



Physical and biochemical characteristics of red and black beans varieties of *Phaseolus lunatus* (L.) consumed in south and east of Côte d'Ivoire

Tchumou Messou¹, Yué Bi Yao Clément^{1*}, Benjamin N'zué Yao², Kouonon Léonie Clémence¹, Tano Kablan¹

¹Laboratory of Biochemistry and Food Technology, Department of Food Science and Technology, Nangui Abrogua University, Abidjan, 02 B.P. 801, Ivory Coast

²Agroforestry, Jean Guédé Lorougnon University, Daloa, Ivory Coast

*corresponding author: yueclement@yahoo.fr

ABSTRACT

To contribute to their valorization, the stage of physiological maturity, the time of harvest and the nutrient content of two cultivars (red and black) of *Phaseolus lunatus* were the subject of this study. These two cultivars are the most consumed in the south and east of Côte d'Ivoire. Seed weights of both cultivars decreased from stage 1 (32 days) to stage 4 (52 days). For major components such as crude protein, fat, vitamins C and B, their values increased while carbohydrates decreased from phase 1 to phase 4. Black cultivars contained more crude protein, fat, vitamins C and B as red cultivars. Both cultivars were found to be rich in macroelements (Na, K, P) and micronutrients (Fe); however, potassium and iron are more abundant in red cultivars. Physiological maturity of both cultivars was reached about 52 days and mature pods can be harvested as a vegetable between 45 and 52 days after pollination. For the best quality of its seed protein, vitamin B and C, black cultivar can be recommended as a good vegetable for human nutrition.

KeyWords

Physiological maturity, pollination, Vegetable pod, Nutrient.

INTRODUCTION

The major constituents of nutrition are calories and protein. On a world scale plant resources provide about 70% and animal about 30% of human protein needs [1]. However, in many developing countries of the tropics plants sources provide up to 88 % of food protein [2]. These legumes are inexpensive source of proteins with high nutritional profile and after cereal important food source for humans [3]. For that reason, consumption of plant protein isolate with special reference to legumes is beneficial [4]. Protein content in legumes ranged from 17 – 40 %, contrasting with that of cereals 7 – 13 % and comparable with meat 18 – 25 % [5]. Lima bean (*Phaseolus lunatus*) is like many other legumes is a rich source of plant protein which compares favorably with other legumes one of which are leading protein source in many parts of the world. It's a new word legume that has been domesticated in areas corresponding to present day Peru and Mexico. Many authors have emphasized their importance for relieving protein malnutrition in the humid tropics [6, 7]. They are many varieties of lima bean [8] of all these varieties the immature and mature seeds are the main products. Reports show that both the seeds and leaves can be eaten as pot herb when they are young and tender [9, 10]. It is a nutritious food stuff which is cultivated primarily for immature vegetables or mature dry seeds [11]. Seed development is the period between fertilizer and maximum fresh weight accumulation and seed maturation begins at the end of seed developments and continues till harvested [12]. Attainment of physiological maturity is a genotypic character which is influenced by environmental factors [13]. Seed yield and quality largely depends on the stage of maturity [14]. Physiological maturity is attained when the seed reaches its maximum dry weight [15] at which nutrients are not flowing into the seed from the mother plant. As such, harvesting of seeds at right stage of maturity is most important since harvesting either at early or late stage results in lower yields with poor quality seeds [16].

This research was carried out to investigate the changes in seed quality of winter rapeseed cultivars at different stages of development and maturity in order to determine the appropriate time for harvest and quality improvement.

MATERIAL AND METHODS

Experimental site, plant material and cropping practice

The red and black cultivars of lima beans used for this research work, has been cultivated from February 2014 to January 2015 at the experimental station of Tomasset (Azaguié, Côte d'Ivoire). The experimental dispositive has been sown on two plots. The plots are separated to 10 m. A plot of 46 m x 10 m composed of twelve holes constitutes for each cultivar. The holes of the

plot were separated to 5 m x 3 m. After the appearance of the first leaves of about two meters guardians were assigned to each plant. Seedlings were rejected after the emergence of way to keep only the strongest plant. After the lifting, tutors of approximately two meters in height were allotted to each plant. A regular weeding is done to avoid any competition between the weeds and the interest of plant.

Pods were harvested at four stages of maturity for the variety: 32 days after pods set (DAP), at 38 days after pods set (DAP), at 45 days after pods set (DAP), at 52 days after pods (DAP). The seed were extracted from each pod, washed and oven-dried (Memmert, Germany) at 60 °C for 72 h [17]. The dried powdered samples obtained were stored in polythene bags at 4°C until for analysis.

Color and weight determination of pods and seeds

The colors of the pods and seeds of *phaseolus lunatus* (L.) were measured using the ICL (International Commission for Lighting) L*, a* and b* color system. The Cie Lab coordinates (L*, a*, b*) were directly read with a spectrophotometer MS/Y-2500 (Hunter lab, In., Reston, VA, USA), calibrated with a white tile. Color values were recorded as L* (Lightness) – the vertical co-ordinate runs from L* = 0 (black) through grey to L* = 100 (white); a* (-a, greenness, +a, redness) – the horizontal co-ordinate, that runs from -a* (green) through grey to +a* (red) and b* (-b, blueness, +b, yellowness) – another horizontal co-ordinate, that runs from -b* (blue) through grey to +b* (yellow) Papadakis et al. [18]; Al-Said et al. [19]. The measurements were repeated on four different pods and seeds randomly selected locations at the surface of each sample.

The weight of pod and seed was measured using an electronic balance (Mettler, Toledo, Switzerland, ± 0.01 g).

Moisture

The moisture content of seed samples was determined by ISTA (1976) [21]. Ten grams (10g) seed samples each of *Phaseolus lunatus* (L.) were taken into moisture cup and put into a pre-heated oven at temperature of 105° C during 24 h. The experiment was made in triplicate. After cooling, the weight of the container with its cover and contents were weighed. The seed samples were cooled in desiccators and weighed. The seed moisture content was determined by dry weight basis and was calculated by the following formula 1:

$$\{(M2-M3) / (M2-M1)\} \times 100 \quad (1)$$

Where, M1 is the weight in gram of the container and its cover, M2 is the weight in gram of the container, its cover and its contents before drying, and M3 is the weight in gram of the container,

its cover and contents after drying.

Crude protein determination by Kjeldahl method

One gram (1g) of dried powdered sample were transferred in temperature resistant glass flask, was heated at 400 °C during 4 h in the presence of a pinch of the mixture of catalyst (Selenium + potassium sulphate (K₂SO₄) and 20 ml of sulphuric acid (H₂SO₄) 95-97 %. 60 ml of distilled water are added to the mineralisât obtained. To this volume, were added 50 ml of soda (40 %, p/v) before being carried to boiling in a distiller of the type LEGALLAISR. The ammonia which got clear was trapped in a dosing mud containing 10 ml of the acido-basic mixture (4 %, p/v) indicating mixed (methyl red + green of bromocrésol) at pH 4,4 -5,8. Proportioning was carried out by a sulphuric solution décimolaire of acid. Crude protein content was calculated by multiplying the nitrogen content by a factor of 6.25.

