Original Research Article

Effect of Ripening Stage on Biochemical Composition of Seeds from three Species of *Canavalia*spp Consumed as Protein Substitutes in Côte d'Ivoire

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ABSTRACT

Aims: The present study was aimed at investigating the nutritional properties of legume-seeds from Canavalia genus as a function of their ripening stage.

Methodology: Seeds of three species of *canavalia*seeds notably *Canavaliagladiata*, *Canavaliaensiformis* and *Canavaliarosea* were obtained from an experimental plot located in Bouaké area's (7°41′00″ N, 5°01′59″ W) in Côte d'Ivoire. The seeds of these three species were collected at different ripening stages. The seeds were sun dried and ground to obtain the crude flour. Chemical composition and functional properties were investigated using standard methods while amino acid profiles were performed by using HPLC analytical methods.

Results: The present study was aimed at investigating the nutritional properties of legume-seeds from Canavalia genus as a function of their ripening stage.Results reveal that the profiles of nutrients in the three Canavalia species varied as a function of ripening stages. The highest fat (3.27 ± 0.01 %), protein (33.20 ± 0.17), fibre (7.51 ± 0.06 %), ash (6.67 ± 0.01 %) and total sugar (25.40 ± 0.05 %) contents were obtained at the last ripening stage. Also, all the studied legume-seeds are excellent sources of proteins mainly consisting of high amounts of essential amino acids such as lysine (100.23 ± 0.04 mg/g prot), leucine (103.51 ± 0.04 mg/g prot), valine (83.68 ± 0.04 mg/g prot), methionine (27.36 ± 0.04 mg/g prot), tryptophan (74.32 ± 0.04 mg/g prot), cysteine (44.24 ± 0.04 mg/g prot), isoleucine (63.61 ± 0.04), threonine (40.22 ± 0.04 mg/g prot) and histidine (17.76 ± 0.04 mg/g prot). As regards mineral contents of these seeds, they were higher at penultimate ripening stage and, they partially meet dietary allowance of infants.

Conclusion: These seeds could be ranked as protein rich food for human and then, used asvaluable substitutes for meat or fish.

Keywords: Physicochemical composition, amino acid profil, legume-seeds, Canavaliagladiata, Canavaliaensiformis, Canavaliarosea

1. INTRODUCTION

Today, in many parts of the world, the exploitation of local resources is certainly a way to achieve the objective of food security[1],particularly for a fast-growing population and facing an increasing demand for protein and other nutrient-rich foods [2].It seems imperative to improve and develop crops such as neglected or underutilized and called orphan or minor crops, by both national and international research programs [3, 4].Many seeds generally

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used as vegetables or leguminous belong to this group of little-known plants. However, it is well known that legume seeds provide less expensive and important protein sources to combat malnutrition, especially in developing countries where protein-rich foods of animal origin are not available for the people in low socio-economic groups. Otherwise, legumes are rich in many nutrients such as proteins, starch, dietary fibre, oils, vitamins, minerals and phytochemicals that are protected by nature. They provide substantial protein intake support to a significant proportion of the world's population, particularly in most developing countries [5].Because of their high nutritional value, abundance of minerals and secondary metabolites, grain legumes have become valuable components of staple and functional foods [6].The Canavalia species are categorized as a member of these crops. The genus of Canavalia is considered the third largest family among flowering plants [7].It comprises approximately 50 species of tropical vines widely distributed in tropical and subtropical regions all over the world [8].

This genus was used traditionally as a food due to its nutritional significance. Sridhar and Seena[9]envisaged a comparative account of nutritional and functional properties of Canavalia species. *Canavaliagladiata* and *Canavaliaensiformis* are the common legume species having the potential to be a rich protein source, like edible legumes. Regarding Canavalia species notably, *Canavaliagladiata* (sword bean otherwise called "magic bean"), *Canavaliaensiformis* (jack bean) and *Canavaliarosea* (bay bean), they can be used to cushion the high cost of proteinaceous feed ingredients in the livestock industry [2].It is pink coloured bean that originated from Asia and Africa. It is a leguminous, annual crop grown as a green manure, or cover crop and used as a fodder for livestock [10].The high crude protein content (20 – 32%) and amino-acid profile of jack bean seed have been reported to make it suitable for use as a substitute for fish feed, while the fully ripened seeds are occasionally used as a coffee substitute [11].Furthermore, the leguminous plant Canavalia species, though not eaten frequently, traditionally possess more medicinal properties yet to be scientifically proven. According to Soetan and Antia[12],jack bean and sword bean are rich in phytochemicals like flavonoids and saponins which should be exploited as medicinal foods for the benefits of human and animals.

Despite the aforementioned important nutritional and medicinal characteristics, in Côte d'Ivoire, Canavalia seeds are legume crops that have not been fully exploited. Thus, to our knowledge, no or very few studies have been devoted to biochemical and nutritional properties of these leguminous seeds.

2. MATERIAL AND METHODS

2.1. RAW MATERIAL COLLECTION AND SAMPLE PREPARATION

The three species of *canavalia*seeds notably *Canavaliagladiata*(sword bean), *Canavaliaensiformis*(jack bean) and *Canavaliarosea*(bay bean) was obtained from an experimental plot located in Bouaké area's (7°41′00″ N, 5°01′59″ W) in Côte d'Ivoire. The seeds of these three species were collected at different ripening stages: 30 days (S1), 40 days (S2), 50 days (S3), 60 days (S4) and 80 days (S5) after fertilization.

