GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Effect of cooking time on the nutritional and anti-nutritional properties of red and black beans of *Phaseolus lunatus* (L.) consumed in south and east of Côte d'Ivoire

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GSC Biological and Pharmaceutical Sciences, 2023, 22(01), 269–281

Publication history: Received on 26 October 2022; revised on 16 December 2022; accepted on 18 December 2022

Article DOI: https://doi.org/10.30574/gscbps.2023.22.1.0459

Abstract

Legume seeds, particularly those of the *Phaseolus lunatus* (L.), are an important source of vegetable protein, fiber and micronutrients in the diet of the Ivorian population. However, these seeds contain anti-nutritive factors that limit their use. This study allowed us to evaluate the effect of cooking time on the nutritional and anti-nutritional parameters of *Phaseolus lunatus* (L.) bean seeds for a better utilization of these seeds. Red and black seeds harvested at stage 4 (52 days) of *Phaseolus lunatus* (L.) bean were cooked at 100 °C for 45, 60 and 75 minutes. The nutritional and anti-nutrional composition was determined on uncooked (control) and cooked samples. The results obtained showed an increase in carbohydrate (64.16 to 71.56%), energy value (29.68 to 36.27%) and fiber (4.62 to 6.05%) followed by a reduction in protein (4.62 to 6.05%), ash (4.62 to 6.05%) and fat (4.62 to 6.05%). The same cooking also caused a reduction in total phenols (59.18 to 72.49%), phytates (33.32 to 39.18%), oxalates (64.64 to 68.91%), tannins (62.07 to 67.59%), cyanidric acid (58.18 to 61.38%) and antioxidant activity (53.71 to 63.94%). The analysis of the seeds cooked at different times (45, 60 and 75 minutes) also shows us that the B vitamins and minerals decrease during cooking. The consumption of *Phaseolus lunatus* bean seeds cooked at 45 minutes could be recommended in human diet due to the decrease of oxalates and phytates to prevent and treat some diseases such as cancer, inflammatory diseases, cardiovascular diseases and neurogenerative diseases.

Keywords: Cooking; Nutritional Value; Bean Cultivars; Nutrition

1. Introduction

Phaseolus lunatus (L.) belonging to the family Fabaceae and genus *Phaseolus*, is a leguminous plant which originates from Peru. Lima bean is the second most economically important of the species of *Phaseolus* and is one of the 12 mostly used legumes in the World [1]. Its cultivation in Africa spans through a wide range of ecological conditions viz warm temperate zones, arid and semi-arid tropical regions [2]. Lima bean is one of the grain legumes that are under-utilized in Ivory Coast [3]. There seeds are commonly consumed among the rural of Ivory Coast [3]. It can either be consumed solely or cooked in combination with cereals such as rice or tuber such as yam [4]. Lima beans can also serve as substitute for the expensive soy meal and groundnut meal which constitute the major portion of conventional protein sources used in composite livestock feeds [5]. Lima beans possess good nutritional profile being a good source of minerals, dietary fibers, carbohydrates and proteins but, it is low in fat [6,2]. Its B-complex vitamins content includes pyridoxine, thiamine, pantothenic acid, riboflavin, and niacin. While its mineral content includes molybdenum, iron, copper, manganese, calcium and magnesium [1; 7]. Lima bean is a cheap source of protein when compared with animal products such as meat, fish, and egg [2]. The seeds contain protein twice as much present in cereals with more balanced profile of essential amino acids including lysine which is lacking in cereals [8]. Lima bean seeds are also rich in bioactive compounds which promote human health [9] and their regular dietary intake contribute to healthy living [8].

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Underutilization of Lima bean is as a result of certain reasons in which its rare popularity, hard seed coat which prolongs its cooking, inadequate processing methods and presence of anti-nutritional factors are inclusive [4 ;10]; [6]). Anti-nutrients are components which interfere with absorption and utilization of important minerals as well as reducing protein digestibility and the nutritive value of foods thereby causing a level of damage to the consumers [6]. Anti-nutritional factors in Lima bean include trypsin inhibitors, phytic acid, oxalate, tannins and cyanide [11]). Utilization of lima beans this way started to reduce due to its hard seed coat which makes the beans to take too long to cook. There is therefore the need to find alternative ways of utilizing this legume which is of nutritional and health benefits. In order to utilize the bean effectively as human food, it is essential to inactivate or remove these anti-nutritional factors. The purpose of this study was to investigate the effect cooking time on the nutritive value and anti-nutritional factors on lima bean.

2. Material and methods

2.1. Materials

Red and black *Phaseolus lunatus (L.)* bean seeds are from pods harvested at stage 4 of maturity (52 days after fertilization) in an experimental field created in Tomasset (Azaguié) Côte d'Ivoire during the year 2014-2015 [3].

Figure 1 Pods (C), black (A) and Red (D) seeds of *Phaseolus lunatus* (L.)