Crude fat

The crude fat was determined by continuous extraction in a soxhlet apparatus for 8 h using hexane as solvent [20]. Five grams (5g) of dried powdered sample was introduced into a cartridge of WHATMAN. 200 ml of hexane were added in a balloon of extraction weighed with vacuum. The balloon containing the hexane (M₁) was deposited on the heating cap (110 °C) during 8 h. After extraction, the balloon was withdrawn from the device of SOXHLET and put at the drying oven with 130 °C during 1h for the total evaporation of solvent. After evaporation, the balloon was weighed again (M₂). The lipid content (TL) was given by the following equation 2:

$$TL (\%) = \frac{(M_2 - M_1)}{5 \text{ g}} \times 100 \quad (2)$$

Total sugars determination

The method described by Dubois et al. [22] was used for the total sugar content determination. The ethanosoluble extract (150 µL) was put in a test tube. To this volume, are added 1 ml of phenol (5%, p/v) and 1 ml of concentrated sulphuric acid (97%). The reading of the optical density was carried out to 490 Nm with spectrophotometer (JASCO V530) against a witness containing 150 µL distilled water instead of the ethanosoluble extract. The optical density was converted into quantity of total sugars thanks to the curved standard obtained starting from a solution of glucose (2 mg/mL).

Reducing sugar

The reducing sugar content was determined according to the method of Bernfeld [23] using 3.5 dinitrosalysilic acid. 1 ml of extract was put in a test tube. To this volume, are added 300 μ l of DNS (acid 3.5 dinitrosalicylic). The mixture was carried to the bath Marie boiling during 5 mn. After cooling during 5 min on the straw mattress, 2 ml of distilled water were added to the reactional medium. The reading of the optical density was carried out at 540 Nm with spectrophotometer (JASCO V530) against a witness containing 150 μ L of distilled water and 300 μ L of DNS. The optical density was converted into quantity of total sugars thanks to the curved standard obtained starting from a solution of glucose (2 mg/mL).

Crude fiber

Crude fiber content was determined according to the gravimetric method of Van Soeest [24]. About 2 ± 0.01 g of dried powdered sample was digested with 0.25 M sulphuric acid and 0.3 M sodium hydroxide solution. The insoluble residue obtained was washed with distilled water and dried in oven (Memmert, Germany) at 100°C until. Carbohydrates and calorific value were calculated using the following formulas by Müller and Tobin [25].

$$\text{Carbohydrates: } [100 - (\% \text{ proteins} + \% \text{ lipids} + \% \text{ ash} + \% \text{ crude fibre})] \quad (3)$$

The energy value of the seed (kJ) was estimated by multiplying the percentages of crude protein, crude lipid and NFE by the factors 2.4, 8.37 and 2.4 respectively [26].

$$\text{Calorific value: } [\% \text{ proteins} \times 2.4 + \% \text{ lipids} \times 8.37 + \% \text{ carbohydrates} \times 3.57] \quad (4)$$

Vitamin C determination

Vitamin C was determined by titration using the method described by Pongraz *et al.* [27]. About 10 g of ground fresh seed of *Phaseolus lunatus* were soaked for 10 min in 40 mL methaphosphoric acid-acetic (2 %, w/v). The mixture was centrifuged at 3000 rpm for 20 min and the supernatant obtained was diluted and adjusted with 50 mL of bi-distilled water. 10 mL of the mixture was titrated to the point with dichlorophenol-indophenol (DCPIP) 0.5 g/L.

Vitamin B determination

All fresh seed of *Phaseolus lunatus* were washed and dried weighed 50 mg and cut into small pieces and extracted with 0.1 NHCl on water bath at suitable temperature and period. All extracts were filtered through 0.40 micron filter and taken into 100 mL volumetric flash and volume was add up for mobile phase. Stock of standard (Sigma Aldrich Analytical grade Rea-

gent) prepared by dissolving 0.01 g of each standard in 100 mL of mobile phase followed by successive dilutions. HPLC equipped with UV detector and supelco discovery C- 18 column (25 cm in length and 0.45 internal diameter) was used for analysis. Mobile phase was 50 mL MK_2HPO_4 and MeOH (70:30) at 1 mL/min flow rate and 10 μL of each sample/standard was injected and monitored at UV 254 nm by Fatim *et al.* [28].

Total Ash

The total ash content was determined by heating 10 g of the dried sample in a silica dish by incinerating in a furnace at 550 °C for 8 h [20].

Mineral analysis

Minerals were analyzed by the method reported by Oshodi [29]. The ash obtained from 1g of sample was dissolved in 10 % HCl, filtered with filter paper and made up to standard volume with dionised water. Flame photometry method reported by AOAC [20] was used to determine sodium and potassium contents of the sample. Calcium, Fe, Mg, Zn and Cu were determined using Atomic Absorption Spectrophotometer (AAS). Phosphorus was estimated colorimetrically (UV-visible spectrophotometer, Model DR 2800/United States).

Statistical analysis

The analysis of variance (ANOVA) was used to determine the differences between treatments. When a difference was observed, the multiple range tests of Newman-Keuls at 5% were performed to separate treatment means. Statistical tests were performed using the STATISTICA software version 7.1

RESULTS AND DISCUSSION

Color of Pods and seeds

Color of Pods and seeds of *Phaseolus lunatus* (L.) at different maturity stages is resumed in **Table 1 and 2**: Color is a primary indicator of maturity or ripeness and is derived from the pigments found in the product [30]. The pods of red and black beans cultivars of *Phaseolus lunatus* (L.) have significantly higher yellowness (*b* value) and lightness (*L* value) during maturation. The visual pods color was used in the study. The first harvest is green, the second harvest (more green than yellow); the third harvest is more yellow than green and the last one is

drowned. Red color intensity (*a* value) of seed was significantly higher and positively in red and black beans during maturation. (*a* value) ranged from (-2.61 - +8.92) red beans and (-3.91 – +3.50 %) in black beans. The seeds of red and black cultivars of *Phaseolus lunatus* (L.) have significantly higher yellowness (*b* value) and lightness (*L* value).

The loss of chlorophyll during maturation and pigments as flavanoids and carotenoids synthesis explained red and black color of seeds [33, 34]. Also, the change in seed and pod color could be a dependable indicator of physiological maturity of *Phaseolus lunatus*. Visual indicators of physiological maturity have been suggested for other Umbelliferae such as seed color in carrot [31] and eryngo [32]. Differences in pod and seed color also lead to differences in the amounts of color pigments in the pod and seed coat. The loss of the green colors of seed, along with change in seed texture are considered as practical and rapid field indicators for seed harvest, which relate to seed dry weight and moisture content [33, 34].

Table 1: Physical Characteristics of pods of *Phaseolus lunatus* (L.) during maturation

Cultivars	Stages of maturity (Days)	L*gs	a*gs	b*gs	C*gs	H*gs
RC	St1(32)	36,33 ± 0,67 ^{ef}	-10,05 ± 0,45 ^g	19,30 ± 0,61 ^{fg}	21,48 ± 0,82 ^b	17,03 ± 0,47 ⁱ
	St2(38)	40,41 ± 0,26 ^{cd}	-9,66 ± 0,33 ^{gh}	22,68 ± 0,61 ^{cd}	24,71 ± 0,33 ^{efg}	12,97 ± 0,17 ^b
	St3(45)	43,20 ± 0,49 ^{ab}	-5,22 ± 0,25 ^b	25,66 ± 0,09 ^b	26,38 ± 0,47 ^e	15,77 ± 0,21 ^c
	St4(52)	35,12 ± 0,37 ^f	-0,28 ± 0,02 ^d	16,72 ± 0,12 ^e	16,81 ± 0,40 ^c	16,27 ± 0,40 ^g
BC	St1(32)	32,42 ± 1,67 ^f	-9,57 ± 0,46 ^h	18,45 ± 1,55 ^{eg}	20,45 ± 0,54 ^g	17,48 ± 0,63 ⁱ
	St2(38)	36,98 ± 1,78 ^{ef}	-9,41 ± 0,66 ^{eh}	19,29 ± 1,29 ^{fg}	21,63 ± 0,32 ^{ef}	8,15 ± 0,34 ^a
	St3(45)	38,36 ± 0,46 ^{de}	-7,43 ± 0,04 ^a	19,76 ± 1,03 ^{fg}	21,96 ± 0,59 ^g	7,24 ± 0,21 ^h
	St4(52)	37,51 ± 0,53 ^{ef}	+3,7 ± 0,25 ^f	19,86 ± 1,37 ^{fg}	20,49 ± 0,39 ^{fg}	8,58 ± 0,18 ^d

