Physicochemical, Sensory and Antioxidant Characteristics of Biscuits made from cooked and germinated lima bean (Phaseolus lunatus) flour.

[1*]Adebayo SF, [2]Okoli EC and [2]Abraham FM

[1]Department of Food Technology, Federal Polytechnic, Ado-Ekiti, Ekiti State Nigeria

[2]Department of Food Science and Technology, Ebonyi State University, Abakaliki, Nigeria

Abstract

The work studied the effect of cooking and germination on the proximate, sensory and antioxidant activities of biscuits baked with composite flour of wheat and lima bean seeds. Germination was done in a controlled dark chamber (100 % humidity and atmospheric temperature), both cooked and germinated sample was dry milled and sieved for baking at 180oC for 20-25 mins and cooled. The result revealed that the crude protein, ash, and fibre content of the germinated sampled biscuits were significantly ($p<0.05$) higher than the cooked samples.Germination increased the phenol, flavonoids, hydroxyl radical, and total antioxidants significantly ($p<0.05$) in the biscuits produced with a germinated samples than the cooked sample. The biscuits produced with a germinated lima bean seed gave higher values of phenol (0.617- 0.788 mgGAE/g); flavonoids (0.575- 1.44 mgRE/100g); DPPH (13.12 to 28.66 µg/ml); hydroxyl radical (59.66- 76.62µg/ml) and FRAP from 0.754- 1.553 Fe2+/mg.While biscuits produced with the cooked sample has phenol content as (0.493- 0.610mgGAE/g); flavonoids (0.364 – 1.279 mgRE/100g); DPPH (7.86 -15.90µg/ml); hydroxyl radical (51.28- 76.42µg/ml) and FRAP (0.717- 1.122 Fe2+/mg). Sensory analysis reveals overall acceptability at 20% substitutionas adequate comparablein terms of crispness, color, texture and taste. Biscuits from lima bean seeds have phytochemicals with potential antioxidant activities for disease prevention.

Keywords: Phaseolus lunatus; Phenolic compounds; Antioxidant; Functional food; Phytochemicals; biscuits.

Introduction

Biscuits are ready to eat cheap and a covenant food product that is consumed among all age groups in many countries. Biscuit belongs to the flour confectionary [1], which could be flat crisp and may be sweetened or unsweetened according to preferences. Biscuits can be made from hard dough, hard sweet dough, or short or soft dough. It is produced by mixing various ingredients like when flour, fat sweetener, and water to form a dough. The dough is formed unlike bread is not allowed to ferment, and then it is baked in the oven [2]. Biscuit is regarded as a form of confectionery dried to very low mixture content. Biscuit is defined as a small thin crisp cake made from unleavened dough and can also be described as a mixture of flour and water together into dough which is rested for a period and then passed between rollers to make a sheet, cut in to shape based on specification. The dough which includes Maries and morning coffee and other types of biscuits may be cream crackers, soda crackers, savory, water biscuit, and digestive and short dough biscuits [3]. There has been a tremendous increase in the consumption of wheat-based products especially biscuits and bread [4] in many parts sub-Sahara Africa, especially in Nigeria. Biscuits are nutritious contributing valuable quantities of iron, calcium, calories, fibre, and some of the b-vitamin to our diet and daily food requirement. Composite flour has the added advantages of improving the nutrient of biscuits and other bakery products especially when cereals are blended with legumes such as Bambara groundnut [5]. The use of plain wheat flour is common today and other ingredients like a groundnut; ground rice and corn-flour are sometimes added to alter the texture [6]. Legumes have been shown to occupy an important place in human nutrition, as it provides a rich source of dietary proteins in Africa and subcontinent and many other developing countries. Most of the legume grains therefore synthesize certain biologically

*Correspondence to: Adebayo SF, Department of Food Technology, Federal Polytechnic, Ado-Ekiti, Ekiti State Nigeria, E-mail: adebayo.stella@yahoo.com

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active substances which are commonly considered as anti-nutritional since they reduce the nutrient utilization and/or food intake of plants or plant products used as human food or animal feed and they play a vital role in determining the use of plants for humans and animals [7]. Besides, legumes contain a rich variety of phytochemicals such as phytosterols, natural antioxidants, and bioactive carbohydrates [8, 9]. Epidemiological and intervention studies indicate that legume consumption is inversely associated with the risk of coronary heart disease [10], type II diabetes mellitus [11], and obesity [12]. Consumption of legumes also results in lower LDL cholesterol and higher HDL cholesterol [13, 14]. Legumes are generally consumed after processing into various products like milling into flour, and used as puffing or roasting into snack foods, grinding into flour for different food preparations [15] or as germinated grounded as used for fufu production.

