COMPARATIVE STUDIES ON THE MINERALS, AMINO ACIDS AND BIOACTIVE COMPONENTS OF SOME *PHASEOLUS LUNATUS* VARIETIES WHOLE SEEDS FLOURS

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Abstract

Whole seeds flour from some varieties of Phaseolus lunatus with accession number NSWP100, NSWP98, NSWP47 and NSWP96 were subjected to standard analytical techniques to evaluate mineral, amino acids and phenolic contents. The results showed a high content of potassium (70.63-374.11 mg/100g); sodium (15.47- 88.46 mg/100g); calcium (14.32-19.85 mg/100g); magnesium (35.81- 70.84 mg/100g) and iron (10.86- 16.90 mg/100g). The Na/K ratio of less than one (0.21- 0.28) was obtained in the varieties. Amino acids profiling showed high content of essential amino acids like lysine (7.21-7.24 g/100g protein); leucine (8.16-8.31 g/100g protein) and phenylalanine (5.61-6.18 g/100g protein) in comparison with FAO/WHO standard. HPLC-DAD result showed that caffeic acid (1.38-3.43 g/100g); ellagic acid (2.69-3.46 g/100g); rutin (1.28-2.53 g/100g); quercetin (1.26-1.59 g/100g) and tocopherol (1.37-1.76 g/100g) are the active polyphenols in the flours. The components underline the potential applications of the flours in food formulation to promote good health in infants and adults.

Keywords: *Phaseolus lunatus*, nutrition, amino acids, HPLC-DAD, phenolics.

IJAFS 2022 (6).12:1782 - 1790
Accepted for Publication
May, 2024

INTRODUCTION

The prevalence of dreadful protein-malnutrition during formative years in children, as a result of low or inadequate quality proteins is a common phenomenon in some African and Asian countries (Stefano *et al.*, 2018). In Nigeria, the menace is of great concern, due to insurgency and other natural phenomenal which has been a major obstacle to food security (Araoye *et al.*, 2023. There have been awareness and sensitization on plant proteins due to the safety concerns of animal products; which has led to the increase in plants protein consumption; hence the quest for new sources of proteins from unexploited legume seeds has increased tremendously over the years (Kouakou *et al*., 2022). Most unconventional seeds legume is capable of preventing notable types of malnutrition (protein malnutrition and hidden hunger); could also help to boost body immunity in this post covid-19 era. The choice of plants constituents is growing in many developed and developing countries alike due to the demand for foods that promote good health (Aguilera *et al.,* 2011). The non-conventional legume seeds components have used in developing countries to ameliorate the menace of food and nutritional insecurity (Stefano *et al.,* 2018). It is important to explore accessible underutilized sources of protein to feed the increasing underfed and malnourished population, especially in third world countries where larger part of the populace live below poverty line.

Plant phenolics, which are generally divided into phenolic acids and flavonoids in either free or conjugated forms; are widely distributed in grain legumes and noted for antioxidant activity (Yunusa *et al.,* 2018). Several literature evidence have suggested that consumption of foods rich in phenolics with antioxidant properties can reduce oxidative stress, as well as improve antioxidant status of the body (Li *et al.,* 2012; Kruk *et al.,* 2022). Attention has now been shifted towards antioxidants contained in legumes due to the reported positive relationship between intake of leguminous diets and lower incidence of non-communicable diseases (cancers, cardiovascular disease, diabetes, inflammation). The significant antioxidants role play by polyphenols in the protective effect of plant-derived foods cannot be over emphasized (Foss *et al.,* 2022), this has become the focus of contemporary nutritional and therapeutic interest in the last decades.

The *Phaseolus lunatus* seeds are called various local names in the southern part of Nigeria; where it is commonly consumed among the underprivileged. The hard-to-cook nature of *P. lunaus* is a major challenge and has discouraged a lot of people from eating it. It is often referred to as food for the aged in South Western Nigeria because of the longer cooking method. There are a large number of *P. lunatus* varieties in Nigeria that have been studied for the quantification of minerals, amino acids and phenolic compounds but the influence of varieties on these parameters have not been evaluated. Hence this study was undertaken to fill this gap.