2.2. Preparation of Lima bean powder

Two hundred (200) grams of each sample of red and black *Phaseolus lunatus* bean seeds from stage 4 (52 days) of maturity were cooked in an IVOIRAL pan containing 2 L of water previously boiled (100°C) on a hot plate (RELPE, Spain). A pre-cooking was carried out to determine the different cooking times. The time 45 minutes is marked by the removal of the film of the seed with a grinding between the two fingers. Fifteen (15) minutes of cooking time interval was considered. Thus, three cooking times were determined (45, 60, 75 min). The seeds are introduced into the IVOIRAL pan, once boiling begins, the timer is started. After each cooking time (45, 60 and 75 min), the seeds are removed and drained for a few minutes. After draining, the seeds were dried in a ventilated oven at 45 °C for 72 h. They were ground using a moulinex type mill. The powders obtained were sieved using an AFNOR 300 µm sieve. The flours obtained were kept in glass bottles previously washed and dried in an oven at 45°C for laboratory analysis

2.3. Biochemical Composition of *Phaseolus lunatus* (L.)

2.3.1. Proximate Analysis of Samples

Moisture, ash, crude protein, crude fat, crude fiber and total sugars were determined respectively by following the standard method [12,13], while Carbohydrate contents were calculated by difference [100- (protein + crude fat + ash + crude fiber)] [14]. In addition to the energy value (EV) was calculated by applying the heat coefficients of [15] according to the following equation: [EV (Kcal/100g) = (4 x Protein %) + (4 x Carbohydrate %) + (9 x Fat %)]. The values of analyses were the means of three determinations.

2.3.2. Vitamin B determination

All fresh seed of *Phaseolus lunatus* (L.) were washed and dried weighed 50mg 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 ReaGSJ: Volume 6, Issue 11, November 2018 ISSN 2320-9186 44 GSJ 2018 www.globalscientificjournal.com 6 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 MK2HPO4 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 [16].

2.3.3. Mineral analysis

Minerals were analyzed by the method reported by [12]. 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 [17] 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).

2.3.4. Amino acid analysis

Amino acid contents of samples were determined using Automatic Amino Acid Analyzer (BIOCHROM 30, serial 103274), according to the method outlined in [18].

Chemical score (CS) of essential amino acids (EAA) was calculated using the following equation according to FAO/WHO scoring pattern [19] following Equation:

$$chemical\ score = \frac{EAA\ in\ test\ potein\ (\frac{g}{100g})}{\text{EAA requierement patern } (\frac{\text{g}}{100\text{g}})}$$

2.3.5. Anti-nutritional factors estimation

Hydrogen cyanide was analysed by the [12]. Ten (10) g of flour were homogenized in 200 mL of distilled water. The trapped distillate was left to stand for 3 hours and filtered through whatman paper. The filtrate obtained was distilled from 20 mL of sodium hydroxide (0.1 N) and 2 mL of KI (0.02 N). The distillate was titrated with silver nitrate AgNO3 (0.02N) until a yellowish haze appears. The tannin content was estimated spectrophotometrically by the procedure described by [20]. One millilitre of methanolic extract is introduced into a test tube to which 5mL of vanillin reagent were added. The tube was left to stand for 20 min in the dark and the absorbance was read with a spectrophotometer at 500 nm against a blank. The blank was prepared for each test by adding 5 mL of distilled water to the test tubes replacing the vanillin reagent. The amount of tannins in the sample was determined using a standard range established from a tannic acid solution (2 mg / mL) under the same conditions as the test. Phytic acid was determined using the procedure described by [21]. One gram of flour was homogenized in 20 mL of HCl (0.65). The mixture obtained is stirred for 12 hours at room temperature. The mixture was centrifuged at 3000 trs/min for 40 minutes. To 0.5 mL of supernatant, 3 mL of Wade's reagent were added. The blank was prepared for each sample with 0.5 mL of distilled water in the test tubes without Wade's reagent. The tubes were left to stand for 20 min in the dark and the optical density was read with a spectrophotometer at 490 nm against a blank. The amount of phytate was determined using a standard range established from a sodium phytate solution (10 mg / mL) under the same conditions as the test. The oxalate was determined using the method of [22]. Two grams of flour were homogenized in 75 mL of H2SO4 (3M). The mixture was stirred magnetically for 1 hour at room temperature. The whole was filtered through Whatman filter paper. Twenty-five milliliters of filtrate were titrated hot with a solution of potassium permanganate (KMnO4, 0.05 M) until the change to persistent pink.

2.3.6. Antioxidant and Phytochemicals Determination

The antioxidant activity in the beans samples was determined using the 1, 1-diphenyl-2-pycrylhydrazyl (DPPH) method, as reported by [23]. The results were expressed as milligrams of Trolox equivalents antioxidant capacity (TEAC g−1) extract. Total phenolic content (TPC) in extracts from the samples was determined by a Folin-Ciocalteu method described by [24], using garlic acid as standard.

2.4. 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.