Lima beans (Phaseolus lunatus) bean sometimes called butter beans, Chad beans and Pakala by the Yoruba tribe of Nigeria are flat- shaped creamy white- colored beans with the potential for good yields in the tropics. Lima It is widely available, thrives in lowland tropical rain forests, and on poor soils where most crops cannot grow well.Lima bean has a crude protein content of about 22% and yields between 3000kg and 5000kg of seeds per hectare would contribute tremendously to the protein intake of the consumers at a low cost to reduce the cost of importation on wheat thereby encouraging the use of our local raw materials. The objective ofthis work is to evaluate the antioxidant potential of biscuit from germinated and cooked lima bean seed flour.

Materials and methods

Sample Preparation

The brown variety of lima bean seed (Phaseolus lunatus) used for this work was obtained from the kings market at Ikere-Ekiti, Ondo State Nigeria. Wheat flour and other ingredients like eggs, vegetable oil, sugar, salt, baking powder, were obtained from the same source. Wholesome lima bean that has been sorted to remove dirt, stone, the shaft was winnowed and were divided into three portions, a part was washed and cooked for about 2hours by normal cooking method until soft. The cooked soft sample was cooled and oven- dried at 50oC for 4hours, cooled, milled, and passes through a 100µm mesh sieve. The raw seed was dried milled and screened. The portion for germination was soaked in 0.1% sodium hypochlorite according to, for 25minutes to prevent mold growth. The seeds were soaked in water after thorough washing for 17 hrs and germinated for 120 hrs by spreading hydrated seeds on jute bags and allowed to germinate at 120hrs. The germinated seed with rootlets and coat were washed and dried at 50oC milled and sieved with 100µm mesh screen, stored in a polyethylene bag for the next analysis.

Biscuit production

Composite biscuit was produce using wheat flour, cooked and germinated lima bean flour at variousproportions of 20:80, 30:70 and 50:50, 100% for the cooked, germinated, and wheat flour as the standard.The ingredients used were wheat flour, sugar, salt, fat, baking powder and flavorant in the proportion as indicated in Table 1. Fat and sugar were mixed in colander mixer until fluffy; other ingredients were added and mixed thoroughly with the cream mixture to form a dough, the dough was hence rolled and cut into a flat shape of 5cm in diameter and baked in the oven at 180oC for 20-25 min. Biscuits were cooled and packaged in polyethylene for the next analysis and biscuit produced from wheat serves as the control.

Wheat	**Lima bean cooked**	**Lima bean germinated**	**Fat**	**Salt**	**Sugar**	**Baking powder**	**Flavourant**	**Codes**
100%	0	0	45	0.6	55	3.5	1	421
0	100%	0	45	0.6	55	3.5	1	672
0	0	100%	45	0.6	55	3.5	1	109
80	20	0	45	0.6	55	3.5	1	280
70	30	0	45	0.6	55	3.5	1	908
50	50	0	45	0.6	55	3.5	1	531
80	0	20	45	0.6	55	3.5	1	211
70	0	30	45	0.6	55	3.5	1	417
50	0	50	45	0.6	55	3.5	1	216

Table 1: Proportion of ingredients use for Lima biscuit

Chemical analysis of biscuit

The proximate composition (moisture, crude protein, crude fat, crude fibre, ash, and carbohydrate) were determined by standard methods as described by [16].

Sensory evaluation of biscuits

Evaluation of the biscuits was carried out by 20 semi-trained panelists recruited from the students of the Department of Food Technology, Federal Polytechnic, Ado–Ekiti. According to, a randomized complete block design was used; each panelist evaluated all the presented samples. The samples were coded and presented in a random sequence to each panelist, six sensory attributes were evaluated (crispness, aroma, taste, color, texture and overall acceptability) using a nine-point hedonic scale for each trait where 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much and 1 = dislike extremely.The evaluation was concluded in one session. The panelists were instructed to rinse their hands with water after every sample not to make comments during evaluation to prevent undue influence on other panelist. Comments were to be freely given about the sample on the given questionnaires and the observations were converted to an equivalent numerical score.