After thoroughly drying in the sun, the pods were thrashed to remove seeds. The seeds,after thoroughly clearing and removal of broken seeds and foreign materials, were stored in airtight plastic containers at room temperature (25°C ± 2°C). The air-dried seeds (nearly 50 g from each accession) were powdered in a Wiley mill to pass a 60-mesh screenand stored in screw-capped bottles at room temperature for further analysis [13].

2.2. Proximate Composition Analysis

Dry matters were determined by drying in an oven at 105ºC during 24 h to constant weight[13].Crude protein was calculated from nitrogen (Nx6.25) obtained using the

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Kjeldahlmethod by AOAC[13].Crude fat was determined by continuous extraction in a Soxhlet apparatus for 8 h using hexane as solvent [13].Carbohydrate content was determined through the method used by Samant and Rege[14].Total ash was determined by incinerating in a furnace at 550ºC [13].Method described by Dubois et al.[15]was used to determine total sugars while reducing sugars were analysed according to the method of Bernfeld[16]using 3.5 dinitrosalycilic acids (DNS).The crude fibre contents were determined according to standard method[13].The energy values of Canavalia seeds were evaluated using formula described by Crisan and Sands[17].Energy value (kcal/100g) = (2.62 × % protein) + (8.37 × % fat) + (4.2 × % carbohydrate)

2.3. Minerals analysis

Minerals were determined employing AOAC[13]method. Flour was digested with a mixture of concentrated nitric acid (14.44 mol/L), sulfuric acid (18.01 mol/L) and perchloric acid (11.80 mol/L) and analysed using an atomic absorption spectrophotometer. The total phosphorus was determined as orthophosphate by the ascorbic acid method after acid digestion and neutralization using phenolphthalein indicator and combined reagent [18].

2.4. Amino acid composition

Total amino acid composition of samples was determined after hydrolysis in 6 M HCl withphenol (1%) at 150°C for 60 min, in Pico-Tag system (Waters, Milford, Mass., U.S.A.). The phenylisothiocyanate (PITC) amino acid derivatives were eluted on HPLC Applied Biosystems Model 172 A (Applera Corp, Foster City, Calif., U.S.A.) equipped with a PTC RP-18 column (2.1 mm × 22 cm). Sodium acetate (45 mM, pH 5.9) and sodium acetate (105 mM, pH4.6; 30%), and acetonitrile (70%) were used as buffers.

2.5. Statisticalanalysis

All analyses were performed in triplicates. Results are expressed as the mean ± standard deviation of several sample with Ky plot (version 2.0 beta 15, ©1997-2001, Koichi Yoshioka) statistical software. The data were statistically analysed by one way analysis of variance (ANOVA). Means were compared by Turkey's test. Differences were considered statistically significant at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. ProximateComposition

The proximate composition of flours from the seeds of three Canavalia species was presented in **Table 1** for different ripening stages. Generally, the results reveal that fat, protein, fibre and ash contents increase as a function of ripening stages while the moisture and carbohydrate contents decrease in the same manner. Thus, at the last ripening stage (stage 5), highest fat, protein, fibre, ash and total sugar contents were obtained. As regards the moisture contents, all the studied seeds exhibited values about 5% at the last ripening stage suggesting that the studied beans are slightly perishable due to low moisture content which inhibits susceptibility to microbial growth and enzyme activity and, therefore could decelerates spoilage [19].

Canavaliaensiformis seeds were found to have the best protein, fibre, ash and total sugar contents with values of 33.20 ± 0.17, 7.51 ± 0.06, 6.67 ± 0.01 and 25.40 ± 0.05 %, respectively. At the same ripening stage, *Canavaliarosea* and *Canavaliagladiata*exhibited protein contents of 32.05 ± 0.11 and25.28 ± 0.29, respectively. These protein contents are higher than those reported for beans (ranged from 20.8 ± 1.3 to 23.5 ± 1.0%) [20], and for

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wild edible mushroom *Termitomycesheimii* (23.75 ± 0.04%) and *Amillariamellea* (21.32 ± 0.86 %) [21, 22].Moreover, crude protein contents obtained in this study were greater than those of some edible insects such as *Analeptestrifasciata*(20.1 %) and *Cirinafordal*arva (20 %) which are categorized as good protein sources [23, 24]. Therefore, Canavalia seeds could be ranked as protein rich food for human and then, used as valuable substitutes for meat or fish especially in developing countries for malnourished children suffering from kwashiorkor (a protein deficiency condition) and for pregnant women.

For crude fats, both *Canavaliarosea* and *Canavaliagladiata* showed the highest content of 3.25 ± 0.01 and 3.27 ± 0.01% (no significant difference) at the last ripening stage. These values are greater than those previously reported for some 52 common bean landraces (range of 0.6 to 2.9%) [25], and Yellow kidney beans had (2.7%) [20]. Los et al.as above reported crude fat contents of 0.7 ± 0.01 to 2.7 ± 0.09% for common beans (*Phaseolus vulgaris* L.). However, these fat contents are low compared to high-fat legumes such as peanut and soybeans which have fat contents of about 25.3% and 22.19%, respectively [27, 28]. In fact, the studied seeds could be useful for preparation of some low-fat foodstuffs and snacks and in preventing hypertension and hypercholesterolemia [6]. Thus, they would be ideal foods for obese persons and recommended as good source of food supplement for patients with cardiac problems or at risk with lipid induced disorders as commented by Pedrosa et al. [29].

