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Research Article

Effect of Functional Property and Anti-Nutritional Factor of Kocho- Flaxseed Flat Bread in Ethiopia

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Abstract

Kocho is one of the main energy sources fermented products of Ensete Vetricosum and the most common food in southern Ethiopia. Study was designed to assess functional property and anti-nutritional factor of kocho-flaxseed flat bread. In order to investigate, two factors flaxseed varieties and blending ratio 95:05, 90:10, and 85:15 of kocho to flaxseed flour with control 100% kocho flour using completely randomized design. Result showed the functional properties were observed to have water absorption capacity (4.08, 4.26 and 4.43 ml/g); oil absorption capacity (2.48, 2.58, 2.40 and 2.65 ml/g); solubility values (3.90, 4.20 and 4.06%); Swelling power (5.03, 5.23 and 6.31%,) in blending of kocho with flaxseed varieties of Belay 96, Jeldu and Kulumusa 1 respectively, having no significant difference ($p > 0.05$) between them and better performance from kocho flour WAC (2.52 ml/g), OAC (2.65 ml/g), solubility (1.50 %) and SP (8.88%). Effect of variety and blending ratios on antinutritional content of kocho breads were observed to significant difference ($P < 0.05$) due to flaxseed variety to have tannin and phytic acid content of Belay96 (6.97, 3.10 mg/g), Jeldu (6.44, 3.01 mg/g) and Kulumusa1 (6.66, 3.07 mg/g) respectively. Similarly, interaction between flaxseed variety and blending ratio had a significant effect ($P < 0.05$) on condensed tannin and phytic acid contents of the breads. hence, anti-nutritional factors were decreased as the quantity of flaxseed flour increased.

Keywords: Kocho; Flaxseed; Kocho-flaxseed bread; Functional property; Anti-nutritional factor

Introduction

Kocho are one of the main energy sources fermented products of Enset (*Ensete ventricosum)* species of the separate genus of banana family and the most common traditional food in southern regions of Ethiopia [1]. It is baked as a form of thin brad with the application of heat. Kocho bread is provide a good source for calcium and iron minerals. It is poor in protein and fat contents and substantially improve by blending with nutrient rich food grains such as flaxseed [2]. Enset plant is cut before flowering, scraped the pseudo stem and leaf midribs, squeezed out the pulp (juice) and stored in a suitable pit for fermentation in several months, and finally baking of fermented steam to prepare a flat-bread. Fermented products of the enset plant are used to make different dishes such as thin, unleavened kocho bread (simply called kocho), bulla porridge (*Genfo*), a thick cooked bulla gruel (*Atmit*), and shredded flake made of a mixture of kocho and bulla (*firfir*). Fermentation decreases various nutrient content such as proteins, carbohydrate etc., possibly due to excessive leaching following the peak of microbial activity and a slight decrease of protein content but, at the same time, improved the quality of the lysine protein as determined by amino acid profiles [3]. The length of fermentation time varies from a few weeks or several months depending on ambient temperature of incubation. The chemical composition of kocho changes during the prolonged fermentation time, the titratable acidity increased and anti-nutritional factors such as tannins and trypsin inhibitors are reduced. Flaxseed is the world potential functional food and has excellent source of source of essential nutrients of alpha-linolenic acid, lignans, high quality protein, soluble fiber and phenolic compounds[4-6]. Functional food can be defined as the food or food ingredients that may provide physiological benefits and helps in preventing and/or curing of diseases. Whole grain, oil, milled or ground flaxseed forms are incorporated in to diet [7]. Blending of flaxseed with kocho flour for flat bread production may enhance its use with improved protein, lipid, Omega-3 fatty acid, dietary fiber and antioxidant potential. It can also decrease certain antinutritional factors like phytates and tannins. Anti-nutrients are chemical substances in food that do not offer nourishment to the body. e.g. phytic acid, oxalates, tannins and hydrocyanic acid. The effect of these anti-nutrients in the body depends on the type and the concentration in which it is present in the food material. Phytic acid lowers the utilization of calcium, magnesium, zinc and iron element due to its ability to form insoluble salts with their ions [5]. Tannins are polyhydric phenols of the hydrolysable tannins (readily hydrolyzed into a mixture of carbohydrates and phenols) and the condensed tannins (insoluble complexes with proteins, carbohydrates and lipids) are leading to a reduction in digestibility of these nutrients. It is also damage to the intestinal tract, toxicity of tannins absorbed from the gut, interference with the absorption of iron and a possible carcinogenic effect [8]. The functional properties of food such as Water Absorption Capacity (WAC), Oil Absorption (OAC), Solubility and Swelling Power (Sp) are very important in food processing industry. Water absorption capacity is a function of water holding ability of the starch and has important implication for viscosity as well in bulking and consistency of products as baking applications [9,10]. Similarly, the reverse was the case for oil absorption capacity and impacts flavor and mouth feel to foods. Solubility and swelling power are properties which provide the evidence of the magnitude of interaction among starch chains within the amorphous and crystalline domains, the extent of this interaction being influenced by amylose/amylopectin