Determination of total polyphenol

The total phenol content of the sample was determined using the method of. The sample (50 µL) was put in test tubes and the volume made up to 500 µL using distilled water. Then, 250 µL of Folin-Ciocalteu reagent was added into the test tube followed by 1.25 ml of 20 % sodium carbonate solution. The tube was vortexed before incubated in the dark for 40 minutes. Absorbanceread at 725 nm using a spectrophotometer.

Determination of total flavonoids

Aluminum chloride colorimetric method was used for flavonoids determination. Each biscuit extracts (0.5 ml of 1:10 g/ml) in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10 % aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It then remains at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 g ml-1 in methanol [17].

Determination of DPPH

DPPH scavenging activity was carried out by the method of [20]. 250 µg/ml of the germinated lima bean flour extract with methanol was dissolved in DMSO (dimethyl sulfoxide) and taken in test tubes in triplicates. Then 5 ml of 0.1 mM ethanol solution of DPPH (1,1, Diphenyl-2-picrylhydrazyl) was added to each of the test tubes and was shaken vigorously. It was allowed to stand at 35oC for 20 minutes. The control was prepared without any extracts. Methanol was be used as base-line corrections in absorbance (OD) of the sample and measured at 517 nm. A radical scavenging activity was expressed as a 1 % scavenging activity and calculated by the following formula.

DPPH radical scavenging activity (%) = [(As-Ao)/As] x 100

Determination of metal ion chelation

The metal chelating activity was measured using a slightly modified version of a previous method.Lima bean sample solution or GSH (final concentration of 1 mg/mL) was combined with 0.05 mL of FeCl2 (2 mM) and 1.85 mL distilled water in a reaction tube. Thereafter, 0.1 mL of 5 mM Ferrozine [3-(2-pyridyl)-5, 6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid sodium salt] solution was added and mixed thoroughly. The mixture was allowed to stand at room temperature for 10 min followed by the removal of 200 µL aliquot of the reaction mixture and added to a clear bottom 96-well plate. The control experiment contained all the reaction mixtures except that distilled water was used to replace the peptide. The absorbance of the sample (As) and control (Ac) was measured using a spectrophotometer at 562 nm and the metal chelating activity of the sample was compared to that of GSH. The percentage chelating effect (%) was calculated using the following equation:

Metal chelating effect (%) = (Ac-As/Ac) ×100

Determination of hydroxyl radical

The hydroxyl radical scavenging assay was modified based on a method described by. Lima bean sample, GSH, and 1,10-phenanthroline (3 mM) were each separately dissolved in 0.1 M sodium phosphate buffer (pH 7.4) while FeSO4 (3 mM) and 0.01% hydrogen peroxide were each separately dissolved in distilled water [18]. An aliquot (50 µL) of the sample, fractions or GSH (equivalent to a final assay concentration of 1 mg/mL) or buffer (control) was first added to a clear, flat- bottom 96-well plate followed by additions of 50 µL of 1, 10-phenanthroline and 50 µL of FeSO4. To initiate a reaction in the wells, 50 µL of hydrogen peroxide (H2O2) solution was added to the mixture, which was then covered and incubated at 37 °C for 1 h with shaking. Thereafter, the absorbance of the mixtures was measured at 536 nm every 10 min for a period of 1 h. The absorbance was also determined for a blank (does not contain peptides or H2O2) and a control (does not contain peptides). The OH• scavenging activity was calculated as shown and described by, below.

OH*scavenging ability (%) = (Absref - Abssam)/Absref ×100

Determination of FRAP

Ferric reducing antioxidant power of the sample extracts was determined as described by. This method was based on the ability of the sample to reduce Fe+3 to Fe+2 ions. FRAP reagent (900 µl), was prepared freshly and incubated at 37 oC, it was mixed with 90 µl of distilled water and 30 µl of seed extract or methanol (for the reagent blank). The seed extracts and reagent blank was incubated at 37 oC for 30 min in a water bath. The final dilution of the test sample in the reaction mixture might be 1/34. The FRAP reagent contained 2.5 ml at 20 mmol/l2,4,6-tripyridyl-triazine (TPTZ) solution in 40 mmol/l HCl plus 2.5 ml of 0.3 mol/l acetate buffer (pH 3.6). After incubation for 6 min at room temperature, reduction of TPTZ to the ferrous complex a blue colour which was measured at a wavelength of 593 nm. FeSO4 was used as a standard.