Concerning carbohydrates, the highest values (decreasing from 74.42 ± 0.08 to 58.17 ± 0.28 %) were obtained with *Canavaliagladiata* seeds and the lowest contents (decreasing from 66.94 ± 0.12 to 49.73 ± 0.22 %) with *Canavaliaensiformis* seeds. Butkute et al.[30]found carbohydrate contents ranged from 38.0 to 46.0 % for seven perennial legume seed species from Lithuania. So, values obtained in the present study were sited in the range of 58.8 to 77.4 % as reported by Los et al**.** [26]for several common bean varieties. These high carbohydrate contents would be due to their low lipid contents. Also, it might imply the uses of these seeds as binder, bulking agent or thickener in soups. These results suggest that the studied legume seeds would be highly nutritious and good for human and animal consumption.

It's noteworthy that the ash content of the sample is of nutritional importance as a previous report indicated that when leaves or vegetables are to be used as food for humans, they should contain about 3.0% ash [31]. As for the ash contents the studied Canavalia seeds, they were all above 3.0% whatever the ripening stage.At the last ripening stage, the ash contents were of 6.67 ± 0.01, 6.05 ± 0.01 and 5.92 ± 0.01, respectively for *Canavaliaensiformis*, *Canavaliarosea* and *Canavaliagladiata*. It indicates that these legume seeds are good sources of mineral elements with values greater than that previously reported (2.1%) by Omowunmi et al.[32]for *Canavaliaensiformis* seeds harvested in Ogun State (Nigeria).

The fibre contents is within 6.38 ± 0.03 – 7.35 ± 0.03%, 6.61 ± 0.03 – 7.39 ± 0.04% and 6.79 ± 0.04 – 7.51 ± 0.06%, respectively for *Canavaliagladiate*, *Canavaliarosea* and *Canavaliaensiformis*. The ripened beans showed greater values of fibres. This ranges ar similar to that found in the literature for legumes that serve as good sources of fibre [33].

The low energy levels provided by the studied Canavalia seeds(less than 400 kcal/l00g DW) give only about 18 % of the daily energy intakes recommended for a 70 kg person. It is well know that the energy content is affected by the proportion of fat, protein and carbohydrate in the vegetables. The present results suggested that Canavalia seeds can be used as a source of low energy diet [21].

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At the last ripening stage, the ash contents were of 6.67 ± 0.01, 6.05 ± 0.01 and 5.92 ± 0.01, for Canavalia ensiformis, Canavalia rosea and Canavalia gladiata respectively.

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Table 1: Proximate composition