Data are represented as Means ±SD (n=3). Means in the column with no common letter differ significantly (P < 0.05) for each seed vegetable. St1 (green pod), St2 (pod more green than yellow), St3 (pod more yellow than green) and St4 (drown pod). RC (Red cultivar) and BC (Black cultivar)

Table 2: Physical Characteristics of seeds of *Phaseolus lunatus* (L.) during maturation

Cultivars	Stages of maturity (Days)	D				
		L*gr	a*gr	b*gr	C*gr	H*gr
RC	St1(32)	52,13 ± 2,01 ^g	-2,61 ± 0,77 ^c	16,10 ± 1,09 ^{de}	16,31 ± 0,47 ^{fh}	7,82 ± 0,34 ^e
	St2(38)	46,55 ± 0,33 ^e	+5,76 ± 0,76 ^b	11,82 ± 0,40 ^b	13,14 ± 0,17 ^b	24,82 ± 0,46 ^g
	St3(45)	44,71 ± 0,76 ^e	+9,75 ± 1,55 ^a	12,37 ± 0,91 ^b	18,54 ± 0,21 ^d	37,49 ± 0,417 ^b
	St4(52)	34,65 ± 0,66 ^c	+8,92 ± 0,38 ^a	14,03 ± 0,33 ^a	16,62 ± 0,40 ^{df}	32,90 ± 0,60 ^a
BC	St1(32)	52,57 ± 0,38 ^{dg}	-3,91 ± 0,32 ^c	16,77 ± 0,87 ^e	17,21 ± 0,63 ^h	13,59 ± 0,41 ^d
	St2(38)	37,25 ± 1,05 ^b	+3,33 ± 0,44 ^c	6,83 ± 0,63 ^c	07,59 ± 0,34 ^e	24,61 ± 0,50 ^g
	St3(45)	28,78 ± 0,76 ^f	+2,85 ± 0,47 ^c	6,50 ± 0,30 ^c	07,09 ± 0,21 ^c	22,93 ± 0,84 ^f
	St4(52)	27,50 ± 0,18 ^f	+3,50 ± 0,41 ^c	7,91 ± 0,28 ^c	08,64 ± 0,18 ^e	22,94 ± 0,32 ^f

ted as Means ±SD (n=3). Means in the column with no common letter differ significantly (P < 0.05) for each seed vegetable. St1 (green pod), St2 (pod more green than yellow), St3 (pod more yellow than green) and St4 (drown pod). RC (Red cultivar) and BC (Black cultivar)

Weight of Pods and seeds

Pods and seeds weight of red beans and black beans increase significantly different (p < 0.05) to 32 at 38 days and decreased after 38 days during maturation (**Figures 1 and 2**). Pod weight ranged from (12.20 ± 0.30 to 14.58 ± 0.38), decreased (14.58 ± 0.38 to 6.86 ± 0.15 g). Seed weight ranged from (1.64 ± 0.05 to 1.91 ± 0.10 g), decreased (1.91 ± 0.10 to 1.12 ± 0.07 g). The decrease of weight pod and seed in the present study, as maturity advanced is probably due to the physiological maturity is usually used to denote maximum dry mass (DM) accumulation in the seed [35].