FRAP = (Ab sample×conc std)/(Ab std×conc sample) (mgAAE/g)

Determination of ABTS

The total antioxidant capacity was determined based on 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonate radical (ABTS•+) scavenging ability of the extracts according to the method described by [24]. ABTS•+ was generated by reacting ABTS aqueous solution (7 mM) with K2S2O8 (2.45 mM, final concentration) in the dark for 16 h and adjusting the absorbance at 734 nm to 0.700 with ethanol. 0.2 ml of appropriate dilution of the extracts was added to 2.0 ml ABTS•+ solution and the absorbance was measured at 734 nm after 15 min. The Trolox equivalent antioxidant capacity (TEAC) was subsequently calculated using Trolox as the standard.

% scavenging ability = (Absref – Abssam)/Absref×1000 (mmol.TEAC/g)

Statistical analysis

All results were expressed as mean ± standard deviation. Analysis of variance (ANOVA) was carried out on data for chemical, sensory and antioxidant followed by Duncan's Multiple Range Test. A p-value below 0.05 was considered significant.

Result and Discussion

The nutritional evaluation of the biscuits was shown in Table 2. The result showed low value 1.31 to 3.94 % for moisture content, indicating stability and shelf life elongation. The protein content was 9.58 to 17.22 %, the highest value recorded in 100% germinated lima bean biscuits and the least value recorded for 20 % substituted with cooked lima bean flour (9.58 %), suggestingthat the biscuits could be of value where protein deficiency is required and in malnourished children. The protein content of the biscuit sample baked with the germinated lima bean is significantly ($p<0.05$) higher than the cooked samples and biscuits baked with wheat flour. The increase in protein content due to the germination of the seed, suggesting that the enzyme has been synthesized during the germination of the seedlings. The decrease in the carbohydrates as observed might be due to an increase in α-amylase activity during germination. This is in agreements with the report of who observed a reduction in the carbohydrate content of African yam bean after germination.The proportion of fat range 13.83 to 19.30 %, the increase might be due to the composition of the baking fat.The mean value for the scores as rated by the consumers revealed that 100% cooked and germinated lima biscuits were brittle and the cooked sample dark in color. Biscuits from wheat flour were crispy and not significantly ($p<0.05$) different with sample substituted at 30 % with germinated lima bean. Samples coded as 50C/50W were less crispy, with coarse texture and very brittle; although tasty but dark in color. Substitution at 20% and 30% show a brighter color in all the biscuits. The astringency and bitterness were fairly reduced in the substituted biscuits but higher in 100% cooked and germinated biscuits respectively. The Addition of germinated lima bean flour up to 20% substitution with wheat did not significantly show a difference from 100% wheat flour which has the highest acceptability. Panelist described biscuits containing 100% cooked and germinated as having a bitter taste and lacking the characteristics crispness and texture of biscuits. However, substitution with 20% lima bean flour was rated as more acceptable. Functional foods and nutritional supplements eliminate certain risks and have preventive effects neutralizing free radicals in preventing certain diseases. Phenolic compounds contribute to the overall antioxidant activities of the plant food; this is shown in this study by an increase in the total phenolic content (TCP) of the extract of biscuits produced from cooked and germinated lima beans, The biscuits produced from the use of cooked and germinated lima bean seeds show a considerable high value of total polyphenol a range of 0.493 to 0.788mgGAE/g; higher values recorded in the germinated samples as shown in graph A. These results show that germination has modifies the quantity and quality of the phenolic compounds in the seed of the legumes, the same trend of increase observed in the 100% germinated Lima biscuits higher than other samples (100% cooked) and wheat.Lipid peroxidation is a common consequence of oxidative stress, Flavonoids act to protect lipids against oxidative damage by various mechanisms has been reported. Therefore the mechanisms of action of flavonoids are through free-radical scavenging or chelating process and protection against oxidation and inhibition of free radical generation [28]. The flavonoid contents of the extract of the biscuit produced from germinated /cooked lima flour ranged 0.364 to 1.720mgRE/100g, highest the in sample(50G/50W) and sample 280 (20C/80W), reflection of highest value shown in the germinated biscuit samples than in cooked respectively. Radical scavenging activity of agricultural produce has been surveyed using DPPH assay which is measured by their ability to quench the DPPH radical as depicted in graph 3.