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ratio, and by the characteristics of amylose and amylopectin in terms of molecular weight/distribution, degree and length of branching, and conformation. Swelling power is a measure of hydration ability of starch granule and it is determined by weighing the swelled starch and retained water. A direct consequence of starch swelling is the increase of solubility, paste clarity and viscosity properties of interest, especially in food industry [11].Therefore, this study was design to assess the effect of functional property and anti-nutritional factor of kocho- flaxseed flat bread prepared from kocho flour blend with flaxseed variety.

Materials and Method

Experimental Area

The study experiment was conducted in 3 x 3 factorial design with three flaxseed varieties and blending ratio (95:05, 90:10, and 85:15) of kocho to flaxseed flour with control 100% kocho flour using completely randomized design and with three replications. The samples were taken from South West Shoa Wonchii Woreda and three flaxseed varieties from Holleta Agricultural Research Center, Ethiopia. Three flaxseed varieties (Kulumusa1, Belay96 and Jeldu) samples were collected from farms at Holleta site with availability, agroecological (Kolla, weyna Dega, and Dega) respectively. The sample were conducted at Haramaya University in Food Science and technology laboratory for functional property and anti-nutritional factors of kocho-flaxseed bread. The Experimental plan is shown in Table 1.

Table 1: Experimental Plan.

Blending Ratio	**Flaxseed Variety**		**y**
Flax (%)	B	J	K
5	B5	J5	K5
10	B10	J10	K10
15	B15	J15	K15

Where: 5 = 5% flaxseed flour, 10 = 10% flaxseed flour,15 = 15% flaxseed flour, K = Kulumsa 1 flaxseed variety, J = Jeldu flaxseed variety, B= Belay 96 flaxseed variety, B= 100% of the kocho flour (control).

Sample preparation and processing

Preparation of flaxseed kocho bread: Kocho flour containing flaxseed of the desired proportion was mixed with potable water in a 1:1 (w/v) ratio and made into dough by hand kneading until a constant consistency was attained. The dough was immediately baked on a pre-heated baking griddle or plate. The baking time and the temperature of the unleaved bread was about 35 min and 155^{o}C respectively.

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Analysis of Anti-Nutritional Factors

Condensed tannins: Condensed tannin was analyzed by vanillin-HCI method of Price et al. [12] using the modified Vanillin-HCI methanol method. The Vanilli-HCI reagent was prepared by mixing equal volume of 8% concentrated HCI in methanol and 1% Vanilli in methanol. The solution of the reagent was mixed just before use. About 0.2 g of the ground sample was placed in small conical flask. Then 10 ml of 1% concentrated HCI in methanol was added. The conical flask was capped and continuously shaken for 20 min and the content then is centrifuged at 2500 rpm for 5 minutes. About 1 ml of the supernatant was pipette in to a test tube containing 5 ml of Vanillin-HCI reagent. Absorbance at 450 nm was read on spectrophotometer after 20 minutes incubation at 30°C blank sample was also analyzed and its absorbance was subtracted from sample absorbance. A standard curve was also be prepared for catechin (0-1.2 mg/ml) Figure 1.

Tannins content was expressed as catechin equivalent as follows:

$$\text{Tannin}(\%) = \frac{c \times 10 \times 100}{200}$$

Where: C = concentration corresponding to the optical density, 10 = Volume of the extract (ml), 200 = Sample weight (mg).