3. Results

3.1. Evolution of the biochemical composition of red and black seeds of beans *Phaseolus lunatus* (L.) as a function of cooking time

Study of the biochemical composition of red and black *Phaseolus lunatus (L.)* bean seeds revealed that the levels of protein, lipid, ash, vitamin C decreased significantly (P<0.05) with cooking time (**Table 1**). Protein levels varied from 21.21 ± 0.18 to 18.52 ± 0.23 mg/100 g dry matter in seeds. Cooked seeds had the same protein loss rates (10.22% and 10.27%, respectively). Red and black *P. lunatus* bean seeds contain lipid levels that range from 2.16 ± 0.08 to 0.70 ± 0.03 mg/100 g dry matter. The lipid reduction rate of the red seed is 50.71% while that of the black seed is 49.07%. The ash contents of *P. lunatus* bean seeds varied from 4.26 ± 0.05 to 2.88 ± 0.04 mg/100 g dry matter during cooking. Vitamin C content varies from 8.63 ± 0.20 -1.02 ± 0.02 mg/100 g fresh matter during this cooking of seeds. The red and black seeds lost 84.13 and 84.70 % of their vitamin C content, respectively. At 45 minutes of cooking, the losses in proteins, lipids, ashes, vitamin C are low whatever the cultivar. 45 minutes of cooking is the optimal time to have seeds of good nutritional quality.

Fiber and carbohydrate levels increase significantly (P<0.05) during cooking of the seeds. *Phaseolus lunatus (L.)* bean seeds contained fiber levels ranging from 5.86 ± 0.12 to 4.62 ± 0.04 mg/100 g dry matter. Seeds of the red cultivar contain a high level of carbohydrates which is 69.63 ± 0.48 to 71.56 ± 0.04 mg/100 g dry matter. At 75 minutes of cooking *P. lunatus* bean seeds, the carbohydrate and fiber levels increase. Cooking time had no effect on total lipid and carbohydrate levels as all these values were not significantly different. The energy value increases significantly (P< 0.05) from 310.02 ± 0.68 to 374.28 ± 0.27 Kcal/100 g dry matter) during cooking in seeds. The time 75 minutes of cooking gives the high levels of carbohydrate and energy values in the seeds.

Table 1 Evolution of the biochemical composition of red and black *Phaseolus lunatus* bean seeds as a function of cooking time (g/100 g dry matter)

Flours	Cooking time (min)	Protein (%)	Lipid (%)	Carbohydrate (%)	Fibers (%)	Ash (%)	Energy (Kcal/100g)	VitaminC (%)
FCR	0	20.63 ± 0.20^{f}	1.60 ± 0.01^{b}	69.63 ± 0.48^{c}	4.62 ± 0.04^{a}	4.26 ± 0.05^{b}	311.25 ± 0.33^{a}	6.43 ± 0.20^{b}
	45	19.59 ± 0.10^{gh}	1.08 ± 0.10^{e}	70.37 ± 0.12^{efh}	5.05 ± 0.13^{de}	3.91 ± 0.02^{fg}	369.60 ± 0.40^{d}	3.70 ± 0.06^{e}
	60	18.90 ± 0.03^{ij}	0.85 ± 0.03^{df}	$71.15 \pm 0.05g^{h}$	5.50 ± 0.20^{bc}	3.63 ± 0.05^{h}	367.85 ± 0.22^{h}	2.30 ± 0.10^{g}
	75	18.52 ± 0.23^{j}	0.70 ± 0.03^{f}	71.56 ± 0.04^{g}	5.83 ± 0.15^{fg}	3.40 ± 0.02^{cd}	366.96 ± 0.31^{hi}	1.02 ± 0.02^{ij}
FCN	0	21.21 ± 0.18^{e}	2.16 ± 0.08^{a}	67.95 ± 0.28^{f}	4.93 ± 0.08^{ae}	3.74 ± 0.05^{fg}	310.02 ± 0.68^{f}	8.63 ± 0.20^{a}
	45	19.70 ± 0.10^{g}	1.68 ± 0.03^{b}	70.09 ± 0.08^{fg}	5.32 ± 0.07^{cd}	3.21 ± 0.02^{de}	374.28 ± 0.27^{a}	4.50 ± 0.04^{d}
	60	19.22 ± 0.06^{ef}	1.35 ± 0.05^{c}	70.82 ± 0.17^{egh}	5.60 ± 0.08^{bcf}	3.00 ± 0.10^{e}	372.31 ± 0.40^{b}	2.40 ± 0.20^{g}
	75	19.03 ± 0.06^{hi}	1.10 ± 0.10^{e}	71.12 ± 0.11^{gh}	5.86 ± 0.12^{fg}	2.88 ± 0.04^{a}	370.59 ± 0.52^{c}	1.32 ± 0.02^{hi}

Mean ± standard deviation, n=3; in columns, means assigned different letters indicate significant difference at the (P<0.0 5) threshold. FCR (red cultivar flour) and FCN (black cultivar flour)

3.2. Evolution of the concentrations of B vitamins in the seeds of the red and black beans of *Phaseolus lunatus* (L.) as a function of cooking time

From the analysis of the results, it appears that the concentrations of vitamins B1 (230.05 ± 0.82-239.05± 0.77µg/100g dry matter), B2 (280.00 ± 0.82 - 420.25 ± 0.50µg/100g dry matter), B6 (2000.03 ±1.32 - 1679.72 ± 0.98µg/100g dry matter) and B9 (575.53 ± 0.81 - 600.00 ± 1.09µg/100g dry matter) of uncooked seeds are significantly higher (P < 0.05) than those of cooked seeds. The concentrations of vitamins B1, B2, B6 and B9 of cooked seeds varied respectively, from (239.97 ± 0.77 to 80.60 ± 1.50µg/100g dry matter); from (420.25 ± 0.50 to 72.20 ± 0.80 µg/100 g dry matter); from (2000.03 ± 1.32 to 403.66 ± 1.66 µg/100g dry matter), from (600.00 ± 1.09 to 120.26 ± 0.46 µg/100 g dry matter) during cooking. The loss rates of vitamins B1; B2, B6 and B9 from red and black seeds during this cooking process varied from (61.48 to 76.06%). The rate of reduction of each B vitamin differs from one cultivar to another. As the cooking time increases, the loss rates of the vitamins (B1, B2, B6 and B9) increase. The vitamins do not have the same heat

sensitivities (**Table 2**). Cooking the seeds for 45 minutes gives high concentrations of B vitamins regardless of the cultivar, but at this cooking time the seeds of the black cultivar contain more B vitamins.