Species	Stage	Parameters								
		Dry matter (%)	Moisture (%)	Fats (%)	Proteins (%)	Carbohydrates (%)	Fibres (%)	Ash (%)	Total sugars (%)	Reducing sugars (%)	Energy value (Kcal)
Canavaliagladiata (CG)	S1	87.24 ± 0.01 ^{c}	12.76 ± 0.01 ^{p}	2.05 ± 0.01 ^{e}	13.67 ± 0.12 ^{a}	74.42 ± 0.08 ^{q}	6.38 ± 0.03 ^{a}	3.47 ± 0.01 c	8.14 ± 0.00 ^{b}	0.98 ± 0.04 ^{a}	370.83 ± 0.11 ^{p}
	S2	90.54 ± 0.01 f	9.46 ± 0.01 ^{k}	2.19 ± 0.01 ^{f}	15.67 ± 0.12 ^{b}	71.52 ± 0.11 ^{p}	6.68 ± 0.01 ^{c}	3.93 ± 0.01 e	14.84 ± 0.00 ^{d}	2.01 ± 0.04 ^{h}	368.51 ± 0.05 ^{m}
	S3	92.56 ± 0.02 ^{h}	7.44 ± 0.02 ^{h}	2.43 ± 0.01 ^{h}	20.11 ± 0.12 ^{d}	66.26 ± 0.12 ^{k}	6.77 ± 0.02 ^{d}	4.43 ± 0.01 g	17.98 ± 0.18 ^{e}	3.58 ± 0.03 ^{k}	367.38 ± 0.07 ^{j}
	S4	94.27 ± 0.00 ^{p}	5.73 ± 0.00 ^{c}	2.79 ± 0.01 ^{k}	24.70 ± 0.14 ^{h}	60.12 ± 0.11 ^{g}	6.86 ± 0.03 ^{e}	5.51 ± 0.01 k	19.77 ± 0.29 ^{ef}	4.08 ± 0.03 ^{m}	364.46 ± 0.12 ^{f}
	S5	95.00 ± 0.00 ^{r}	5.00 ± 0.00 ^{a}	3.27 ± 0.01 ^{n}	25.28 ± 0.29 ^{j}	58.17 ± 0.28 ^{f}	7.35 ± 0.03 ^{k}	5.92 ± 0.01 n	20.97 ± 0.10 ^{f}	1.65 ± 0.03 ^{e}	363.27 ± 0.13 ^{d}
Canavaliarosea (CR)	S1	86.43 ± 0.01 ^{b}	13.57 ± 0.01 ^{q}	1.85 ± 0.02 ^{c}	19.09 ± 0.18 ^{c}	69.30 ± 0.20 ^{n}	6.61 ± 0.03 ^{b}	3.15 ± 0.01 a	5.41 ± 0.00 ^{a}	1.52 ± 0.00 ^{d}	370.17 ± 0.09 ^{n}
	S2	88.90 ± 0.01 d	11.10 ± 0.01 ^{n}	2.01 ± 0.02 ^{d}	22.09 ± 0.18 ^{f}	65.22 ± 0.23 ^{j}	7.01 ± 0.05 ^{f}	3.67 ± 0.01 d	7.71 ± 0.01 ^{b}	1.67 ± 0.00 ^{e}	367.32 ± 0.21 ^{j}
	S3	91.77 ± 0.00 ^{g}	8.23 ± 0.00 ^{j}	2.47 ± 0.01 ^{j}	27.53 ± 0.18 ^{k}	58.26 ± 0.17 ^{f}	7.16 ± 0.04 ^{h}	4.57 ± 0.01 h	11.22 ± 0.08 ^{c}	2.01 ± 0.03 ^{h}	365.42 ± 0.15 ^{h}
	S4	92.58 ± 0.00 ^{j}	7.42 ± 0.00 ^{g}	2.89 ± 0.01 ^{m}	30.88 ± 0.16 ^{n}	53.87 ± 0.17 ^{d}	7.25 ± 0.07 ^{j}	5.11 ± 0.01 j	15.14 ± 0.16 ^{d}	2.94 ± 0.04 ^{j}	365.03 ± 0.25 ^{g}
	S5	94.23 ± 0.01 ^{n}	5.77 ± 0.01 ^{d}	3.25 ± 0.01 ^{n}	32.05 ± 0.11 ^{q}	51.25 ± 0.10 ^{b}	7.39 ± 0.04 ^{k}	6.05 ± 0.01 ^{p}	19.07 ± 0.25 ^{e}	1.34 ± 0.02 ^{c}	362.49 ± 0.15 ^{c}
Canavaliaensiformis (CE)	S1	85.79 ± 0.01 ^{a}	14.21 ± 0.01 ^{r}	1.67 ± 0.02 ^{a}	21.28 ± 0.07 ^{e}	66.94 ± 0.12 ^{m}	6.79 ± 0.04 ^{d}	3.31 ± 0.01 ^{b}	5.54 ± 4.04 ^{a}	1.31 ± 0.02 ^{c}	367.93 ± 0.14 ^{k}
	S2	89.34 ± 0.00 ^{e}	10.66 ± 0.00 ^{m}	1.71 ± 0.01 ^{b}	24.28 ± 0.07 ^{g}	62.75 ± 0.11 ^{h}	7.08 ± 0.03 ^{g}	4.17 ± 0.01 ^{f}	10.30 ± 0.01 ^{c}	1.51 ± 0.00 ^{d}	363.55 ± 0.11 ^{e}
	S3	92.81 ± 0.00 ^{k}	7.19 ± 0.00 ^{f}	2.03 ± 0.01 ^{e}	29.72 ± 0.07 ^{m}	55.37 ± 0.10 ^{e}	7.19 ± 0.02 ^{hj}	5.69 ± 0.01 ^{m}	16.12 ± 0.18 ^{d}	1.76 ± 0.03 ^{f}	358.64 ± 0.08 ^{b}
	S4	93.81 ± 0.01 ^{m}	6.19 ± 0.01 ^{e}	2.29 ± 0.01 ^{g}	31.54 ± 0.12 ^{p}	52.70 ± 0.13 ^{c}	7.35 ± 0.03 ^{k}	6.11 ± 0.01 ^{q}	21.40 ± 0.24 ^{f}	1.89 ± 0.02 ^{g}	357.63 ± 0.11 ^{a}
	S5	94.56 ± 0.00 ^{q}	5.44 ± 0.00 ^{b}	2.89 ± 0.01 ^{m}	33.20 ± 0.17 ^{r}	49.73 ± 0.22 ^{a}	7.51 ± 0.06 ^{m}	6.67 ± 0.01 ^{r}	25.40 ± 0.05 ^{g}	1.10 ± 0.04 ^{b}	357.74 ± 0.24 ^{a}

Values are mean ± standard deviation of three measurements (n = 3). The different case letters (a, b, c) in the column indicate significant differences ($p < 0.05$) in the respective values. S1: Stage 1; S2: Stage 2; S3: Stage 3; S4: Stage 4; S5: Stage 5.

3.2. Mineral Composition

The variation of mineral contents as a function of ripening stages is given in **Table 2**. Significant differences ($p < 0.05$) were noted in mineral composition as a function of Canavalia species and, ripening stages. Results reveal that highest mineral contents were obtained at the penultimate ripening stage (stage 4) for all the studied legume-seeds. *C. rosea* seeds contained the highest concentration of potassium (43506.67 ± 60.28µg 100 g^{-1}), magnesium (27656.67 ± 30.55µg 100 g^{-1}), manganese (9.65 ± 0.39µg 100 g^{-1}), zinc (107.45 ± 0.22µg 100 g^{-1}) and iron (973.67 ± 4.16µg 100 g^{-1}), while calcium (9669.37 ± 0.54µg 100 g^{-1}) and copper (74.67 ± 4.04µg 100 g^{-1}) contents were greater in *C. gladiata* seeds. These values are higher than those previously reported by Nkwocha et al.[34]for the same legume-seeds harvested in the Kaduna state (Nigeria).