Phytic acid: Phytic acid was Analyzed through phytate phosphorus (Ph-P) analysis according to Wheeler and Ferrel [13]. About 0.25 g of flour sample was extracted with 12.5 mL of 3% trichloroacetic acid for 45 min in a water bath with vortex mixing at an ambient temperature (23^{o}C) and centrifuged (4000 rpm/10 min). The supernatant was used for phytate estimation. About 4 mL of FeCl3 was added to 10 mL of the sample solution and centrifuged. The clear supernatant was carefully decanted and the precipitate was washed by 20 mL of 3% TCA, 0.2 M of HCl and 20 mL distilled water. The precipitate was digested by concentrated H2SO4 and H2O2 (30%) to converts phosphorus in to phosphate. The phosphate generated was analyzed by measuring the absorbance of phosphomolybdate blue generated on addition of ammonium molybdate [(NH4)6Mo7O24.4H2O]. Stock P solutions were prepared by taking 0.1 mg KH2PO4 in to 250 mL H2O. From the stock solution series of solutions (0, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ppm) was prepared for calibration curve (Figure 2). The absorbance at 822 nm was read using spectrophotometer (6505 uv/vis spectrophotometer, Model 6505, U.K, and GENWAY). The absorbance for sample was subtracted from the blank and phosphorus level was estimated from the calibration curve. Then phytate was estimated from phytate phosphorus (i.e., phytate = P x 3.55). Phytate: mineral molar ratios were calculated using the molecular weight of IP6=660. Phytic acid content was calculated by the following formula:

$$phytic\, acid(mg/g) = \frac{(\frac{\mu g}{mL}) \times 100 \times 3.55}{Sample\, mass(mg)}$$

Where: µg/mL is the concentration of sample used for absorbance reading

Analysis of Functional Property

Water absorption capacity (WAC): To 1 g of the sample, 10 ml of distilled water was added in a 25 ml centrifuge tube and agitated on a vortex mixer for 2 minutes. It was centrifuged at 4000 rpm for 20 minutes. The supernatant was decanted and discarded. The adhering drop of water was removed and the tube reweighed again [14].

$$\text{WAC}(\%) = \frac{\text{(Weight of tube+Sediment)-Weight of empty tube}}{\text{Weight of sample}}$$

Oil absorbing capacity (OAC): To 1 g of the sample, 10ml of vegetable oil was added in a 25 ml centrifuge tube and agitated on a vortex mixer for 2 minutes. It was centrifuged at 4000 rpm for 20 minutes. The supernatant was decanted and discarded. The adhering drops of water were removed and the tube reweighed again [14].

$$\text{OAC}(\%) = \frac{(\text{Weight of tube} + \text{Sediment}) - \text{Weight of empty tube}}{\text{Weight of sample}}$$

Solubility and Swelling Power: Solubility and swelling powers of the samples were determined in triplicate. 1 g of each sample was suspended in 10 ml of deionized water and heated at 90°C for

1 hour in a bath under constant stirrings. Then resulted suspension was cooled to 30°C and centrifuged at 4000 rpm for 15 minutes. The supernatant was decanted into aluminum dishes, and the swollen granule was weighed. The supernatant was dried at 110°C for 12 hours and the weight of dry solids was determined by Anderson et al [15].

The solubility was determined using the following formulas:

$$So(\%) = \frac{\text{Weight of dried supernatant}}{\text{Weight of fresh sample}} \times 100$$

The swelling power was determined using the following formulas:

$$Sp(\%) = \frac{\text{Weight of sediment}}{\text{Weight of fresh sample} \times (100 - So)}$$

Where: so is solubility, SP swelling power

Statistical Analysis

Triplicate data were analyzed using two factors analysis of variance (ANOVA) using SAS (Version 9.1; SAS Institute, Cary, NC, USA). DMRT was used for mean separation at P < 0.05.

Result and Discussion

Anti-nutritional Factors

The anti-nutritional factors of the kocho flour and of the flaxseeds are presented in Table 2. The Tannin content of kocho and those of Belay 96, Jeldu and Kulumusa1 were 11.98, 3.05, 2.77 and 2.39 mg/g, respectively. These values were statistically (P < 0.05) different from each other. Likewise, the phytic acid content of the flaxseed varieties and of the kocho flour were significantly (P < 0.05) different from each other. Kocho flour and flaxseed varieties Belay 96, Jeldu and Kulumusa exhibited phytic acid values of 4.85, 0.70, 0.57 and 0.62 mg/g, respectively. This is in agreement with the work of Tewodros etal [16] who reported the level of phytic acid of flaxseed flour.