2. MATERIALS AND METHODS

2.1 Preparation of seeds flour

Four varieties of lima beans (*Phaseolus lunatus* L. WALP) seeds with accession number NSWP100, NSWP98, NSWP47 and NSWP96 were obtained from the Institute of Agricultural Research and Training (IAR&T), Ibadan, Nigeria. The beans were cleaned and dried in an air oven (Memmert, model ULM 600 II, Germany) at 60±2 ^oC for 36 h; followed by cracking in a hammer mill (Trojan TGS 228 E); ground in a laboratory blender (HGBTWTS3, Torrington, CT, USA) and sieved through 100 mm mesh size (British Standard) and kept in airtight containers at 4 ^oC prior to use.

2.2 Determination of mineral elements

The mineral composition of the whole meal flours was determined using the dry aching procedure, an official method of AOAC (2012). The parameters determined were potassium, sodium, calcium, magnesium, iron, phosphorus, manganese, zinc and copper.

2.3 Amino acid composition

The Bidlingmeyer *et al.* (1984) procedure which involved an HPLC system was used to determine the amino acid profiles. The profiling was carried out after the flour samples were hydrolyzed for 24 h with 6 M HCl; the cysteine and methionine contents were determined after performic acid oxidation and tryptophan content after alkaline hydrolysis. The result was expressed as g aa/100 g protein.

2.4 HPLC-DAD quantification of phenolics

The phenolic fingerprinting with HPLC, C_{18} column (4.6 mm x 150 mm) packed with 5 µm diameter particles were used. The mobile phase was a combination of A (acetic acid, 2%) and B (methanol) and which composition gradient was set at 5% of B until 2 min and changed to obtain 25%, 40%, 50%, 60%, 70% and 100% B at 10, 20, 30, 40, 50 and 80 min. The following antioxidant compounds were identified in the samples by comparing their retention time and UV absorption spectrum with those of commercial standards. Confirmation of the chromatography peaks was done by comparing the retention time with those of reference standards and by DAD spectra (200 to 500 nm) (Boligon *et al.,* 2012).

2.5 HPLC-DAD estimation of β-carotene and tocopherol

Tocopherol and β-carotene were carried out at reverse phase chromatographic under gradient conditions using C_{18} column (4.6 mm × 150 mm) packed with 5 µm diameter particles. A mixture of ACN: H_2O (9:1, v/v) with 0.25% triethylamine (A) and EtAc with 0.25% triethylamine (B) served as the mobile phase. The gradient started with 90% A at 0 min to 50% A at 10 min. The percentage of A decreased from 50% at 10 min to 10% A at 20 min. Signals were detected at 450 nm, following the method described by Janovik *et al.* (2012) with little modifications. Solutions of standards references (tocopherol and β-carotene) were prepared. Samples were analyzed and quantified in the samples by comparison of retention times and UV spectra with the standard solution.

2.6 Statistical analysis

All results are expressed as mean ± standard deviation. Analysis of variance (ANOVA) was performed using Statistical Software (SPSS version 20). Differences in means were determined using Duncan's multiple range tests.

3. RESULTS AND DISCUSSION

3.1 Mineral composition

The mineral content of the whole meal flours is depicted in Table 1. Significant (p < .05) differences were observed in the mineral compositions. The highest concentration of potassium (374.11 mg/100g) and sodium (88.46 mg/100g) was found in NSWP96 and NSWP98 respectively while NSWP100 had the least value (7.53 mg/100 g) for both varieties. Potassium is essential for a number of functions in human body including lowering of blood pressure in people with hypertension (Alexander *et al.*,2016). The sodium/potassium ratio (Na/K) of less than one (<1) was obtained in all the varieties (0.22, 0.28 and 0.21 and 0.21 respectively) which fall within the recommended value for health improvement in people with hypertension (Ogunka-Nnoka *et al.,* 2017). Therefore, the consumption of diet rich in NSWP100, NSWP98, NSWP47 and NSWP96 could ameliorate the incidence of high blood pressure/hypertension (Perez and Chang, 2014).