Potassium was the most abundant macro-mineral and its composition ranged from 999.85 ± 30.65 to 35400.00 ± 45.83 µg 100 g^{-1} in *C. gladiata*, 564.85 ± 30.65 to 43506.67 ± 60.28µg 100 g^{-1} in *C. rosea* and 452.21 ± 6.98 to 13610.00 ± 45.83 µg 100 g^{-1} in *C. ensiformis* seeds. It partially meets requirements for infants (500-700 mg/ 100 g DW) than adults and pregnant lactating women (2000 mg/ 100 g DW) [35]. In the same manner, magnesium, manganese, zinc, iron, calcium and copper contents in the studied seeds partially meet dietary allowance of infants. It would be recommended to consume these legume-seeds with the addition of mineral-rich foods such as meat, fish or even caterpillars to meet the daily nutrient needs as describe by Akpossan et al[36]and Assiélou et al.[37].

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Table 2: Mineral composition

Species	Stage	Parameters								
		Calcium (ug/100 g DW)	Phosphorus (ug/100 g DW)	Magnesium (ug/100 g DW))	Potassium (ug/100 g DW)	Sodium (ug/100 g DW)	Manganese (ug/100 g DW)	Zinc (ug/100 g DW)	Copper (ug/100 g DW)	Iron (ug/100 g DW)
Canavaliagladiata (CG)	S1	1239.37 ± 0.64 ^{d}	967.33 ± 41.88 ^{b}	234.33 ± 3.51 ^{a}	999.85 ± 30.65 ^{c}	0.97 ± 0.07 ^{b}	0.00 ± 0.00 ^{a}	9.89 ± 0.50 ^{b}	1.89 ± 0.12 ^{a}	18.37 ± 0.15 ^{ab}
	S2	2033.37 ± 0.64 ^{f}	1878.33 ± 41.88 ^{d}	4340.33 ± 29.87 ^{h}	10195.95 ± 30.65 ^{f}	9.01 ± 0.13 ^{d}	0.50 ± 0.02 ^{b}	14.24 ± 0.11 ^{c}	8.98 ± 0.04 ^{cd}	23.33 ± 0.08 ^{bc}
	S3	7588.47 ± 76.61 ^{p}	3483.33 ± 35.12 ^{e}	9523.33 ± 29.87 ^{k}	18161.00 ± 53.51 ^{m}	12.37 ± 0.35 ^{f}	1.55 ± 0.05 ^{d}	42.67 ± 1.53 ^{e}	27.50 ± 0.54 ^{g}	109.80 ± 0.19 ^{d}
	S4	9669.37 ± 0.64 ^{r}	9536.67 ± 28.87 ^{n}	21297.33 ± 12.22 ^{q}	35400.00 ± 45.83 ^{p}	47.60 ± 0.36 ^{k}	4.11 ± 0.10 ^{f}	99.95 ± 1.00 ^{h}	74.67 ± 4.04 ^{n}	465.91 ± 3.91 ^{j}
	S5	4746.47 ± 81.00 ^{k}	4200.00 ± 55.68 ^{f}	12390.00 ± 29.31 ^{n}	19500.00 ± 55.68 ^{n}	32.1 ± 0.30 ^{h}	3.10 ± 0.11 ^{e}	62.33 ± 1.15 ^{f}	39.80 ± 0.74 ^{h}	186.88 ± 2.90 ^{f}
Canavaliarosea (CR)	S1	699.39 ± 0.62 ^{a}	627.67 ± 15.95 ^{a}	1007.31 ± 11.09 ^{c}	564.85 ± 30.65 ^{b}	0.97 ± 0.05 ^{b}	0.00 ± 0.00 ^{a}	9.83 ± 0.61 ^{b}	1.45 ± 0.15 ^{a}	18.32 ± 0.07 ^{b}
	S2	1039.39 ± 0.62 ^{c}	1439.33 ± 41.88 ^{c}	4190.00 ± 36.06 ^{g}	2287.55 ± 30.65 ^{d}	6.64 ± 0.32 ^{c}	0.99 ± 0.04 ^{c}	27.23 ± 1.62 ^{d}	5.01 ± 0.17 ^{b}	23.94 ± 0.34 ^{c}
	S3	3020.00 ± 70.49 ^{g}	5153.33 ± 45.09 ^{g}	11190.00 ± 36.06 ^{m}	14723.33 ± 49.33 ^{k}	14.43 ± 0.49 ^{g}	2.96 ± 0.25 ^{e}	40.67 ± 1.53 ^{e}	13.88 ± 0.19 ^{e}	204.00 ± 4.36 ^{g}
	S4	6201.47 ± 3.07 ^{m}	9900.00 ± 45.83 ^{p}	27656.67 ± 30.55 ^{r}	43506.67 ± 60.28 ^{r}	84.43 ± 0.31 ^{n}	9.65 ± 0.39 ^{m}	107.45 ± 0.22 ^{j}	65.95 ± 0.04 ^{m}	973.67 ± 4.16 ^{n}
	S5	3433.13 ± 57.91 ^{j}	7556.67 ± 20.82 ^{k}	13173.33 ± 56.86 ^{p}	35956.67 ± 61.10 ^{q}	41.08 ± 0.16 ^{j}	7.78 ± 0.16 ^{j}	76.98 ± 1.75 ^{g}	46.88 ± 0.12 ^{j}	741.67 ± 3.06 ^{m}
Canavaliaensiformis (CE)	S1	879.39 ± 0.62 ^{b}	907.33 ± 41.88 ^{b}	294.67 ± 3.51 ^{b}	452.21 ± 6.98 ^{a}	0.00 ± 0.00 ^{a}	0.25 ± 0.02 ^{d}	5.53 ± 0.11 ^{a}	7.70 ± 0.28 ^{c}	13.25 ± 0.07 ^{a}
	S2	1949.39 ± 0.62 ^{e}	3546.33 ± 41.88 ^{e}	2035.23 ± 42.39 ^{d}	3891.21 ± 14.32 ^{e}	0.00 ± 0.00 ^{a}	0.27 ± 0.04 ^{d}	9.89 ± 0.61 ^{b}	10.31 ± 0.28 ^{d}	21.95 ± 0.09 ^{bc}
	S3	3292.47 ± 12.53 ^{h}	6890.00 ± 45.83 ^{h}	3238.00 ± 20.30 ^{f}	10923.33 ± 35.12 ^{g}	10.13 ± 0.55 ^{e}	5.07 ± 0.11 ^{g}	13.13 ± 0.35 ^{c}	23.40 ± 0.35 ^{f}	317.33 ± 3.06 ^{h}
	S4	8606.47 ± 11.72 ^{q}	7043.33 ± 45.09 ^{j}	5730.00 ± 26.46 ^{j}	13610.00 ± 45.83 ^{j}	93.77 ± 0.60 ^{p}	8.59 ± 0.09 ^{k}	78.00 ± 4.58 ^{g}	63.90 ± 0.13 ^{k}	597.33 ± 6.43 ^{k}
	S5	7466.47 ± 57.56 ^{n}	8880.00 ± 20.00 ^{m}	2453.33 ± 15.28 ^{e}	11996.67 ± 65.06 ^{h}	70.87 ± 0.75 ^{m}	7.03 ± 0.15 ^{h}	40.99 ± 1.02 ^{e}	46.51 ± 0.50 ^{j}	164.17 ± 3.82 ^{e}