Effect of flaxseed Variety and Blending Ratios on Antinutritional Content of Kocho Breads

The data of the tannin and phytic acid contents of the breads are shown in Table 3. Significant (P <0.05) differences were observed in tannin values due to variety with values of 6.97, 6.44 and 6.66 mg/g for breads having variety Belay96, Jeldu and Kulumusa1, respectively. These values are also significantly (p < 0.05) lower than that in the control sample. A decrease in tannin content was observed in the breads with increase in the flaxseed proportion. That was because flaxseeds generally had less tannin than kocho. The tannin content of the three varieties were reduced highly and the reasons for this reduction were processing condition and fermentation process which were known to dramatically reduce the condensed tannin levels of food [17,18]. The phytic acid content of the breads, also shown, differed significantly (P < 0.05) due to flaxseed variety. The values were 3.10, 3.01, and 3.07 mg/g for those substituted with varieties Belay96, Jeldu and Kulumusa1, respectively. The control sample showed higher (3.63 mg/g) value than the composite breads. Increase in proportion of flaxseed resulted in low of phytic acid content. Such phytic acid destruction is nutritionally important since it makes the minerals (Fe and Zn) bio available. Previous studies [19] on 100% grain teff injera also indicated a destruction of 91% to 93% phytic acid.

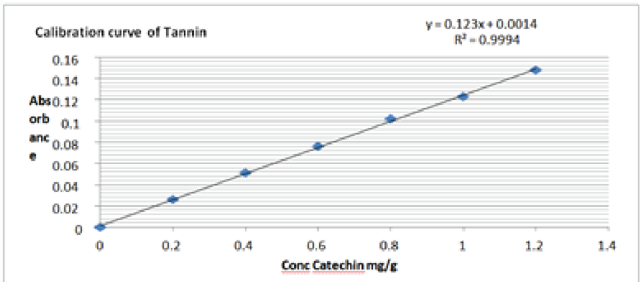

Figure 1: Calibration curve for catechin mg/g.

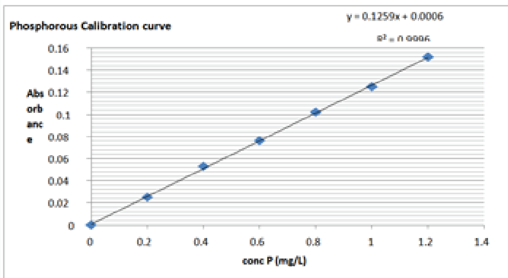

Figure 2: Calibration curve for catechin mg/g.

Functional Properties of Kocho Flour and Flaxseed Flours

Data of functional properties of kocho flour and flaxseed flour are presented in Table 4. The water absorption capacity of the flaxseed powder of Belay 96, Jeldu and Kulumusa 1 were determined to be 4.08, 4.26 and 4.43 ml/g, respectively, having no significant difference (p > 0.05) among them. These values, however, were significantly (p < 0.05) higher than the 2.52 ml/g of kocho flour. The oil absorption capacity of the flaxseed samples and of the kocho flour also exhibited significant differences among each other with value of 2.48, 2.58, 2.40 and 2.65 ml/g for Belay 96 flaxseed, Jeldu flaxseed, Kulumusa flaxseed and Kocho flour, respectively. Values of Jeldu flaxseed (2.58 ml/g) and

Table 2: Anti-nutritional contents of kocho and flaxseed flour.

Sample	Tannin (mg/g)	Phytic acid (mg/g)
B	3.05±0.03b	0.70±0.01b
J	2.77±0.12c	0.57±0.02d
K	2.39±0.01d	0.62±0.01c
Con.	11.98±0.19a	4.85±0.01a
CV	1.98	0.72
LSD	0.19	0.02

CV= Coefficient of Variation, LSD= Least Significance Difference; Con: Kocho flour; B: Belay96 flaxseed variety; J: Jeldu flaxseed variety; K: Kulumusa1 flaxseed variety. Values are Means ± standard error. Means followed by the same letter in a column are not significantly different at p<0.05 level of significance.

Table 3: Effect of main factors (variety and blending ratio) on anti-nutritional content of flaxseed substituted kocho bread.