Table 1: Mineral composition of *P. lunatus* whole meal flours (mg/100 g)

Elements	NSWP100	NSWP98	NSWP47	NSWP96
Copper	0.80 ±0.03a	0.51±0.02b	0.27± 0.01c	0.25 ±0.02c
Zinc	2.30 ±0.08c	1.84±0.32d	3.08± 0.20b	4.18 ±0.27a
Manganese	0.15 ±0.01	0.17± 0.01	0.18± 0.01	0.15 ±0.02
Calcium	19.85±0.11a	17.87±0.68c	14.32±0.49d	19.37±0.50b
Potassium	70.63±0.65d	320.67±0.74c	357.80±0.50b	374.11±0.54a
Sodium	15.47±0.10d	88.46± 0.89a	75.30±0.36c	79.37±0.53b
Magnesium	35.81±0.48d	56.64±0.34c	70.84± 0.24a	64.41 ±0.19b
Iron	16.90±0.52a	16.89± 0.49a	12.72± 0.46b	10.86 ±0.15c
Phosphorus	15.34±0.21b	13.72±0.23c	12.14±0.05d	16.32±0.20a
Na/K	0.22±0.01b	0.28±0.03a	0.21±0.01b	0.21±0.01b
Ca/P	1.29±0.01	1.30±0.01	1.18±0.01	1.19±0.01

Values are means of triplicate determinations ± Standard deviation. Means with different letters in the same row are significantly different ($p \leq 0.05$).

Calcium (Ca) (14.32 to 19.85 mg/100g); which varied significantly among the varieties; helps in the formation of vital human part such as bones and teeth during the formative years, and the flours can serve as good sources of Ca. Magnesium (Mg) was significantly higher in NSWP47 (70.84 mg/100 g) and lowest (35.81 mg/100 g) in NSWP100. Magnesium is important in Ca metabolism; regulating blood pressure as well as insulin release in the body (Oyedeji *et al.*, 2021). Phosphorus also varied between 12.14 and 16.32 mg/100 g. The flours from all the varieties were high in phosphorus. The calculation of Ca/P ratio is used as an index of calcium measurement in the bone; where a Ca/P ratio above 1 (>1) in a diet is considered as good and otherwise when is below 1(<1) (Nieman *et al.*,1992). The Ca/P ratio in the present study ranged between 1.18 (NSWP47) to 1.30 (NSWP98) which underline the varieties as good sources of minerals for bone formation to support growth and development especially in infants.

Iron (Fe) is the most concentrated among the trace elements determined with a range of 10.86 to 16.90 mg/100 g with the highest value observed in NSWP100. Iron is important in hemoglobin circulation during respiration in human (Chirwa-Moonga *et al.*, 2020). Zinc (Zn) and Copper (Cu) were in range of 1.84 to 4.18 and 0.25 to 0.80 mg/100 g, respectively. Zinc; known as antiviral mineral helps to boost body immunity against infection and diseases (Achi *et al.*, 2017).