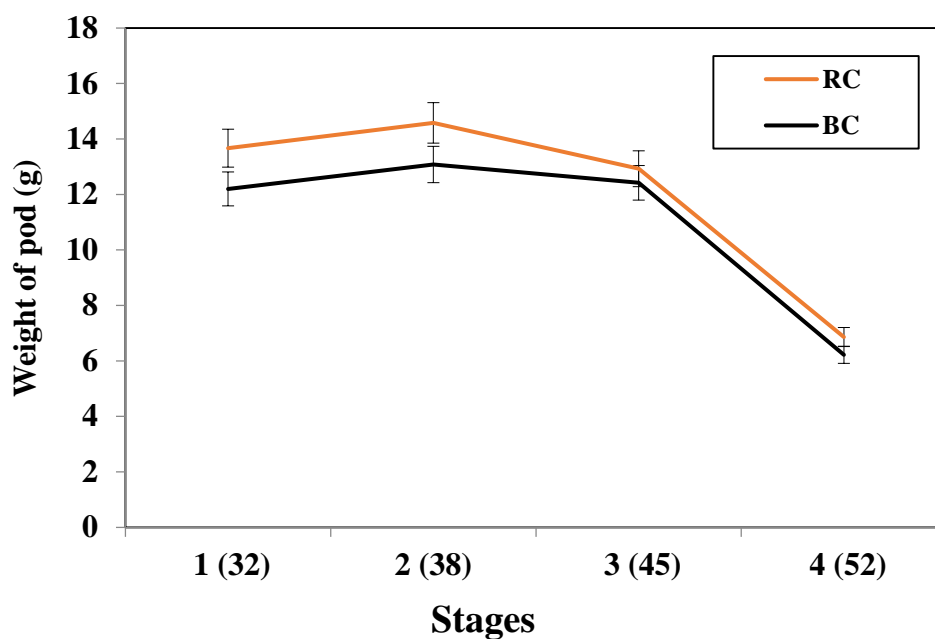


Figure 1: Weight of red and black beans pods during maturation

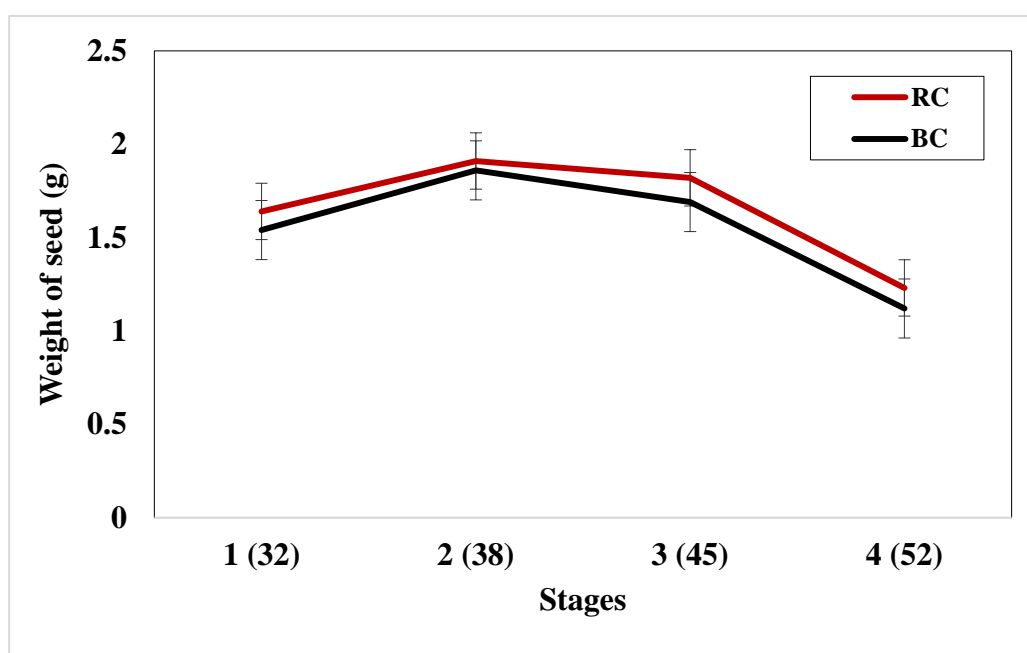


Figure 2: Weight of red and black seeds during maturation

Nutritive proprieties

The proximate composition of seed *Phaseolus lunatus* during maturation is resumed in **Table 3**. The moisture, carbohydrate and Energy in seed of the two cultivars decreased during to Stage1 at Stage 4. The moisture and carbohydrate decreased generally differ significantly ($P < 0.05$) in the seed of *Phaseolus lunatus* during maturation.

Moisture

The decrease in seed moisture ranged from (69.97 – 33.02 %) red beans and (72.54 – 35.80 %) in black beans (**Table 3**). This decrease in seed moisture at early developmental stages was a result of the increase in dry matter. Also, the decrease of moisture in the present study, as maturity advanced is probably due to the utilization of water in various metabolic activities and removal of water by desiccation caused by environment [36]. This loss of moisture was also observed by [37] during similar studies with (*Anethum graveolens* L.).

Carbohydrates

The carbohydrates decreased during maturation. Carbohydrates ranged from (73.60 – 69.45 %) red beans and (72.91 – 67.94 %) in black beans (**Table 3**). The decrease in seed carbohydrates at early developmental stages was a result of the seed desiccation. The decrease in carbohydrate content of seeds of *Phaseolus lunatus* can be attributed to the transformation of starch into soluble sugars under the action of phosphorylase enzyme during maturation [38] (**Figure 3 and 4**). The result obtained of the stage 4 for different varieties of *Phaseolus lunatus* was similar of the different varieties of *Vigna mungo* had a higher range of carbohydrate (61.24 – 64.43 %), than peanut and soybeans [40]. The high carbohydrate contents of *Vigna mungo* enable this vegetable to act as a good source of calories which would be antimarasumus, especially infant nutrition [39].

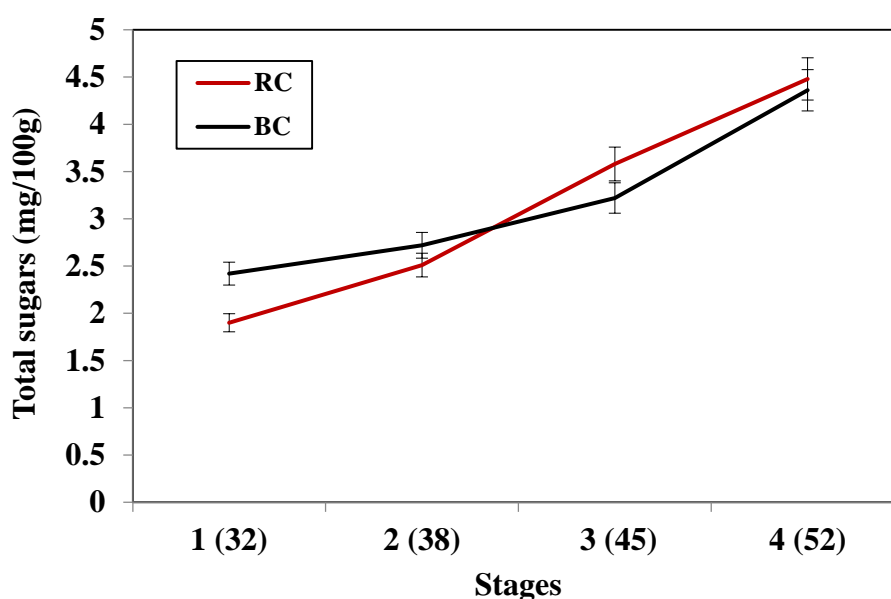


Figure 3: Total sugar of red and black beans seeds during maturation

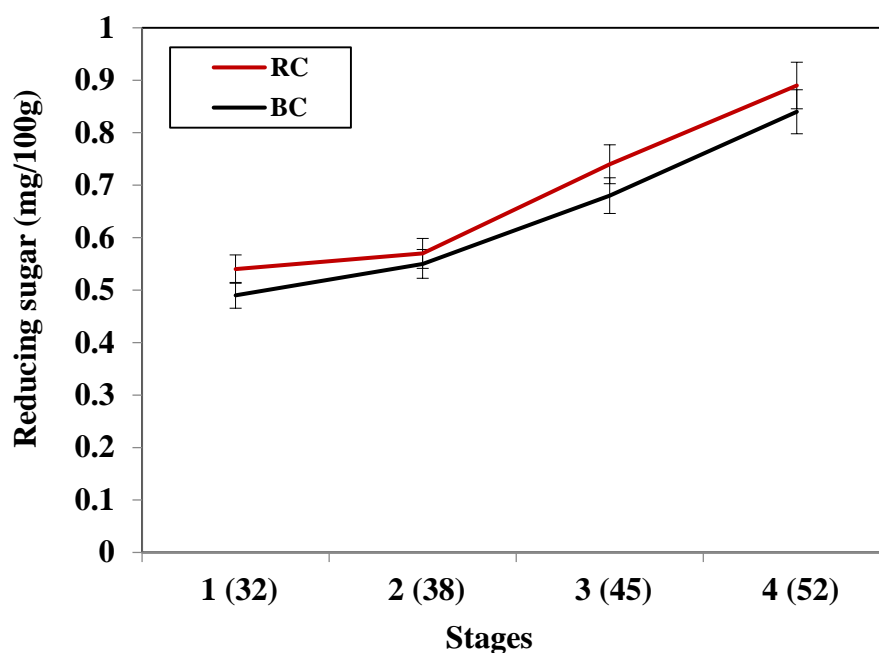


Figure 4: Reducing sugar of red and black beans seeds during maturation

Energy

No significant differences were noted among decreased Energy in seed of the three cultivars from Stage 1 to Stage 4 (**Table 3**). Energy ranged from (314.54 – 311.59 %) red beans and (312.51 – 310.51 %) in black beans. The decrease energy at early developmental stages was a result of decreased carbohydrates. The caloric value was due to its low-fat content and decreasing of carbohydrate content. The range in calorific values was comparable to energy values of cowpea, green gram, horse gram, moth beans and peas [40] which are in the range of 1318–1394 KJ 100 g⁻¹ DM.

Crude proteins

Crude proteins ranged from (17.21 - 20.60 %) red beans and (17.21 – 21.21 %) in black beans (**Table 3**). Black beans of *Phaseolus lunatus* was found to be higher (21.21 %) amount of crude protein when compared to certain vegetables such as *Cicer arietinum* (20.70 %) and lowest *Vigna mungo* (23.60 %) and *Vigna radiata* (24.50 %) as reported by Bravo et al. [41]. To meet the protein demands in developing countries where animal protein is grossly inadequate, considerable attention is being paid to less consumed protein sources, especially in legumes which are considered as protein tablets [42]. The crude proteins levels of the studied samples suggest

its usefulness as alternative source of protein. The proteins have as a role, the replacement of cells died in the adults, a good growth of nourrissons and children, a good development of the foetus among pregnant women and a good secretion of the mother's milk during breast feeding Cheer *et al.* [43].