Table 2 Evolution of the concentrations of B vitamins (B1, B2, B3, B6 and B9) in red and black bean Phaseolus lunatus (L.) as a function of cooking time (µg/100g dry matter

Flours	Cooking time (min)	B1	B2	B3	B6	B9
FCR	0	230.05 ± 0.82^{b}	280.00 ± 0.82^{b}	ND	1679.72 ± 0.98^{b}	575.53 ± 0.81^{b}
	45	160.00 ± 2.00^{e}	150.93 ± 0.60^{e}	ND	905.00 ± 1.00^{e}	281.16 ± 0.36 ^{e}
	60	116.20 ± 1.70^{i}	108.66 ± 1.36^{h}	ND	663.33 ± 1.52^{i}	201.50 ± 0.50^{k}
	75	88.60 ± 0.40^{k}	90.43 ± 0.51^{j}	ND	506.61 ± 1.33^{k}	137.76 ± 0.24^{i}
FCN	0	239.97 ± 0.77^{a}	420.25 ± 0.50^{a}	ND	2000.03 ± 1.32^{a}	600.00 ±1.09^{a}
	45	170.33 ± 1.70^{d}	206.60 ± 0.52^{c}	ND	1008.86 ± 1.02^{d}	305.53 ± 0.50^{d}
	60	120.66 ± 1.52^{g}	143.66 ± 0.35^{f}	ND	865.26 ± 0.64^{f}	202.00 ± 1.00^{k}
	75	87.53 ± 0.50^{k}	116.33 ± 0.35^{g}	ND	687.00 ± 1.00^{h}	151.26 ± 0.65^{h}

Mean ± standard deviation, n=3; in columns, means assigned different letters indicate significant difference at the (P<0.0 5) threshold. FCR (red cultivar flour) and FCN (black cultivar flour)

3.3. Mineral composition of red and black *Phaseolus lunatus* (L.) bean seeds as a function of cooking time

Analysis of the seeds cooked at different times (45, 60 and 75 minutes) shows that the levels of minerals such as sodium, potassium, phosphorus, magnesium, calcium, iron and zinc decrease during cooking (**Table 3**). These levels vary respectively from (75.27 ± 0.72 to 64.22 ± 0.23 mg/100g dry matter) for sodium, from (1592.27 ± 6.38 to 852.50 ± 0.50 mg/100g dry matter) for potassium, from (250.48 ±0.67 to 132.18 ± 0.12mg/100g dry matter) for phosphorus from (140.56 ± 0.20 - 81.12 ± 0.13 mg/100g dry matter) for Magnesium, from (359.32 ± 0.89 to 180.50 ± 0.40mg/100g dry matter) for Calcium, from (2.98 ± 0.06 to 1.05 ± 0, 05 mg/100g dry matter) for copper, (12.56 ± 0.28 to 4.90 ± 0.01mg/100g dry matter) for iron and (1.70 ± 0.01 to 0.06 ±0.01mg/100g dry matter) for zinc. Mineral levels in uncooked seeds (FNC) were significantly higher (P < 0.05) than in cooked seeds. Thus, the loss rates of minerals in red and black seeds to during cooking vary respectively from (9.30 to 11.60%) for sodium, (34.71 to 46.48%) for potassium, (42.12 to 42, 57%) for phosphorus, (35.46 to 42.27%) for magnesium, (34.98 to 40.03%) for calcium, (48.36 to 49.84%) for iron, (63.28 to 64.42%) for copper and (57.64 to 66.66%) for zinc. The results show that the losses of minerals in cooked P. lunatus bean seeds are statistically different from each other. Thus, the minerals do not have the same sensitivities to heat. Seeds cooked at 45 minutes contain high mineral concentrations.

3.4. Evolution of phytochemical parameters and antioxidant activity of red and black *Phaseolus lunatus* (L.) bean seeds according to cooking time