Factors	Tannin (mg/g)	Phytic acid (mg/g)
Flaxseed variety		
B	6.97±0.13b	3.10±0.09b
J	6.44±0.13d	3.01±0.08d
K	6.66±0.12c	3.07±0.06c
Con.	8.96±0.01a	3.63±0.01a
Blending ratio		
5%	6.84±0.20b	3.18±0.01b
10%	6.69±0.21c	3.03±0.01c
15%	6.54±0.23d	2.97±0.04d
Con.	8.96±0.01a	3.63±0.01a
CV	0.117	0.003
LSD	0.008	0.001

Values are Means± standard deviation Means with different letters after data within a column represents significant differences at P ≤ 0.05. Factor= Effect of variety and blending, CV=Coefficient of Variance LSD=Least Significant Difference. B, J, and K= Belay96, Jeldu and Kulumusa flaxseed varieties, respectively; 05%, 10%, and 15% = flour flaxseed substitution levels, respectively. Con.= control sample (100% kocho bread).

kocho flour (2.65 ml/g) were not different from each other while both are significantly ($p < 0.05$) higher than the 2.48 and 2.40 ml/g of the Belay 96 and Kulumusa flaxseed varieties, respectively. The flaxseed powders had shown water absorption capacity values of twice as much. On the other side all three flaxseed flours had solubility values of 3.90, 4.20 and 4.06% and significantly the same ($P > 0.05$) whereas all three of them are significantly greater than the 1.50% of kocho. The flaxseed flour solubility increased this is might be an increased in lipids had the potential to reduce the starch retro-gradation by amylose-lipid complex formations. The swelling power values of the three-flaxseed powder were also shown in the same table and exhibit statistical difference at $p < 0.05$. The highest value (8.88%) was recorded for kocho sample with flaxseed kulumusa1 showing swelling power of 6.31%, being significantly lower than that of kocho. Flaxseed varieties Belay 96 and Jeldu had values of 5.03 and 5.23%, respectively, and were statistically the lowest. The two had no difference between.

Interaction Effects of Flaxseed Variety and Blending Ratio on Anti nutritional Contents of Kocho Composite Bread

The flaxseed variety and blending ratio interactions had a significant effect ($P < 0.05$) on condensed tannin and phytic acid contents of the breads (Table 5). For the blended products the highest value (7.12 mg/g) was recorded for samples having 5% flaxseed. Belay96 and Kulumusa1 varieties and the lowest (6.29 mg/g) happened to those with 15% flaxseed of Jeldu variety. Similarly, the highest phytic acid (3.22 mg/g) was of breads having only 5% flaxseed of Belay96 variety and the lowest (2.92 mg/g) was of samples having 15% flaxseed of Jeldu variety. All composite breads showed lower phytic acid than the control sample.

Conclusion

The study intended to assessed the functional property and anti-nutritional factor of kocho- flaxseed flat bread prepared from blending of kocho flour with flaxseed varieties. Blending ratio was the most significant factor that had an effect on functional property and anti-nutritional factors of kocho-flaxseed flat bread. The interaction effect of variety and blending ratios showed significant influence on antinutritional content of kocho-flaxseed blended bread. The result obtained in the analysis of anti-nutritional content is reduced and rising the blending 15% flaxseed variety will have improved nutritional as well as functional property. Therefore, consumption of flaxseed variety blending with kocho flour in bread production will have complement dietary content.

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Table 4: Functional properties of kocho and flaxseed flours.

Sample	WAC (ml/g)	OAC (ml/g)	Solubility (%)	SP (%)
B	4.08±0.28a	2.48±0.05bc	3.90±0.10a	5.03±0.43c
J	4.26±0.18a	2.58±0.15ab	4.20±0.17a	5.23±0.02c
K	4.43±0.20a	2.40±0.01c	4.06±0.20a	6.31±0.39b
Con.	2.52±0.04b	2.65±0.07a	1.50±0.5b	8.88±0.29a
CV	5.17	3.41	8.44	5.14
LSD	0.37	0.16	0.54	0.61

Values are Means± standard deviation with different letters after data within a column represents differences at $P \leq 0.05$. CV= Coefficient of Variation; LSD = Least Significance Difference; Con = kocho flour; B = Belay96 flaxseed flour; J = Jeldu variety flaxseed flour; K = Kulumsa1 variety flaxseed flour; WAC = Water Absorption Capacity; OAC = Oil Absorption Capacity; SP = swelling Power.

Table 5: Interaction effects of flaxseed (variety and blending ratio) on anti-nutritional content of kocho bread.