Ascorbic Acid

As observed in **Table 3**, ascorbic acid content significantly varied among samples harvested during different stages and it ranged from (3.10-6.43 mg/100g) red beans and (5.23± 0.14 - 8.63±0.20 mg/100g) at stage 1 to at stage 4. According to the results of [44], vitamin C levels in the kale leaves ranged from 77 to 133 mg 100 g⁻¹ and they depend on the variety and degree of plant maturity. Singh *et al.* [45] stated that vitamin C content ranged from 9.66 to 52.90 mg 100 g⁻¹ and that the harvesting stage of the samples might be another important source of variation. [46] gave that climatic conditions might also alter vitamin C level. According to Maorun *et al.* [47], ascorbic acid content varied significantly during maturation and showed a growing trend as maturity advances. [48], reported that maturity is among the major factors that define the compositional quality of fruits and vegetables.

Crude fat

Changes in lipid ranged from (1.05 – 1.60 %) red beans and (1.1 – 2.17 %) in black beans during seed development were observed in (**Table 3**). Crude fat content of white bean was comparable to the range of 1.3-2.3 g/100 g reported for some lima bean varieties Bello-Perez *et al.* [49]; Granito *et al.* [50]; [51] and a range of 0.66-1.27 g/100 g reported for several other food grains [52]. Lipids are an important component of diet and several functions in the human body. Lipids are a concentrated source of energy and supplies per unit weight more than twice the energy furnished by either proteins or carbohydrates [53].

Total fibers

The total fibers ranged from (4.1 – 5.01 %) red beans and (4.93 – 5.70 %) in black beans (**Table 3**). The presence of fibers in the diet is necessary for digestion and for elimination of wastes. The contraction of muscular walls of the digestive tract is stimulated by fibers, thus counteracting constipation Rao *et al.* [41]. The total fibers level of presently investigated was compared to certain legumes like cowpea and kidney bean [54], different varieties of *Vigna mungo* [55]; Co9, Co11 and Co12 varieties of *Lablab purpureus* [56].

Total ash

The ash content of seeds vegetables increased during maturation ranged from (2.37 – 4.25 %) red beans and (3.42 – 3.86 %) in black beans (**Table 3**). Values obtained with *phaseolus lunatus* cultivars were lower than the ash content of investigated *Mucuna* varieties/species (4.78-5.30%) [53].

The ash content of investigated of red and black beans (above 4 %) would be important to the extent that it contains the nutritionally important mineral elements. Similar values were reported in the case of *Cassia obtusifolia* by Vijayakumari *et al.* [57].



Table 3: Proximate composition in seed vegetables of red and black cultivars of *Phaseolus lunatus* (L.) consumed in South and east Côte d'Ivoire during maturation (g/100 g dry weight basis)

Cultivars	Stages of Maturity (Days)	Moisture (%)	Proteins (%)	Lipids (%)	Carbohydrates %	Fibers (%)	Ash (%)	Vitamin C	Energy (Kcal/100g)
RC	St1(32)	69,97 ± 0,37a	17,21 ± 0,29a	1,05 ± 0,02a	73,60 ± 0,45a	6,05 ± 0,18ab	2,37 ± 0,10a	3,10 ± 0,11 ^{bc}	314,54 ± 2,50a
	St2(38)	59,55 ± 0,58b	18,55 ± 0,25b	1,20 ± 0,06a	72,33 ± 0,72a	5,01 ± 0,27a	2,86 ± 0,44a	4,70 ± 0,28f	313,68 ± 2,58a
	St3(45)	47,26 ± 0,01c	19,41 ± 0,28c	1,34 ± 0,02e	71,72 ± 0,97a	4,70 ± 0,26a	2,83 ± 0,50 a	6,20 ± 0,17 ^{eg}	312,64 ± 0,94a
	St4(52)	33,02 ± 0,06d	20,60 ± 0,20d	1,60 ± 0,02f	69,45 ± 2,18e	4,10 ± 0,26ba	4,25 ± 0,20 b	6,43 ± 0,20 ^{dg}	311,59 ± 1,02a
BC	St1(32)	72,54 ± 0,94a	17,51 ± 0,13a	1,1 ± 0,3d	72,91 ± 0,10a	5,20 ± 0,20a	3,42 ± 0,44a	5,23 ± 0,14 ^f	312,51 ± 1,46a
	St2(38)	61 ± 1,13b	18,46 ± 0,02b	1,6 ± 0,6a	71,23 ± 0,22ac	5,15 ± 0,31a	3,86 ± 0,15a	6,76 ± 0,23 ^{dg}	311,90 ± 4,34a
	St3(45)	57,26 ± 0,01c	19,70 ± 0,02c	1,83 ± 0,15a	69,29 ± 0,50cf	5,70 ± 0,30a	3,47 ± 0,20 a	7,4 ± 0,21 ^d	310,78 ± 1,53a
	St4(52)	35,80 ± 0,62d	21,21 ± 0,02d	2,17 ± 0,28e	67,94 ± 0,43ef	4,93 ± 0,11a	3,74 ± 0,12 a	8,63 ± 0,20 ^a	310,51 ± 1,56a

Data are represented as Means ±SD (n=3). Means in the column with no common letter differ significantly (P < 0.05) for each seed vegetable. St1 (green pod), St2 (pod more green than yellow), St3 (pod more yellow than green) and St4 (drown pod). RC (Red cultivar) and BC (Black cultivar)

Mineral composition

Changes in mineral element contents in *Phaseolus lunatus* at the investigated fruit development stages are shown in **Table 4**. Sodium, potassium, iron and copper increased at stage 1 to stage 4 during maturation in seeds of red and black beans. Minerals ranged from ($46.12 \pm 0.87 - 75.27 \pm 0.72$ mg/100g); ($909.23 \pm 0.75 - 1592.90 \pm 6.38$ mg/100g), ($6.36 \pm 0.17 - 12.56 \pm 0.28$ mg/100g) and ($1.29 \pm 0.11 - 2.98 \pm 0.06$ mg/100g), respectively. Similar result was also observed by Al-Maiman and Ahmad [62] who reported that these minerals were found in fruit aril during development. Phosphorus, magnesium and calcium decreased during maturation. This cultivar analyzed in this investigation contained relatively high amounts from phosphorus ($470.55 \pm 0.40 - 250.48 \pm 0.67$ mg/100g); magnesium ($155.72 \pm 0.72 - 140.56 \pm 0.20$ mg/100g) and calcium ($539.50 \pm 0.70 - 359.32 \pm 0.89$ mg/100g). Changes in the mineral profile of beans can be explained by different factors, including genotypic variability in absorption of minerals from soil [63], effect of fertilizers on metallic composition of plants [64], and levels of soil salinity Carbonell-Barrchina *et al.* [65].

The iron contents of the studied seed vegetables were higher than recommend dietary allowance for males (1.37 mg/day) and females (2.94 mg/day) Siddhuraju *et al.* [66]. According to Geissler *et al.* [67]; iron plays numerous biochemical roles in the body, including oxygen binding in hemoglobin and acting as an important catalytic center in many enzymes as the cytochrome oxidase. Thus, the selected of red and black cultivars of *Phaseolus lunatus* of this study could be recommend in diets for reducing anemia which affects more than one million people worldwide [68].