The result showed that biscuits baked with germinated lima bean had higher DPPH free radical scavenging ability (13.12 to 28.66 µg/ml) than the cooked sample (7.86 to 15.90 µg/ml). The higher value of DPPH radical scavenging could be due to hydroxyl groups existing in the phenolic compounds chemical structure.FRAP assay measures the antioxidant effect of any substance in the reaction medium and the method is based on the ability of the sample to reduce Fe3+ to Fe2+. The reducing power of the biscuits baked with germinated Lima beans was found to be higher than of the biscuits from cooked and wheat flour samples. This means that the presence of higher antioxidants in the germinated sample caused a higher reducing power compared with the cooked sample and wheat sample respectively graph 4. This result is in agreement with the works of that discover higher antioxidant levels in germinated African yam bean than the un-germinated African yam bean seed.The data on the metal ion chelating effects of the biscuits extracts as presented in Table 4 indicates that germinated produced biscuits at 20 % substitution showed the highest level of chelating activity 98.36 µg/ml, the value significantly decreased, wheat (82.56µg/ml) and in cooked biscuits (88.88µg/ml) respectively. Furthermore, the extract of biscuit showed a significant Fe2+chelating effect and could afford protection against oxidative damage. The total antioxidant activity of the biscuit extract as presented in Table 4 indicates that the germinated samples have scavenging activity than the cooked and sample from wheat. Free radicals (ABTS) scavenging activity of the studied biscuits might be due to the presence of phenolic compounds. Furthermore the binding between phenolics and protein matrix might account for the enhancement of antioxidant capacity since phenolics protein interaction can stabilize protein further increase its antioxidant capacity during germination Hagerman et al., 1998. The hydroxyl radical respectively were higher in the germinated flour sample of lima bean seed biscuits than in the cooked. Several lines of research have shown the involvement of free radicals in many disorders like neurodegenerative diseases, cancers. This shows that the biscuits produced with the germinated lima bean have greater antioxidant potential than the biscuits from cooked sample flour which could make the biscuit baked from germinated flour of lima beans of more health benefit serving as a protector against degenerative diseases. This study has shown that chemical analysis, sensory parameters, and antioxidant quality parameters of lima bean seed flour have been improved by germination processes, Therefore the use of germinated sample flour from lima beans will be of a health benefit than cooking of the sample.

Blends of flour	Moisture content (%)	Fat content (%)	Ash content (%)	Crude Fibre (%)	Protein content (%)	Carbohydrate (%)
Cooked	2.19 ± 0.19c	13.83 ± 0.64d	2.56 ± 0.57a	3.45 ± 0.20 a	12.27 ± 0.19 a	65.70 ± 1.23ab
Germinated	2.73 ± 0.38b	17.04 ± 0.18b	3.71 ± 460a	1.99 ± 0.19 b	17.22 ± 0.03 e	57.31 ± 4.71ab
50C/50W	2.16 ± 0.17c	12.76 ± 0.27e	0.94 ± 0.05a	0.40 ± 0.20 f	11.30 ± 0.10f	72.44 ± 0.28 a
50G/50W	3.94 ± 0.56a	19.30 ± 0.26a	1.29 ± 0.56a	0.58 ± 0.02 f	12.32 ± 0.08 e	62.57 ± 0.69ab
30G/70W	2.62 ± 0.33bc	16.77 ± 0.37b	1.91 ± 0.02a	1.45 ±0.11cd	13.24 ± 0.14c	64.01 ± 1.61 ab
30C/70W	1.31 ± 0.01d	15.24 ± 0.01c	2.20 ± 0.54a	1.25 ±0.12de	12.73 ± 0.07d	67.27 ± 0.53 ab
20C/80W	1.54 ± 0.57d	14.94 ± 0.01c	2.91 ± 0.00a	3.64 ± 0.11a	9.58 ± 0.07g	67.39 ± 0.55 ab
20G/80W	3.85 ±0.20a	16.51 ± 0.47b	1.92 ± 0.00a	1.69 ± 0.10c	12.61 ± 0.20d	63.42 ± 19.05 b
Wheat flour	1.42 ± 1.68d	15.26 ± 0.54c	0.97 ± 0.01a	0.41 ± 0.19f	14.67 ± 0.06b	67.27 ± 0.47ab

Table 2: Chemical composition of biscuits produced from cooked and germinated lima bean flour

Conclusion

Based on the findings obtained in this study, the germination process caused an increase in phenol, flavonoid contents as well as an increase in the antioxidant properties of composite biscuits baked with germinated lima bean than the cooked sampled lima bean seed.

Acknowledgements

None

Conflict of Interest

None

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