]Overall percentage reduction of aflatoxin levels due to processing; [f]Values are LOD/2.

following stages in triplicates: bulk melon seeds, visibly bad (mouldy, discoloured and unhealthy-looking) seeds were removed, fine melon powder obtained from visible clean seeds, mushroom (bought from local market) added to thicken/bind the melon dough during the kneading step, molded melon cake obtained from the kneading process, cake sauce obtained after the boiling process, and the final product (boiled melon cake). The triplicate samples were composited and quartered to obtain representative samples for liquid chromatography tandem mass spectrometric (LC-MS/MS) analysis. All samples were ground to powder and along with cake sauce, were kept at -20°C prior to analysis.

Determination of aflatoxin levels in food samples

Five grams of each representative sample were analyzed for the presence of aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2) by LC-MS/MS. The LC-MS/MS method including extraction and chromatographic separation details is as described by Malachova et al. (2014). Spiking and recovery details are as reported by Ezekiel et al. (2016).

Data analysis

Data analysis was performed using SPSS® 17.0 (Windows version, SPSS, IL, USA). The percentage reduction of toxins from raw material to finished product was calculated.

RESULTS AND DISCUSSION

Aflatoxins B_1, AFB_2 and AFG_1 were the only quantified aflatoxins in samples obtained from each stage during the processing of melon seeds into melon cake (Table 1). AFG_2 was not detected in the melon seeds used in this study unlike previous studies (Ezekiel et al., 2016; Somorin et al., 2016). The concentrations of aflatoxins in the starting material (melon seeds) and processed products as well as the overall reduction in aflatoxin types at the end of processing are given in Table 1. The levels of aflatoxins [AFB_1 (39.5 µg/kg), AFB_2 (3.5 µg/kg) and AFG_1 (1.97 µg/kg) and total aflatoxins (sum of AFB_1, AFB_2 and AFG_1; 44.9 µg/kg)] in raw melon seeds were significantly reduced by the various processing steps applied in the food processing. As indicated in Figure 1, hand sorting of visibly bad seeds reduced the total aflatoxin level in the bulk melon seed by 32.4%, while milling the seeds further reduced the total aflatoxin levels by 13.7%. The findings, which agree with previous reports on the effectiveness of sorting and milling in aflatoxin/mycotoxin reduction in maize (Bullerman and Bianchini, 2007; Matumba et al., 2015), confirm that these two processes are critical first-line measures towards the reduction of aflatoxin exposure during food processing. Perhaps, the substitution of automated sorting techniques equipped with UV/fluorescent sensors for hand sorting, which may be most feasible with up-scaling of this traditional melon seed processing method, may further reduce the toxin content in the starting material.

The absence of aflatoxins in the mushroom samples used during the preparation of melon cake confirms our previous report, which showed no regulated mycotoxins were present in mushrooms (Ezekiel et al., 2013). The addition of mushroom (a thickener) and condiments (for flavour and aroma) followed by a kneading step, which results in extracted oil, yielded a further reduction of 24.4% of the total aflatoxin level in the starting material. The toxin loss is most likely shared between the oil extract (Bordin et al., 2014) which we could not analyse and the additives (condiments). Many condiments (e.g. spices) have shown promise to reduce aflatoxin levels in food materials- e.g. the essential/volatile oils of turmeric were reported to decrease AFB_1 and AFB_2 levels by 99.9 and 99.6%, respectively (Ferreira et al., 2013). A further 23.3% reduction was recorded after the extended cooking (boiling) of the moulded cakes for 60 to 90 min, to leave a final 2.3 and 0.5 µg/kg total aflatoxin levels in the melon cake and cake sauce, respectively. This kind of extended cooking in the presence of liquid which contains spices may have resulted in the toxin decrease; a fact that agrees with the suggestions of Park et al. (2005) and Park and Kim (2006) for moderate reduction of aflatoxins under normal cooking and pressure cooking of rice. Overall and in view of the fact that the cake and sauce are usually combined in the soup making process, the percentage reduction for the aflatoxins influenced by the entire processing method was appreciably high (82 to 95% for AFB_1, AFB_2 and AFG_1; 94% for total aflatoxins) (Table 1). Traditional processing of melon seeds, which had AFB_1 and total aflatoxins levels several times higher than the European Union regulated limits of 2 and 4 µg/kg, respectively, reduced aflatoxins levels below the regulated limits in the final products (melon cake and sauce) (AFB_1 = 1.87 µg/kg; total aflatoxins = 2.8 µg/kg). This suggests that consumption of melon cake and sauce may not pose a high risk of exposure to aflatoxins.

Conclusion

Melon seeds are highly prone to aflatoxin contamination and, this study has shown that traditional processing of melon seeds to melon cake and sauce is able to reduce aflatoxin levels and may consequently reduce aflatoxin exposure among melon seed consumers. In view of the overwhelming toxicological evidence available for aflatoxins in literature and the increasing exposure evident in sub-Saharan Africa, which cannot be attributed only to maize and groundnut (Abia et al., 2013; Ezekiel et al., 2014), it is recommended that aflatoxin mitigation approaches to lower human exposure via consumption of contaminated foods be adopted, from the pre-harvest through post-harvest to the food processing stage.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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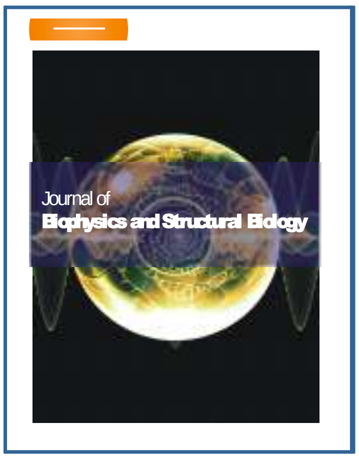

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