Values are mean ± standard deviation of three measurements (n = 3). The different case letters (a, b, c) in the column indicate significant differences ($p < 0.05$) in the respective mineral concentrations. S1: Stage 1; S2: Stage 2; S3: Stage 3; S4: Stage 4; S5: Stage 5.

3.3. Amino acids profile

Amino acid profiles are indicators of proteins nutritional qualities and functionalities. Essential/nonessential amino acid content is one of the parameters that provide important nutritional details about the protein quality of legumes [38]. The amino acid profiles of the three Canavalia species as a function of ripening stages is shown in**Table 3**. Overall observation revealed significant differences differences ($p < 0.05$) in amino acid amounts as a function of Canavalia species and, ripening stages. Generally the highest values were obtained at penultimate or last ripening stage.

The essential amino acids detected were methionine, valine, tryptophan, lysine, leucine, tyrosine, cysteine, isoleucine, threonine and histidine. Among them, the highest amounts were obtained with lysine, leucine and valine. Thus, *C. ensiformis* seeds contained 103.51 ± 0.04 mg/ g protein of leucine and 100.23 ± 0.04 mg/ g protein of lysine, while *C. rosea* seeds exhibited 83.68 ± 0.04 mg/ g protein of valine. The amino acid amounts determined in this study were higher than those reported for *C. ensiformis* in Nigeria [39], three jack bean accessions [33] and for pigeon pea [40]. The relatively high content of essential amino acids in the studied Canavalia seeds make them potential supplement in cereal-based diets due to the fact that cereal grain-based diets have been reported to be deficient in some essential amino acid such as lysine [41]. Furthermore, as argued by Millar et al.[42], the amino acid content of the three studied legume-seeds meet dietary allowance for adults [43].

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Comment [H92]: your discussion of result is more of a literature search. Discussion of result should be strictly narrowed down to your research finding as it involve this experimental studies and not relative to some literature discoveries.. Seperate your research findings from your literature.