3.2 Amino acid composition

An important factor in establishing human protein requirement on habitual diets is the quality of the dietary proteins gauge by essential amino acid content (National Institute of Nutrition, 2009). The results demonstrated a high level of essential amino acids as depicted in Table 2. The highest total essential amino acid (TEAA) 42.55 g/100 g protein was recorded in NSWP98 while NSWP100 recorded the lowest TEAA (41.11 g/100 g protein); these values are adequate to meet up with the body recommended daily requirement for different stages of development (39%-infants, 26%-children and 11%-adults) (FAO/WHO/UNU, 1985). In terms of the essential amino acid, all the varieties exhibited higher amounts of valine, isoleucine, tryptophan, leucine, lysine, phenylalanine and histidine compared to FAO/WHO (2007) requirements for (2–5 years old) child; they are also adequate for adults. However, the high lysine content of the *P. lunatus* varieties is a very important nutritional attribute, which underline it significant supplementary protein to cereal-based diets which are known to be deficient in lysine. The most concentrated essential amino acid in all the varieties were leucine (8.16-8.31g/100 g protein), higher than 6.90 g/100 g protein obtained in marama beans flour (Amonsou *et al.,* 2012) and lysine (6.85-7.25 g/100 g protein). The most concentrated non-essential amino acids were glutamic and aspartic acids with NSWP47 and NSWP96 varieties having the highest values respectively, this could be attributed to botanical variation. Consumption of diet rich in glutamine is beneficial during catabolic stress, such as trauma, sepsis and post-surgery recovery also an important incitement for enterocytes and immune cells (Sayles *et al.,* 2016).

Table 2: Amino acid composition of *P. lunatus* whole meal flours (g/100 g protein)

Amino acid	**NSWP100**	**NSWP98**	**NSWP47**	**NSWP96**	**FAO/WHO/UNU****	
					Child	**Adult**
ASX	13.25±0.10c	13.35±0.15b	13.51±0.18a	13.17±0.25d	-	-
THR*	4.38±0.10b	4.44±0.11a	4.14 ±0.12c	4.37±0.11b	3.40	0.90
SER	7.41±0.15c	7.58±0.12b	7.80 ±0.21a	7.22 ±0.01d	-	-
GLX	14.13±0.15c	14.50±0.25b	14.60±0.11a	14.63±0.01a	-	-
PRO	4.06±0.05a	3.45±0.10b	3.16 ±0.01d	3.31±0.12c	-	-
GLY	4.28±0.10c	4.24±0.10c	4.42 ±0.15a	4.32±0.01b	-	-
ALA	4.17±0.15c	4.44±0.10b	4.55 ±0.11a	4.52±0.01a	-	-
CYS	0.58±0.01a	0.33±0.00c	0.41 ±0.18b	0.49±0.01a	-	-
VAL*	4.67±0.10c	5.24±0.13a	4.73 ±0.05b	4.74±0.01b	3.50	1.50
MET*	1.15±0.01b	0.86±0.00d	1.07 ±0.28c	1.23±0.01a	2.70	1.70
ILE*	3.91±0.01c	4.56±0.10a	3.82 ±0.18d	4.22±0.02b	2.80	1.30
LEU*	8.24±0.15b	8.16±0.10c	8.22 ±0.11b	8.31±0.23a	6.60	1.90
TYR	2.98±0.01d	3.33±0.10a	3.16 ±0.10b	3.06±0.14c	-	-
PHE*	5.81±0.10b	6.18±0.30a	5.89 ±0.01b	5.61±0.10c	6.30	1.90
HIS*	5.28±0.10a	4.60±0.10c	5.30 ±0.01a	5.20±0.15ab	1.90	1.60
LYS*	7.24±0.12	7.24±0.15	7.24 ±0.01	7.21±0.18	5.80	1.60
ARG	7.52±0.10a	6.56±0.10d	7.09 ±0.28c	7.39±0.10b	-	-
TRP*	1.43±0.10a	1.22±0.01b	1.14 ±0.02c	1.17±0.02c	1.10	0.50
TEAA	41.11±0.02a	42.50±0.01b	41.55±0.02c	42.06±0.01a		

Values are means of triplicate determinations ± Standard deviation. Means with different letters in the same row are significantly different ($p \leq 0.05$).

Key

ASX - aspartic acid + asparagine; GLX - glutamic acid + glutamine.*= Essential amino acids, **= FAO, 2007.