The ratios of sodium to potassium (Na/K) and calcium to phosphorus (Ca/P) are also shown in **Table 4**. Na/K ratio in the body is of great concern for prevention of high blood pressure. Na/K ratio less than one is recommended. Na/K ratio of red and black bean ranged from 0.04 to 0.05 during maturation. Hence, in the present study, red and black beans seed of *Phaseolus lunatus* would probably reduce high blood pressure disease because they had Na/K less than one. Food is considered 'good' if the Ca/P ratio is above 1 and 'poor' if it is 1. Ca/P ratio of red and black beans ranged from 1.12 to 1.43 during maturation. It appears that the Ca/P ratio of white seed was comparable about the ratio Ca/P of *M. pruriens* var *utilis* white seeds [69]. The Ca/P ratio in the present study indicated that seed of white beans would serve as good sources of minerals for bone formation.

Table 4: Mineral composition in seed vegetables of red and black cultivars of *Phaseolus lunatus* (L.) consumed in South and east Côte d'Ivoire during maturation (g/100 g dry weight basis)

Cultivars	Stages of maturity (Days)	Na	K	P	Mg	Fe	Ca	Cu	Zn	Na/K	Ca/P
RC	ST1(32)	56,81 ± 0,15 ^j	1043,3 ± 1,25 ^h	569,67 ± 0,89 ^a	160,14 ± 0,66 ^a	9,13 ± 0,18 ^c	669,32 ± 0,76 ^a	1,29 ± 0,11 ^c	0,16 ± 0,03 ^{gh}	0,5	1,17
	ST2(38)	63,40 ± 0,40 ^e	1359,20 ± 1,07 ^d	430,35 ± 0,25 ^d	157,43 ± 0,66 ^f	10,78 ± 0,11 ^{dc}	529,69 ± 0,30 ^g	1,93 ± 0,03 ^d	0,05 ± 0,01 ^f	0,4	1,23
	ST3(45)	69,12 ± 0,12 ^c	1450,50 ± 1,15 ^c	340,46 ± 0,80 ^g	146,45 ± 0,10 ^e	10,86 ± 0,08 ^d	479,33 ± 0,87 ^h	1,14 ± 0,02 ^c	0,08 ± 0,01 ^{fg}	0,4	1,40
	ST4(52)	72,65 ± 0,32 ^b	1592,9 ± 6,38 ^a	250,48 ± 0,67 ^k	140,56 ± 0,20 ^g	12,56 ± 0,28 ^a	359,32 ± 0,89 ⁱ	2,86 ± 0,07 ^b	0,18 ± 0,01 ^{gh}	0,4	1,43
BC	ST1(32)	46,12 ± 0,87 ^h	909,23 ± 0,75 ^j	470,55 ± 0,40 ^c	155,72 ± 0,72 ^f	6,36 ± 0,17 ^f	539,50 ± 0,70 ^b	1,96 ± 0,02 ^d	0,16 ± 0,03 ^{gh}	0,4	1,14
	ST2(38)	55,60 ± 0,40 ⁱ	1086,80 ± 1,05 ^g	410,83 ± 0,97 ^e	139,63 ± 0,55 ^g	6,66 ± 0,24 ^{fg}	460,18 ± 0,86 ^c	1,81 ± 0,05 ^d	0,19 ± 0,01 ^g	0,4	1,20
	ST3(45)	67,62 ± 0,37 ^d	1209,2 ± 0,64 ^e	320,55 ± 0,64 ⁱ	135,95 ± 1,12 ^c	7,80 ± 0,10 ^e	359,47 ± 0,74 ⁱ	1,38 ± 0,06 ^c	0,30 ± 0,01 ^e	0,4	1,20
	ST4(52)	75,27 ± 0,72 ^a	1519,50 ± 1,40 ^b	230,18 ± 1,13 ^l	128,46 ± 0,55 ^d	9,49 ± 0,27 ^{bc}	301,03 ± 1,00 ^f	2,98 ± 0,06 ^b	1,70 ± 0,01 ^a	0,5	1,30

Data are represented as Means ± SD (n=3). Means in the column with no common letter differ significantly (P < 0.05) for each seed vegetable. St1 (green pod), St2 (pod more green than yellow), St3 (pod more yellow than green) and St4 (drown pod). RC (Red cultivar) and BC (Black cultivar)

Vitamin B

The concentrations of thiamine (B1), riboflavin (B2), Pyridoxine (B6) and Folate (B9) are presented in **Table 5**. Thiamine (B1) and riboflavin (B2) varied significantly ($P < 0.05$) at different stages. Pyridoxine (B6) and Folate (B9) was not varied significantly ($P < 0.05$) at different stages. Pyridoxine (B6) was found higher than thiamine (B1), riboflavin (B2) and Folate (B9). It ranged from $1650 \pm 2.22 \mu\text{g}/100\text{g}$ at stage 1 to $2000.03 \pm 1.32 \mu\text{g}/100\text{g}$ at stage 4. The black seeds recorded high B6 Vitamin ($1900\text{--}2000.03 \mu\text{g}/100\text{g}$) content. Vitamin content also depends on the stage of maturity of fruits and vegetables, as to reap, the crop before maturity is a common practice of farmers to get economical benefits, while some studies have also suggested the HPLC methods less compatible for vitamin finding than other essays Toma *et al.* [60]. So, comparison of vitamin determination methods is recommended. Vitamins are one of the indispensable organic components of vegetables and fruits nutrients. In this study we have selected only water-soluble B complex (B1, B2, B6, B9) vitamins which are considered necessary for cellular metabolism especially carbohydrates metabolism. The higher Pyridoxine (B6) content in the red and black seed of *Phaseolus lunatus* may be recommended for consumption because the daily permissible range for adults is up to $1000 \mu\text{g}/\text{day}$ Alexander *et al.* [61].

Table 5: B-Vitamins composition in seed vegetables of red and black cultivars of *Phaseolus lunatus* (L.) consumed in South and east Côte d'Ivoire during maturation ($\mu\text{g}/100 \text{ g}$ dry weight basis)

Cultivars	Stages of maturity (Days)					
		B1	B2	B3	B6	B9
RC	ST1(32)	$180,05 \pm 0,94^i$	$240,00 \pm 1,46^h$	ND	$1650,00 \pm 2,22^d$	$500,45 \pm 1,46^b$
	ST2(38)	$190,05 \pm 1,13^e$	$258,00 \pm 2,48^g$	ND	$1500,00 \pm 1,58^h$	$500,75 \pm 0,95^b$
	ST3(45)	$200,02 \pm 1,39^h$	$270,02 \pm 0,68^f$	ND	$1600,00 \pm 1,36^e$	$499,13 \pm 0,43^b$
	ST4(52)	$230,05 \pm 0,82^b$	$280,00 \pm 0,90^e$	ND	$1679,72 \pm 0,98^c$	$575,53 \pm 0,81^a$
BC	ST1(32)	$180,00 \pm 0,74^i$	$400,00 \pm 0,50^d$	ND	$1900,00 \pm 1,42^a$	$599,93 \pm 0,86^a$
	ST2(38)	$200,02 \pm 0,66^h$	$403,27 \pm 1,25^c$	ND	$1800,00 \pm 1,10^b$	$600,35 \pm 1,00^a$
	ST3(45)	$220,35 \pm 0,75^c$	$409,75 \pm 1,10^b$	ND	$2000,05 \pm 0,46^g$	$599,60 \pm 0,49^a$
	ST4(52)	$239,97 \pm 0,77^a$	$420,25 \pm 0,50^a$	ND	$2000,03 \pm 1,32^g$	$600,00 \pm 1,09^a$