Table 3: Amino acidcomposition

Species	Stage	Arginine (mg/g proteins)	Methionine (mg/g prot)	Valine (mg/g prot)	Alaline (mg/g prot)	Tryptophane (mg/g prot)	Proline (mg/g prot)	Lysine (mg/g prot)	Glutamic acid (mg/g prot)
Canavaliagladiata (CG)	S1	20.36 ± 0.04 ^{a}	9.27 ± 0.04 ^{e}	0.00 ± 0,00 ^{a}	6.32 ± 0.04 ^{a}	0,00 ± 0,00 ^{a}	30.57 ± 0.04 ^{e}	48.33 ± 0.04 ^{a}	55.62 ± 0.04 ^{h}
	S2	27.97 ± 0.04 ^{b}	17.39 ± 0.04 ^{h}	0.00 ± 0.00 ^{a}	12.87 ± 0.04 ^{b}	3.27 ± 0.04 ^{b}	39.31 ± 0.04 ^{g}	61.51 ± 0.04 ^{c}	77.11 ± 0.04 ^{n}
	S3	33.76 ± 0.04 ^{c}	24.32 ± 0.04 ^{p}	6.88 ± 0.04 ^{b}	18.93 ± 0.04 ^{c}	9.35 ± 0.04 ^{c}	50.92 ± 0.04 ^{p}	72.68 ± 0.04 ^{g}	87.29 ± 0.04 ^{p}
	S4	42.18 ± 0.04 ^{f}	23.76 ± 0.04 ^{n}	10.44 ± 0.04 ^{c}	23.77 ± 0.04 ^{e}	14.16 ± 0.04 ^{e}	62.15 ± 0.04 ^{r}	84.53 ± 0.04 ^{n}	98.35 ± 0.04 ^{q}
	S5	40.74 ± 0.04 ^{e}	27.36 ± 0.04 ^{q}	12.58 ± 0.04 ^{d}	22.34 ± 0.04 ^{d}	13.48 ± 0.04 ^{d}	59.37 ± 0.04 ^{g}	80.17 ± 0.04 ^{j}	110.54 ± 0.04 ^{r}
Canavaliarosea (CR)	S1	51.35 ± 0.04 ^{g}	2.58 ± 0.04 ^{b}	52.72 ± 0.04 ^{f}	68.53 ± 0.04 ^{m}	22.67 ± 0.04 ^{f}	20.16 ± 0.04 ^{b}	51.65 ± 0.04 ^{b}	32.15 ± 0.04 ^{d}
	S2	59.62 ± 0.04 ^{h}	7.94 ± 0.04 ^{d}	60.48 ± 0.04 ^{h}	81.58 ± 0.04 ^{n}	30.22 ± 0.04 ^{g}	28.55 ± 0.04 ^{c}	69.11 ± 0.04 ^{e}	44.71 ± 0.04 ^{g}
	S3	70.17 ± 0.04 ^{k}	11.84 ± 0.04 ^{g}	72.61 ± 0.04 ^{k}	100.22 ± 0.04 ^{r}	37.59 ± 0.04 ^{j}	34.41 ± 0.04 ^{f}	82.84 ± 0.04 ^{m}	57.36 ± 0.04 ^{j}
	S4	85.59 ± 0.04 ^{n}	17.33 ± 0.04 ^{h}	80.02 ± 0.04 ^{m}	98.78 ± 0.04 ^{q}	44.36 ± 0.04 ^{k}	40.72 ± 0.04 ^{h}	80.77 ± 0.04 ^{k}	70.92 ± 0.04 ^{k}
	S5	92.34 ± 0.04 ^{r}	22.47 ± 0.04 ^{m}	83.68 ± 0.04 ^{q}	97.22 ± 0.04 ^{p}	50.34 ± 0.04 ^{n}	45.92 ± 0.04 ^{k}	79.39 ± 0.04 ^{h}	73.21 ± 0.04 ^{m}
Canavaliaensiformis (CE)	S1	39.51 ± 0.04 ^{d}	0.00 ± 0.00 ^{a}	37.66 ± 0.04 ^{e}	25.28 ± 0.04 ^{f}	32.12 ± 0.04 ^{h}	17.63 ± 0.04 ^{a}	68.15 ± 0.04 ^{d}	13.67 ± 0.04 ^{a}
	S2	60.57 ± 0.04 ^{j}	6.11 ± 0.04 ^{c}	52.91 ± 0.04 ^{g}	41.51 ± 0.04 ^{g}	49.23 ± 0.04 ^{m}	28.68 ± 0.04 ^{d}	71.23 ± 0.04 ^{f}	17.15 ± 0.04 ^{b}
	S3	73.14 ± 0.04 ^{m}	10.02 ± 0.04 ^{f}	70.17 ± 0.04 ^{j}	50.49 ± 0.04 ^{h}	61.25 ± 0.04 ^{p}	41.15 ± 0.04 ^{j}	88.13 ± 0.04 ^{p}	29.98 ± 0.04 ^{c}
	S4	87.21 ± 0.04 ^{p}	18.77 ± 0.04 ^{j}	81.40 ± 0.04 ^{p}	60.77 ± 0.04 ^{j}	74.32 ± 0.04 ^{r}	48.16 ± 0.04 ^{n}	100.23 ± 0.04 ^{r}	38.47 ± 0.04 ^{e}
	S5	88.70 ± 0.04 ^{q}	20.91 ± 0.04 ^{k}	80.63 ± 0.04 ^{n}	62.35 ± 0.04 ^{k}	72.77 ± 0.04 ^{q}	46.38 ± 0.04 ^{m}	97.68 ± 0.04 ^{q}	42.87 ± 0.04 ^{f}

Values are mean ± standard deviation of three measurements (n = 3). The different case letters (a, b, c) in the column indicate significant differences ($p < 0.05$) in the respective mineral concentrations. S1: Stage 1; S2: Stage 2; S3: Stage 3; S4: Stage 4; S5: Stage 5.