The cooking study of *Phaseolus lunatus* (L.) bean seeds shows a significant decrease (P<0.05) in all phytochemicals and antioxidant activity (Table IV). Phytochemicals ranged from (803.90 ± 0.90 to 268.10 ± 0.08 mg /100g dry matter, total phenolics), (124.37 ± 0.30 to 73.23 ± 0.32 mg/100g dry matter, phytates), (560.50 ± 0, 50 to 140.20 ± 0.72 mg/100g, oxalates), (128.67± 1.02 to 56.12 ± 0.13 mg/100g dry matter, tannins), (6.37 ± 0.03 to 1.68± 0.02 mg/100g dry matter, hydrocyanic acid) in the seeds during cooking. The levels of phytochemicals in uncooked seeds (FNC) were significantly higher (P<0.05) than in cooked seeds. Red and black seeds show losses in total phenolics (62.65 - 62.01%), phytates (36.95 - 39.18%), oxalate (68.01 - 64.64%), tannins (53.07 - 48.28%), cyanidric acid (58.48 - 57.71%) respectively. Seeds of both cultivars cooked at 45 minutes show low losses in phytochemicals and antioxidant activity compared to 60 and 75 minutes cooking time. The antioxidant activity of *Phaseolus lunatus* (L.) bean seeds decreased significantly (P < 0.05) with cooking time (Table 4). Antioxidant activity ranged from (85.06 ± 0.90 to 35.30±0.36% dry matter for red seeds) and from (95.30 ± 0.71 to 40.30±0.05% dry matter for black seeds. The antioxidant activity of uncooked seeds (FNC) was significantly higher (P < 0.05) than that of cooked seeds. Seeds of both cultivars gave high antioxidant activities at 45 minutes of cooking but these decreased with cooking time.

Table 3 Evolution of mineral concentrations of *Phaseolus lunatus (L.)* bean seeds as a function of cooking time (mg/100g dry matter)

Flours	Cooking time (min)	Sodium	Potassium	Phosphorus	Magnésium	Iron	Calcium	Cooper	Zinc
FCR	0	72.65 ± 0.32^{h}	1592.90 ±6.38^{a}	250.48 ±0.67^{b}	140.56 ± 0.20^{f}	12.56 ±0.28^{a}	359.32 ± 0.89^{a}	2.86 ± 0.07^{b}	0.18 ± 0.01^{de}
	45	68.34 ± 0.43^{i}	1150.22±0.23^{d}	196.57 ± 0.50^{e}	106.32 ± 1.14^{c}	9.26 ± 0.14^{c}	300.69 ± 0.27^{k}	1.71 ±0.01^{cd}	0.12 ± 0.02^{de}
	60	66.33 ± 0.59^{b}	966.99 ± 0.01^{h}	164.09 ±0.09^{k}	90.45± 0.48^{h}	7.35 ± 0.15^{de}	268.67 ± 0.29^{d}	1.31 ±0.08^{ef}	0.08 ± 0.01^{e}
	75	64.22 ± 0.23^{c}	852.50 ± 0.50^{i}	145.14 ±0.15^{h}	81.12 ± 0.13^{i}	6.30 ± 0.20^{fh}	233.60 ± 0.40^{f}	1.05 ± 0.05^{f}	0.06 ± 0.01^{e}
FCN	0	75.27 ± 0.72^{a}	1519.50±1.40^{b}	230.18 ± 1.13^{c}	128.46± 0.35^{a}	9.49 ± 0.27^{c}	301.03 ± 1.00^{k}	2.98 ± 0.06^{b}	1.70 ± 0.01^{a}
	45	71.90 ± 0.26^{h}	1238.34 ±0.36^{c}	185.13 ± 0.13^{f}	102.72 ± 0.23^{d}	6.97 ± 0.11^{ef}	223.84 ± 0.96^{g}	1.86 ± 0.14^{d}	1.15 ± 0.15^{b}
	60	69.62 ± 0.54^{i}	1100.92 ±0.08^{f}	150.33 ±0.15^{g}	91.97 ± 0.24^{g}	5.86 ± 0.04^{h}	203.81 ± 0.19^{i}	1.36 ± 0.04^{f}	0.90 ± 0.10^{c}
	75	68.27 ± 0.30^{i}	992.09 ± 0.34^{g}	132.18 ± 0.12^{j}	82.90 ± 0.13^{e}	4.90 ± 0.01^{g}	180.50 ± 0.40^{j}	1.06 ± 0.06^{f}	0.72 ± 0.07^{c}

Mean ± standard deviation, n=3; in columns, means assigned different letters indicate significant difference at the (P<0.0 5) threshold. FCR (red cultivar flour) and FCN (black cultivar flour)

Table 4 Evolution of phytochemical characteristics (mg/100 g) and antioxidant activity (%) of red and black *Phaseolus lunatus* (L.) bean seeds as a function of dry matter cooking time

Flours	Cooking time (min)	Polyphenols					
		Totals	Phytic acid	Oxalates	Tannins	cyanidric Acid	Antioxydant. Activity
FCR	0	803.90 ± 0.90^{b}	124.37± 0.30^{a}	560.50 ± 0.50^{a}	128.67± 1.02^{b}	6.37 ± 0.03^{a}	85.06 ± 0.90^{ab}
	45	510.42 ± 0.25^{d}	102.50 ± 0.50^{d}	346.92 ±0.23^{d}	101.20 ± 0.11^{e}	4.20 ± 0.02^{d}	60.46 ± 1.20^{cd}
	60	400.81 ± 0.24^{f}	85.23 ± 0.75^{i}	240.60 ± 0.40^{g}	89.37 ± 0.59^{hi}	3.23 ± 0.03^{f}	44.80 ± 0.20^{cd}
	75	300.22 ± 0.25^{j}	78.41 ± 0.52^{f}	178.93 ± 0.30^{j}	60.38 ± 0.46^{j}	2.60 ± 0.36^{c}	35.3 ± 0.36^{d}
FCN	0	705.85 ± 0.95^{c}	120.23 ± 0.92^{b}	396.53 ±0.61^{c}	108.42 ±1.14^{c}	4.26 ± 0.06^{d}	95.30 ± 0.71^{a}
	45	470.23 ± 0.06^{g}	96.16 ± 0.04^{e}	252.84 ±0.63^{f}	90.36 ± 0.25^{h}	3.06 ± 0.07^{f}	66.57 ± 0.45^{bc}
	60	350.36 ± 0.39^{h}	85.70 ± 0.43^{i}	183.65 ± 1.21^{i}	82.20 ± 0.20^{f}	2.24 ± 0.04^{g}	50.56 ± 0.41^{cd}
	75	268.10 ± 0.08^{k}	73.23 ± 0.32^{h}	140.20 ± 0.72^{l}	56.12 ± 0.13^{g}	1.68 ± 0.02^{e}	40.30 ± 0.05^{cd}