Data are represented as Means \pm SD ($n=3$). Means in the column with no common letter differ significantly ($P < 0.05$) for each seed vegetable. St1 (green pod), St2 (pod more green than yellow), St3 (pod more yellow than green) and St4 (drown pod). RC (Red cultivar) and BC (Black cultivar)

CONCLUSION

Results revealed that maximum accumulation in seed was achieved around 52 DAF and this indicates that the Physiological Maturity (PM) of vegetable *Phaseolus lunatus* (L.) varieties was around 52 DAA in the two varieties studied. The results of chemical analysis showed that Protein, Carbohydrates, fibers and Vitamin C contents vary appreciably in dark pod of the two varieties. But the amounts of these nutrients were fairly good around 52 DAF in all the two varieties with the highest protein content was in the red variety indicating a good variety. It may be concluded that 52 DAF may be PM and vegetable *Phaseolus lunatus* pod may be harvested between 45 and 52 DAA for good nutrients in the two varieties indeterminate. From the result above, the seed of *Phaseolus lunatus* could serve as a supplementary diet for the Ivorian population, supplying the body. Hence, the studied seed vegetables could contribute to the alleviation of protein-energy malnutrition and micronutrient deficiencies if they are consumed in enough.

REFERENCES

- [1] Rachie K.O. and silvester P. Grain legumes Foods Crops of the Lowland Tropics, Oxford University Press, Oxford., pp: 44-74, 1997.
- [2] Oke O.L. A case for vegetables protein in developing countries. World Review of Nutrition and Dietetics, (23):259-295, 1975.
- [3] Amarteifio J.O, Munthali D.C, Karikare SK, Iqbal A and Khalil N.A. The composition of Lima bean Nutrition quality of important food legumes grown in Botswana. Nutr., 2005 (57): 173-176.
- [4] Doyle J.J. Phylogeny of the legume family: An approach to understanding the origins of nodulation. Annu Rev Ecol Syst., 1994 (25): 325-349.
- [5] Genovese, Lajolo Proximate composition and functional properties of winged bean (*Psophocarpus tetragonolobus*). Nig J Nutri Sci., 2001 (13): 36-38.
- [6] Baudoin J.P. Observations on some interspecific hybrids involving *Phaseolus lunatus* L. Bull. Resch. Argon. Gemboux. 1981.
- [7] Katunga K. and J.P Baudoin. Meiotic analysis of the F1 hybrids and study of F2 progenies in four

interspecific combinations with *Phaseolus lunatus* L. Bull. Resch. Argon.Gemboux., 1990.

[8] Darbie M.G., Williams T.K. and George B. Lima beans, commercial vegetable production. Georgia Extension services publication, Circular., 1999 pp: 13-17.

[9] Daisy E.K. Food legumes TPI crop product digest No 3. Tropical Product Institute, London., 1979.

[10] Van de Maessen, L.G.J and S. Sadikin. Plant resources of South Eastern Asia., 1989 (1):56-60.

[11] Lyman S.M, Baudoin J.P and Hildago R. Lima beans (*Phaseolus lunatus*) In: Grain Legume Crops. RJ Summerfield and EH Roberts (Eds.). London: Williams Collins sons & Co Ltd LondonUnited Kingdom., 1985: P.477-519.

[12] Mehta C.J., Kuhad M.S., Sheoran I.S. and Nandwal A.S. Studies on seed development and germination in chickpea cultivars. Seed Res., (1993) 21(2): 89-91.

[13] Mahesha CR, Channaveeraswami AS, Kurdikeri MB, Shekhargouda M. and Merwade MN. Storability of sunflower seeds harvested at different maturity dates. Seed Res., 2001, 29 (1): 98-102.

[14] Kumar V., Shahidhan S.D., Kurdikeri M.B., Channaveeraswami A.S. and Hosmani R. M. Influence of harvesting stages on seed yield and quality in paprika (*Capsicum annuum* L.). Seed Res., 2002, 30 (1): 99-103.

[15] Harrington J.F. Seed storage and longevity. In: Seed Biology, VII. (T.T. Kozlowski, ed.). Academic Press, New York., 1972, pp. 145-245.

[16] Khatun A., Bhuiyan M.A.H. and Dey T. Nitrogen uptake and protein yield in lentil as affected by seed collection from different parts of plants. Bangladesh J. Agril. Res., 2009, Vol. 34.

[17] Chinma C.E. and Igyor M.A. Micro-nutriments and anti-nutritional content of select tropical vegetables grown in south- east, Nigeria. Nig. Food., 2007, 25.111-115.

[18] Papadakis S.E., Abudal-Malek S., Kamden R.E. and Yam K.L. A versatile and inexpensive techniques for measuring colour of foods. Food Technol., 2000, 54 (12): 48-51.

[19] Al-Said F.A., Opara U.L. and Al-Yahyai R.A. Physico-chemical and textural quality attributes of

pomegranate cultivars (*Punica granatum* L.) grown in the Sultanate of Oman. *J. Food Eng.*, 2009, 90:129–134.

[20] AOAC, Official Methods of Analysis. Washington, DC: Association of Official Analytical Chemists 15th edn., 1990.

[21] ISTA (International Seed Testing Association). International Rules for Seed Testing. *Seed Sci. Tech.* 1976, 4: 3-49.

[22] Dubois M., Gilles K.A., Hamilton J.K., Rebers P.A. and Smith F. Colorimetric method for determination of sugars and related substances. *Anal Chem.*, 1956, 28 350–356.

[23] Bernfeld P. Amylase α and β methods in enzymology 1. S. P. Colowick and N.O. K, edition academic press, Inc New-York., 1955, 149-154 p.

[24] Van Soest P.J. Use of detergent in the analysis of fibrous seeds. A rapid method for the determination of fiber and lignin. *J. Ass. Offc Agr Chem.*, 1963, 46:829-835.

[25] Müller H.G. and Tobin G. Nutrition and Food Processing. London: Croom Helm Ltd., 1980.

[26] FAO. Food energy-methods of analysis and conversion factors. FAO Ed, Rome. 2002, 97.

[27] Pongraz G., Weiser H. and Matzinger D. Tocopherols-Antioxydant. *Fat Sci. Technol.*, 1971, 97: 90-104.

[28] Ismail F., Talpur F.N. and Memon A.N. Determination of Water Soluble Vitamin in Fruits and Vegetables Marketed in Sindh Pakistan. *Pak J Nutri.*, 2013, 12 (2): 197-199.

[29] Oshodi A.A. Proximate composition, nutritionally valuable minerals and functional properties of *Adenopus breviflorus* benth seed flour and protein concentrate. *Food Chem.*, 1992, 45: 79-83.

[30] Shewfelt R.L. Quality of minimally processed fruits and vegetables. *J Food Quality.*, 1987, 10: 143–148.

[31] Rubatzky V.E., Quiros C.F. and Simon P.W. Carrots and related vegetable Umbelliferae. Crop production science in Horticulture series 10. CABI Publishing. 1999.

[32] Ekong B. and Samutprakarn S. Seeds physiological maturity in dill (*Anethum graveolens* L.) *Kasetsart J.*, 2006, 42: 1-6.