3.3 Phenolics, β-carotene and tocopherol contents

The antioxidants activity of most plant bioactive has been associated with active polyphenol components, such as flavonoids and phenolic acids that are known to possess powerful antioxidant activities (Pandey and Rizvi, 2009). HPLC-DAD fingerprinting has some advantages over other methods of phenolics content determination. The HPLC-DAD analysis, showed positive results for phenolic acids (gallic, chlorogenic, ellagic and caffeic acids); flavonoids (quercetin, rutin and kaempferol); β-carotene and tocopherol (Table 3). The result showed ellagic acids as the predominant phenolics in the flours irrespective of the varieties. Ellagic acid composition in the flour's ranges between 2.69 - 3.72 g/100 g. The level of phenolics content varied significantly ($p \leq 0.05$) between varieties (Table 3). Caffeic acid was the highest in NSWP96 (3.43 g/100 g) and the lowest in NSWP98 (1.38 g/100 g), while gallic acid was relatively low in all studied varieties.

Table 3: Phenolics, β-carotene and tocopherol composition of *P. lunatus* flours (g/100 g)

Compounds	NSWP100	NSWP98	NSWP47	NSWP96
Gallic acid	0.71±0.03a	0.15±0.11c	0.40±0.09b	0.83±0.12a
Chlorogenic acid	1.39±0.01a	ND	0.45±0.09b	ND
Caffeic acid	2.78±0.03c	1.38±0.02d	2.91±0.01b	3.43±0.04a
Ellagic acid	3.46±0.02b	2.69±0.03d	3.72±0.04a	2.95±0.02c
Rutin	1.83±0.02b	2.53±0.04a	1.28±0.01c	1.79±0.01b
Quercetin	1.42±0.02b	1.26±0.01b	1.59±0.04a	1.30±0.02b
Kaempferol	0.65±0.07c	1.41±0.02b	1.35±0.03b	1.76±0.01a
Tocopherol	1.53±0.02b	1.76±0.01a	1.37±0.03c	1.58±0.02b
β-Carotene	0.42±0.01a	0.27±0.11c	0.39±0.00b	0.43±0.01a

Values are means of triplicate determinations ± Standard deviation. Means with different letters in the same row are significantly different ($p \leq 0.05$). ND-Not detected

Rutin, the most abundant flavonoids varied from 1.28 g/100 g (NSWP98) to 1.83 g/100 g (NSWP100). The quercetin content (1.26 -1.59 g/100 g) has been reported to exhibit considerable antioxidant activity in the lipophilic phase and capable of protecting low-density lipoprotein from oxidation (Kruk *et al.,* 2022).

Another class of phenolics often encountered in foods are tocopherol (1.37-1.76 g/100 g) and β-carotene (0.27-0.43 g/100g). Tocopherol concentration and β-carotene were observed to be more in NSWP98 and NSPW96 flours respectively. Tocopherol is an important vitamin in human health as an antioxidant, plays important role in maternal health and child development (Bastani *et al.,* 2011). It has been implicated in the prevention of placental aging, endothelial vascular damage, which increases the occurrence of high-risk infections during pregnancy (Houston, 2018). *β*-carotene on other hand is a naturally occurring, provitamin A; the most prominent member of the carotenoids group and its antioxidant properties have been studied in human subjects (Loft *et al.,* 2008). A large number of studies have also reported cultivar-dependence of phenolics content in some other legumes (Abd-ElGawad *et al.,*2020; Madrera *et al.,* 2021; Lin *et al.,* 2022) due to geographical origin, climatic conditions and plant species (Giampieri *et al.,* 2022). The variation in minerals, amino acid and bioactive composition of the studied varieties could be attributed to the genetic variations and climatic condition.

CONCLUSIONS

Botanical distribution influences the levels of mineral and amino acids composition as well as phenolics concentration in the studied *P. lunatus* varieties. The NSWP100 had the highest level of TEAA; NSWP96 variety stands out for its high contents of phenolic compounds. The mineral content, essential amino acids content coupled with phenolics in *P. lunatus* flour studied underline the varieties potential application in food supplementation programme to ameliorate protein-energy malnutrition and usefulness as nutraceutics to promote good health.

ACKNOWLEDGMENTS

This research was supported by the Federal Government of Nigeria through Post-Graduate Scholarship Award.

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