Mean ± standard deviation, n=3; in columns, means assigned different letters indicate a significant difference at the (P<0.05) threshold. FCR (red cultivar flour) and FCN (black cultivar flour)

3.5. Anti-nutritional/mineral and mineral/mineral ratios of red and black *Phaseolus lunatus (L.)* bean seeds according to cooking time

Investigation of anti-nutritional/mineral and mineral/mineral ratios of *Phaseolus lunatus* bean seeds as a function of cooking time showed that the Phytate/Iron and Phytate/Ca ratios of raw seeds (9.67-0.18) are lower than cooked seeds (12.44-0.33) of red and black cultivars. On the other hand, Oxalate/Ca and Oxalate/ Ca+Mg ratios obtained in raw seeds are higher than in cooked seeds. Oxalate/Ca and Oxalate/ (Ca+Mg) ratios ranged from (0.82 to 0.84) and (0.63 to 0.67) in uncooked seeds and from (0.76 to 1.15) and (0.53 to 0.85) in cooked seeds of red and black cultivars, respectively. Na/K and Ca/P ratios vary from (0.04 to 0.05) and (1.14 to 1.17) in uncooked seeds and then from (0.05 to 0.07) and (1.12 to 1.63) in cooked seeds of red and black cultivars, respectively. Cooking the seeds causes an increase in Phytate/mineral ratios and then a decrease in Oxalate/mineral ratios. Seeds of red and black cultivars have Na/K ratios below 1 and Ca/P ratios above 1. Phytate/Ca and Oxalate/Ca ratios are less than 2.5 regardless of cultivar type (Table 5).

Table 5 Anti-nutritional/mineral and mineral/mineral ratios of *Phaseolus lunatus* (L.) bean seeds as a function of cooking time

Flours	Cooking time (min)	Phytate/Iron	Phytate/Ca	Oxalate/Ca	Oxalate/Ca+Mg	Na/K	Ca/P
FCR	0	9.67	0.18	0.84	0.67	0.05	1.17
	45	11.06	0.34	1.15	0.85	0.05	1.52
	60	12.14	0.33	0.89	0.66	0.06	1.63
	75	12.44	0.33	0.76	0.56	0.07	1.6
FCN	0	12.66	0.20	0.82	0.63	0.04	1.14
	45	13.79	0.42	1.12	0.77	0.05	1.2
	60	14.11	0.40	0.90	0.62	0.06	1.35
	75	14.94	0.40	0.77	0.53	0.06	1.36

Mean ± standard deviation, n=3; in columns, means assigned different letters indicate a significant difference at the (P<0.05) threshold. FCR (red cultivar flour) and FCN (black cultivar flour)

4. Discussion

The decrease in protein content of red and black *Phaseolus lunatus (L.)* bean seeds during cooking is only significant after 45 min of cooking time. This result could be explained by protein denaturation after 45 min of cooking, i.e., breaking of peptide bonds and certainly of protein disulfide [25]. Also, during cooking, there is disintegration of the crude protein into amino acids and therefore the heat treatment induces changes in the structure of the proteins, which can inactivate the anti-nutrients, thus increasing the digestibility and the biological values of the protein of the bean [26]. However, different results on reduction of protein content during processing such as cooking have been reported in two cultivars of beans (*Vigna unguiculata* and *Vigna angustifoliata*) [27]. These results are in agreement with [28] who reported that the crude protein content of cooked millet is lower than that of uncooked millet. According to [29], the protein requirement of infants is estimated at 9 g/day. With a protein content that varies from 19.03 to 21.21%, these bean flours could partially cover the protein needs of infants.

The decrease in lipid content from (2.16 to 0.70%) of red and black *Phaseolus lunatus (L.)* bean seeds during cooking would be due to lipid leaching phenomena during cooking [30]. The results of the present study are in agreement with those of [310] who found the loss of fat in the flour of cooked *Mucuna spp* seeds. Similarly, [31] observed the same similarities in their work on *Vigna sesquipedalis* bean. Beans are not a good source of lipid. Low lipid content in the beans is an advantage, as this will reduce the risk of heart attack and increased blood cholesterol level [21].