- [33]- Demir I. Development of seed quality during seed development in okra. Acta Hort., 1994, 362: 125-131.
- [34] Elias S.G and Copeland L.O. Physiological and harvest maturity of canola in relation to seed quality. Agron J., 2001, 93: 54-58.
- [35] Copeland L.O. and McDonald M.B. Seed vigor and vigor test. In Principles of Seed Sci. Technol. (ed.) Chapman and Hall, 1995, p. 157.
- [36] Sreeramulu N., Tesha A.J. and Kapuya J.A. Some biochemical changes in developing seeds of bambarra groundnut (*Voandzeia subterranea* Thouars). Ind. J. Plant Physiol., 1992, 35: 191-194.
- [37] Egli, D.B. Seed Biology and the Yield of Grain Crops. CABI International. Wallingford, UK, 1997, 178p.
- [38] Germain P et Linden G. Activités enzymatiques. In: Deymier, B., Multon, J.L., Simon, D (eds) Analyse des constituants alimentaires. Techniques d'Analyse et de contrôle dans les industries agrolimentaires, Tec. Et Doc Lavoisier, Paris., 1981, 4: 211-244.
- [39] Vadivel V. and Janardhanan K. Chemical composition of the underutilized legume *Cassia hirsuta* L. Plant Foods Hum. Nutri., 2000, 55: 369-381.
- [40] Rao N., Deosthale B.S. and Pant Y.G. Nutritive Value of Indian Foods. Hyderabad, India: National Institute of Nutrition, Indian Council of Medical Research., 1989.
- [41] Bravo L., Siddhuraju P. and Sauvo-Calixto F. Composition of under exploited Indian pulses. Comparison with common legumes. Food Chem., 1999, 64: 185-102.
- [42] Salunkhe, D.K. Legumes in human nutrition: current status and future research needs. Current Sci., 1982, 57: 387-394.
- [44] Korus, A. Level of vitamin C, polyphenols and antioxidant and enzymatic Activity in three varieties of kale (*Brassica Oleracea* L. var. *acephala*) at different stages of maturity. Int. J. Food Properties, 2010, 14:1069-1080.
- [45] Singh J., Upadhyay A.K., Prasad K., Bahadur A. and Rai M. Variability of carotenes, vitamin C, E and phenolic in Brassica vegetables. J. Food Comp. Anal., 2007, 20: 106–112.

- [46] Podsedek A. Natural antioxidant and antioxidant capacity of Brassica vegetables. Food Sci.Technol., 2007, 40 (1): 1-11.
- [47] Maorun H., Zhiping Z., Yuying Y., Jing Y. and Linchun M. Antioxidant properties and involved compounds of daylily flowers in relation to maturity. Food Chem., 2009, 114: 1192-1197.
- [48] Lee S.K. and Kader A.A. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest Bio Technol., 2000, 20: 207-220.
- [49] Bello-Pe´rez L.A., Sa´yago-Ayerdi S.G., Cha´vez-Murillo C.E., Agama-Acevedo E. and Tovar J. Proximal composition and in vitro digestibility of starch in lima bean (*Phaseolus lunatus*) varieties. J. Sci. Food Agric., 2007, 87: 2570–2575.
- [50] Granito M., Brito Y. and Torres A. Chemical composition, antioxidant capacity and functionality of raw and processed (*Phaseolus lunatus*). J. Sci. Food Agric., 2007, 87: 2801–2809.
- [51] Apata D.F. and Ologhobo A.D. Biochemical evaluation of some Nigerian legume seeds. Food Chem., 1994, 49: 333–338.
- [52] Barampama Z. and Simard R.E. Nutrient composition, protein quality and antinutritional factors of some varieties of dry beans (*P. vulgaris*) grown in Burundi. Food Chem., 1993, 47: 159–167.
- [53] Tresina P.S. and Mohan V.R. Comparative assement on the nutritional and antinutritional attributes of the underutilized legumes, *Canavalia gladia* (JACQ.) DC, *Erythrina indica* LAM and *Abrus precatorius* L. Trop Subtrop Agroecos., 2012, 15: 539 – 556.
- [54] Singh J.N., Kumar R., Kumar P. and Singh P.K. Status of dietary fibers in new millennium-A review. Ind J Nutri Diet., 2000, 37: 261-273
- [55] Tresina P.S., Kala K.B., Mohan V.R. and Vadivel V. The biochemical composition and nutritional potential of three varieties of *Vigna mungo* (L.) Hepper. Advances in Bioresearch, 2010, 1: 6-16.
- [56] Kala KB, Tresina S.P, Mohan VR and Vadivel V. Nutrient and chemical evaluation of raw seeds of five varieties of *Lablab purpureus* (L.) Sweet. Advances in Bioresearch, 2010, 1: 44-53.
- [57] Vijayakumari K. Siddhuraju P. and Janardhanan K. Nutritional and anti-nutritional properties of certain underexploited legume seeds. Int Food Sci Nutri., 1993, 44: 181-189.

- [58] Kalidass C. and Mohan, V.R. Nutritional composition and anti-nutritional of factors of little-known species *Vigna*, Tropical and Subtropical Agroecosystems., 2012, 15: 525 – 538.
- [59] Kalidass C. and Mahapatra A.K. Evaluation of the proximate and phytochemical compositions of an underexploited legume *Mucuna pruriens* var. *utilis* (Wall ex Wight) L.H. Bailey. *Int Food Research J.*, 2014, 21(1): 303-308.
- [60] Toma R.B. and Tabeckia M.M. HPLC analysis of B-vitamins in rice and rice products. *J. Food Sci.*, 1979, 44:263-266.
- [61] Alexander M., Emanuel G., Golin T., Pinto J.T. and Rivlin R.S. Relation of riboflavin nutriture in healthy elderly to intake of calcium and vitamin supplements: Evidence against riboflavin supplementation. *Am. J. Clin. Nut.*, 1984, 39: 540-546.
- [62] Al-Maiman S.A. and Ahmad D. Changes in physical and chemical properties during pomegranate (*Punica granatum* L) fruit maturation. *Food Chem.*, 2002, 76: 437– 441.
- [63] Vadivel V. and Janardhanan K. Nutritional and anti-nutritional attributes of the underutilized legume, *Cassia floribunda* Cav. *Food Chem.*, 2001, 73: 209-215.
- [64] Sadiq M. and Hussain G. Effect of chelate fertilizers on dry matter and metallic composition of bean plants in a pot experiment. *J Plant Nutri.*, 1994, 17 (9): 1477-1488.
- [65] Carbonell-Barrchina A.A.; Burlo F. and Mataix J. Response of bean micronutrient nutrition to arsenic and salinity. *J Plant Nutri.*, 1998, 21(6): 287-299.
- [66] Siddhuraju P., Becker K. and Makkar H.S. Chemical composition, protein fractionation, essential amino acid potential and antimetabolic constituents of an unconventional legume, Gila bean (*Entada phaseoloides* Merrill.) seed kernel. *J. Sci. Food Agri.*, 2001, 82: 192 – 202.
- [67] Geissler C.A., Powers H.J. *Human Nutrition*. Elsevier, Churchill, Livingston, 2005.
- [68] Trowbridge F. and Martorell M. Forging effective strategies to combat iron deficiency. Summary and recommendations. *J. Nutri.*, 2002, 85: 875-880.
- [69] Adeyeye E. and Fagbohun E.D. Proximate, mineral and phytase profiles of some selected spices found in Negeria. *Pakistan J Sci Industr Research.*, 2005, 48 (1): 14 – 22.