Treatment	Condensed Tannin (mg/g)	Phytic Acid (mg/g)
B*05%	7.12±0.01b	3.22±0.01b
B*10%	6.97±0.01c	3.07±0.01e
B*15%	6.82±0.01d	3.02±0.01g
J*05%	6.59±0.01f	3.12±0.01d
J*10%	6.44±0.01h	2.97±0.01i
J*15%	6.29±0.02i	2.92±0.01j
K*05%	6.81±0.01d	3.18±0.01c
K*10%	6.66±0.01e	3.03±0.01f
K*15%	6.51±0.01g	2.98±0.01h
Con.	8.96±0.02a	3.63±0.01a
CV	0.081	0.003
LSD	0.009	0.001

Values are Means± standard deviation with different letters after data within a column represents differences at $P \leq 0.05$. Treatment=Interaction of variety and blending CV=Coefficient of Variance LSD=Least Significant Difference, B, J, and K= Belay96, Jeldu and Kulumusa1 flaxseed varieties, respectively; 05%, 10%, and 15% = flour flaxseed substitution levels, respectively Con.=control sample (100% kocho bread).

References

1. Yirmaga MT. Improving the Indigenous Processing of Kocho, an Ethiopian Traditional Fermented Food. J Nutr Food Sci. 2013;3(1).
2. Daba T, Shigeta M. Enset (Ensete Ventricosum) Production in Ethiopia: Its Nutritional and Socio-Cultural Values. Agric Food Sci Res. 2016;3(2):66-74.
3. Andeta AF. Fermentation of enset (Ensete ventricosum) in the Gamo highlands of Ethiopia: Physicochemical and microbial community dynamics. Food Microbiol. 2018;73:342-50.
4. Goyal A, Sharma V, Upadhyay N, Gill S, Sihag M. Flax and flaxseed oil: an ancient medicine & modern functional food. J Food Sci Technol. 2014;51(9):1633-53.
5. Kajla P, Sharma A, Sood DR. Flaxseed—a potential functional food source. J Food Sci Technol. 2015;52(4):1857-71.
6. Al-Dmoor HM. Flat bread: Ingredients and fortification. Quality Assurance Safety Crops Foods. 2012;4:2-8.
7. Tarpila S. The effect of flaxseed supplementation in processed foods on serum fatty acids and enterolactone. Eur J Clin Nutr. 2002;56(2):157-65.
8. Acid P, Mohan VR. Antinutritional Factors Antinutritional Factors in Legume Seeds : Characteristics and Determina- tion Reutilization of cereal processing by-products in bread making Usage of Tomato (Lycopersicum escu- lentum Mill .) Seeds Health. 2016.
9. Julianti E, Rusmarilin H, Ridwansyah, Yusraini E. Functional and rheological properties of composite flour from sweet potato, maize, soybean and xanthan gum. J Saudi Soc Agric Sci. 2017;16:171-7.
10. Hadnađev TD, Torbica A, Hadnađev M. Rheological properties of wheat flour substitutes/alternative crops assessed by Mixolab. Procedia Food Sci. 2011;1:328-34.
11. Guo L, Wang S, Zhu C, Hu J, Zhang J, Du X. The Impacts of the Entanglement Concentration on the Hydrodynamic Properties of Kudzu and Lotus Rhizome Starch Aqueous Solutions. J Food Nutr Res. 2016;4:750-9.
12. Price ML, Van Scoyoc S, Butler LG. A Critical Evaluation of the Vanillin Reaction as an Assay for Tannin in Sorghum Grain. J Agric Food Chem. 1978.
13. Wheeler E, Ferrel ER. A method for Phytic Acid Determination in Wheat and Wheat Fractions. Cereal Chem. 1971.

14. Mepba HD, Akpapunam MA, Berepubo NA. Production of non-fermented beverage from ripe banana: chemical content and sensory characteristics. Discov Innov. 1990.

15. Anderson RA, Conway HF, Peplinski AJ. Gelatinization of Corn Grits by Roll Cooking, Extrusion Cooking and Steaming. Starch. 1970.

16. Girma T, Bultosa G, Bussa N. Effect of Grain Tef (Eragrostis tef (Zucc.) Trotter) Flour Substitutions with Flaxseed on Mineral Content, Antioxidant Activity, Phytic Acid Content and Microbial Quality of Injera. Sci Technol Arts Res J. 2013;2:51.

17. Athanasiadou S, Kyriazakis I, Jackson F, RL Coop. The effects of condensed tannins supplementation of foods with different protein content on parasitism, food intake and performance of sheep infected with Trichostrongylus colubriformis. Br J Nutr. 2001;86(6):697-706.

18. Soares S, Mateus N, De Freitas V. Effect of Condensed Tannins Addition on the Astringency of Red Wines. Chem Senses. 2012;37(2):191-8.

19. Biliaderis CG. The Structure and Interactions of Starch with Food Constituents. Canadian J Physiology Pharmacology. 1991;69(1):60-78.