The ash content decreases from 4.26 to 2.88% in the seeds of both cultivars of *P. lunatus* during cooking. Low ash content in the bean might be due to leaching of salts and minerals into the cooking water [6]. Similar results were also reported on soybean (glycine maximum) and *P. lunatus* bean, when samples were subjected to the autoclave sterilization process [32].

The crude fiber contents of *P. lunatus* bean seeds increased from (4.62 to 5.86%) during cooking. The high rate of crude fiber in the processed sample could be explain by the fact that heat treatments can have variable effects on crude fiber and that cooking causes disruption of the cellular components of beans (cellulose, hemicellulose, lignin, pectin and gums). The cooking process results in interactions between proteins and lipids and it leads to qualitative and quantitative changes in the composition of total fiber of cooked foods compared to that of raw foods [33]. This increase could be explained by the formation of protein-fiber complexes formed after chemical modification induced by cooking as observed by [34] in his work on lentil (*lens culinaris*) seeds. Also, this increase in fiber in cooked *P. lunatus* bean seeds is similar with the work of [35] who showed that cooking increases the content of soluble fiber and decreases the content of insoluble fiber. Consumption of *P. lunatus* bean seeds may contribute to the reduction of risk of hypertension, constipation, diabetes, colon and breast cancer.

Carbohydrate levels in the seeds of both *Phaseolus lunatus* bean cultivars increase during cooking. The carbohydrate levels of *P. lunatus* bean seeds ranging from 67.95 to 71.56% are lower than the carbohydrate levels of millet seeds (71.95% to 77.13%) [28]. This increase is due to the determination of the carbohydrate levels which is done by the mathematical difference. *P. lunatus* bean seeds are an important source of carbohydrates. The carbohydrate content of the seeds of the two cultivars of *P. lunatus* bean makes them an energy food that can contribute to food security in developing countries [36], particularly in Côte d'Ivoire.

The energy value of *Phaseolus lunatus* bean seeds increases with the cooking time (respectively 310.02 in raw seeds and 374.28 Kcal/100g dry matter in cooked seeds) during 75 minutes. This increase is similar to that observed in *Dioscorea alata* raw and cooked for 90 min (357.65 and 370.01 Kcal/100g dry matter respectively) [37]. Seeds of both *Phaseolus lunatus* bean cultivars could be used in part as energy meals in porridges for infants and children with energy requirements ranging from 547 to 1092 kcal/day [38]. The vitamin C content decreases from 8.62 to 1.02% during cooking. This loss in Vitamin C during cooking is due to the high activity of ascorbinase between 40 and 70 °C [39]. This observation would generally be explained by enzymatic oxidation during seed cooking [40]. The work of [41], *Phaseolus lunatus (L.)* bean seeds, when cooked for 45 minutes, contain high concentrations of B vitamins. Indeed, whatever the cultivar, the contents of thiamine (B1), riboflavin (B2), pyridoxine (B6) and folate (B9) in the meals of red and black cultivars of *P. lunatus* beans undergo a significant reduction ($P< 0.05$) during cooking. The loss of vitamins is explained by their leaching into the cooking water under the effect of heat. [42] showed a decrease in B vitamins during cooking of bean seeds (kidney) and carrot. Similarly, [43] showed high loss of thiamine, riboflavin and ascorbic acid in vegetables subjected to microwave cooking. [44] also reported that microwave and pressure cooking decreased the thiamine content of eight legumes studied. *Phaseolus lunatus (L.)* bean seeds of both cultivars cooked at 45 minutes contain high concentrations of minerals but decrease significantly ($P<0.05$) with cooking time from (45 to 75 minutes). The loss of minerals is due to the degradation of anti-nutritional factors such as phytate which traps 60-80% of these minerals in seeds compared to 20-34% in fruits and tubers [45] with iron being trapped by tannin [46]. [47] & [48] have shown that the denaturation of anti-nutritional factors by heat during cooking of *P. lunatus* bean seeds will release minerals in their matrices which will then be diffused in the cooking water 49[49] hence their decrease. The Na/K and Ca/P ratios obtained in the seeds of both cultivars are less than or greater than 1. The Na/K ratio is very important for the body because sodium and potassium regulate high blood pressure and muscle contraction. Howed the loss of vitamin C in leafy vegetables under heat. A food product is a good source of Ca and Fe if the Ca/P ratio is greater than 1 [50]. Considering the iron content obtained with *phaseolus* seeds, this bean can be recommended in human diet according to the work of [51] who showed that the recommended content in human diet is 1.37 mg/day (Men) and 2.94 mg/day (Women). Seeds of *P. lunatus* bean cultivars can be recommended in human diet to combat anemia in the world [52].