Species	Stage	Leucine (mg/g prot)	Serine (mg/g prot)	Tyrosine (mg/g prot)	Cysteine (mg/g prot)	Isoleucine (mg/g prot)	Aspartic acid (mg/g prot)	Threonine (mg/g prot)	Histidine (mg/g prot)	Glycine (mg/g prot)
Canavaliagladiata (CG)	S1	38.29 ± 0.04^{a}	16.71 ± 0.04^{a}	17.54 ± 0.04^{j}	5.73 ± 0.04^{a}	22.41 ± 0.04^{b}	33.53 ± 0.04^{b}	14.39 ± 0.04^{e}	0.00 ± 0.00^{a}	4.32 ± 0.04^{a}
	S2	57.27 ± 0.04^{c}	24.77 ± 0.04^{d}	26.29 ± 0.04^{m}	15.11 ± 0.04^{d}	37.71 ± 0.04^{g}	69.37 ± 0.04^{h}	25.66 ± 0.04^{k}	1.11 ± 0.04^{b}	10.63 ± 0.04^{b}
	S3	64.15 ± 0.04^{f}	32.22 ± 0.04^{g}	36.13 ± 0.04^{n}	17.17 ± 0.04^{e}	49.23 ± 0.04^{k}	80.55 ± 0.04^{k}	32.47 ± 0.04^{m}	4.43 ± 0.04^{e}	18.15 ± 0.04^{c}
	S4	71.44 ± 0.04^{h}	40.63 ± 0.04^{j}	42.47 ± 0.04^{p}	20,73 ± 0.04^{j}	58.63 ± 0.04^{p}	91.84 ± 0.04^{m}	38.11 ± 0.04^{q}	11.24 ± 0.04^{h}	23.41 ± 0.04^{f}
	S5	69.21 ± 0.04^{g}	42.53 ± 0.04^{k}	44.76 ± 0.04^{q}	18.40 ± 0.04^{f}	63.61 ± 0.04^{q}	97.35 ± 0.04^{n}	36.71 ± 0.04^{n}	15.76 ± 0.04^{j}	20.33 ± 0.04^{d}
Canavaliarosea (CR)	S1	50.43 ± 0.04^{b}	19.21 ± 0.04^{b}	0.00 ± 0.00^{a}	13.15 ± 0.04^{c}	22.63 ± 0.04^{c}	42.33 ± 0.04^{d}	3.49 ± 0.04^{a}	0.00 ± 0.00^{a}	41.39 ± 0.04^{h}
	S2	58.11 ± 0.04^{d}	28.19 ± 0.04^{e}	2.53 ± 0.04^{c}	20.44 ± 0.04^{h}	30.29 ± 0.04^{e}	50.15 ± 0.04^{e}	10.02 ± 0.04^{c}	0.00 ± 0.00^{a}	57.18 ± 0.04^{n}
	S3	71.72 ± 0.04^{j}	40.55 ± 0.04^{h}	9,33 ± 0.04^{e}	31.50 ± 0.04^{m}	44.88 ± 0.04^{h}	54.76 ± 0.04^{f}	17.28 ± 0.04^{f}	1.81 ± 0.04^{c}	64.35 ± 0.04^{p}
	S4	86.37 ± 0.04^{m}	51.27 ± 0.04^{n}	15.41 ± 0.04^{h}	40.78 ± 0.04^{p}	54.11 ± 0.04^{m}	70.44 ± 0.04^{j}	23.62 ± 0.04^{g}	7.77 ± 0,04^{f}	72.71 ± 0.04 q
	S5	88.22 ± 0.04^{n}	58.27 ± 0.04^{r}	15.22 ± 0.04^{g}	44.24 ± 0.04^{r}	58.39 ± 0.04^{n}	68.61 ± 0.04^{g}	24.44 ± 0.04^{h}	11.22 ± 0.04^{h}	75.29 ± 0.04^{r}
Canavaliaensiformis (CE)	S1	60.03 ± 0.04^{e}	20.51 ± 0.04^{c}	0.00 ± 0,00^{a}	11.17 ± 0.04^{b}	10.71 ± 0.04^{a}	0.00 ± 0.00^{a}	9.21 ± 0.04^{b}	0.00 ± 0.00^{a}	21.38 ± 0.04^{e}
	S2	72.11 ± 0.04^{k}	29.24 ± 0.04^{f}	1.02 ± 0.04^{b}	18.75 ± 0.04^{g}	23.74 ± 0.04^{d}	0.00 ± 0.00^{a}	13.88 ± 0.04^{d}	4.29 ± 0.04^{b}	30.11 ± 0.04^{g}
	S3	89.78 ± 0.04^{p}	42.72 ± 0.04^{m}	6.77 ± 0.04^{d}	30.69 ± 0.04^{k}	31.43 ± 0.04^{f}	0.00 ± 0.00^{a}	24.58 ± 0.04^{j}	10.13 ± 0.04^{g}	42.39 ± 0.04^{j}
	S4	100.02 ± 0.04^{q}	55.67 ± 0.04^{q}	13.18 ± 0.04^{f}	39.26 ± 0.04^{n}	48.60 ± 0,04^{j}	40.55 ± 0.04^{c}	37.19 ± 0.04^{p}	17.76 ± 0.04^{m}	52.26 ± 0.04^{m}

	S5	103.51 ± 0.04 r	54.47 ± 0.04 p	21.13 ± 0.04 k	43.76 ± 0.04 q	30.29 ± 0.04 e	0,00 ± 0,00 a	40.22 ± 0.04 r	16.27 ± 0.04 k	51.55 ± 0.04 k

Table4: Amino acid composition in different species

4. CONCLUSION

The present study reveals that the profiles of nutrients in the three Canavalia species variedas a function of ripening stages. The highest fat, protein, fibre, ash and total sugar contents were obtained at the last ripening stage especially in *C. ensiformis* seeds. So, all the studied legume-seeds are excellent sources of proteins mainly consisting of high amounts of essential amino acids such as lysine, leucine, valine, methionine, tryptophan, cysteine, isoleucine, threonine and histidine. As regards mineral contents of these seeds, they were higher at penultimate ripening stage and, they partially meet dietary allowance of infants.

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Comment [H94]: Maintain this format:
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