Phaseolus lunatus bean seeds cooked at 45 minutes contain high concentrations of phytochemicals but decrease in the seeds during cooking to 75 minutes. The loss of total polyphenols, which is 67.73% during cooking, is due to cell rupture facilitating the release of these compounds into the cooking water. This decrease in total polyphenols of *P. lunatus* bean seeds is similar to the results obtained by [53] who showed that cooking of common bean seeds causes the release of 90% of polyphenols into the cooking water. Also, cooking *munga bean* for 30 min showed a 73% reduction of polyphenols [54]. Phytates decreased by 41.11% during 75 min of cooking. The loss of phytate observed in the seeds during cooking is similar to that observed by [55]. These authors showed a reduction of phytates in legume seeds during heat treatment. For these authors this loss is related to the thermolabile character of phytic acid and to the formation of insoluble complexes between phytate with other components, such as proteins and minerals [55]. Similarly [56] showed that the apparent decrease in phytic acid content of legume seeds is due to leaching in cooking water. The phytate reduction rate (41.11%) in *P. lunatus* bean seeds is similar to that found by [57] which is 42.90% phytate reduction of *Artocarpus altilis* fruit after 20 minutes of cooking. Also, [58] reported a 17% reduction after 45 min of cooking common bean seeds. Knowledge of the phytate content of a food is important because high levels can cause adverse effects on digestibility [59]. This is because phytate forms complexes with certain minerals (copper, zinc,

manganese, iron and calcium) making them unavailable to the body. The rate of reduction of tannins in cooked P. lunatus bean seeds is 56.38% during 75 minutes.

This decrease could be explained by the destruction of the bonds formed with the proteins giving an insoluble complex [60] followed by their leaching in the cooking water as shown in the work of [61] on cooking Faba bean seeds. This reduction in tannin content in *Phaseolus lunatus* seeds could also be explained by the thermolability of tannins [62]; [55] which are destroyed by heat. The rate of tannin reduction in cooked *P. lunatus* bean seeds is similar to the results of [63] , [64] and [65] who showed that tannins in seed legumes decrease during certain treatments (such as microwave cooking, autoclaving and blanching). These results are contrary to those of who showed [66] an increase in tannin levels during cooking, autoclaving and bleaching of bean (*Delichos lablab*) seeds. The variation in tannin content and reduction rate depends on the plant material and the cooking time and temperature pair. The reduction rate of total oxalates in *P. lunatus* bean seeds is 74.97% during 75 minutes of cooking. This high rate of reduction would be due to the damage of the plant cells during cooking and the leaching of oxalates in the cooking water. The rate of reduction of total oxalates in cooked *P. lunatus* bean seeds during 75 min is higher than the results of who observed a 50% reduction after 60 min of cooking for *Artocarpus heterophyllus* seeds. This difference could be explained by the high fast thermal conductivity of the pulp compared to *P. lunatus* bean seeds. A reduction of oxalate in a feed increases the bioavailability of essential minerals and reduces the risk of irritation of the digestive system, especially the stomach and kidneys [67]. The lethal level of oxalate in food is between 2000 and 5000 mg oxalate/100 g food [69;70]. With low levels of oxalate observed in *P. lunatus* bean seeds, their consumption would therefore be safe. The rate of loss of hydrocyanic acid is 73.63% after 75 minutes of cooking *Phaseolus lunatus* bean seeds. Moist heat reduces the hydrocyanic acid level by almost half. This high reduction rate is due to the thermolability of hydrogen cyanide [71]; [72]; [67]. The loss of hydrocyanic acid is by volatilization during cooking and the remaining rate is converted to thiocyanide or other compounds [73]. Our results are similar to those of [71]; [72 & [67] on *Phaseolus lunatus* seeds which showed a 68, 37% reduction in hydrocyanic acid as a function of cooking time and temperature. The consumption of P. lunatus seeds cooked at 45 minutes could be recommended in human diet due to the decrease of oxalates and phytates to prevent and treat some diseases such as cancer [74], inflammatory diseases [75], cardiovascular diseases [76] and neurogenerative diseases [77]. The antioxidant activity of the seeds of both *Phaseolus lunatus* cultivars decreased significantly ($P < 0.05$) with cooking time. It ranged from 85.06 ± 0.90 to 95.30±0.30% dry matter in uncooked seeds (FNC) and was significantly higher ($P < 0.05$) than in cooked seeds (35.30 ± 0.36 to 40.30±0.05% dry matter). Red and black seeds showed losses in antioxidant activity (58.49 and 57.71%), respectively. Seeds of both cultivars give high antioxidant activities at 45 minutes of cooking but the black cultivar shows the highest antioxidant activity at this cooking time.

5. Conclusion

Heat treatment by cooking *Phaseolus lunatus* (L.) bean seeds in water at 100°C for 45, 60 and 75 minutes results in a decrease in nutritional factors except for carbohydrates, energy value and fiber which increase. On the other hand, the other nutritional factors and all phytochemical parameters decrease during cooking. The energy value is higher at 45 minutes of cooking in the seeds of the black cultivar. At 60 minutes cooking time, carbohydrate and fiber levels are higher in the black cultivar seeds. Although at 75 minutes cooking time the maximum of anti-nutritional factors and some nutritional factors are removed, the 45 minutes cooking time can be recommended for cooking the seeds of both cultivars of *P. lunatus* bean. With this time, the cooked seeds contain high levels of nutrients even if the reduction of anti-nutritional factors is not at its maximum, the remaining levels are low and safe.

Compliance with ethical standards

Acknowledgments

The authors are very grateful to the Laboratory of Food Biochemistry and Processing of Tropical Products of the University Nangui Abrogoua, Abidjan (Côte d'Ivoire) and the Biochemistry Laboratory of the Professional High school of Yopougon (Abidjan), Côte d'Ivoire.

Disclosure of conflict of interest

The authors declare no conflict of interest, financial or otherwise.

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