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Effects of Different Processing Methods on the Iron, Zinc and Other Nutrients Content of Biofortified Beans (*Phaseolus vulgaris L.*)

Marlyne-Josephine Mananga[1], Youmbi Nenkam Audrey Darline[2], Demasse Mawamba Adelaide[2], Youogo Marlène[2], Kouandjoua Ndjigoui Brice Didier[1], Kana Sop Marie Modestine[2,*]

[1]Department of Biochemistry, Laboratory for Food Science and Metabolism,
University of Yaounde I, Yaounde, Cameroon: PO Box 812 Yaoundé
[2]Department of Biochemistry, Laboratory of Biochemistry,
University of Douala, Douala, Cameroon, PO Box 24157 Douala
*Corresponding author: kanamod@yahoo.com

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Abstract Iron deficiency anemia remains a real public health problem among young children in Cameroon. To reduce it, iron biofortification of legumes was developed to improve the iron nutritional status of the children. This study aimed to assess the effect of different processing methods on the iron, zinc and other nutrients content of biofortified bean cultivars (*Phaseolus vulgaris L.*) from Cameroon. Firstly, a survey was done in the city of Douala on different processes applied to the bean seeds before cooking. The raw and cooked sample of seeds were analysed for proximate and antinutrients composition, using AOAC methods for macronutrients content and atomic absorption spectrophotometry for minerals. The traditional bean cultivar served as control. From the results of the survey, beans treatment were divided into four groups: raw, boiled, soaked and boiled, boiled with limestone. The results showed that different processing methods led to an increase in protein (19.53%-27.66%), and crude fibre (4.46%-7.99%) but a decrease in carbohydrate (62.23%-53.28 %), lipids (5.48%-3.32%) contents. The biofortified bean showed statistically significant differences in iron and zinc contents compared to the traditional bean. Soaked and boiled biofortified bean had higher mineral contents compared to the traditional bean. Processing improved significantly ($p<0.05$) the nutritional value of the beans by reducing the antinutrient contents. Boiling with limestone was found to have the highest level of reduction effect on the tannin, oxalate, phytate and saponin. Biofortified bean cultivar could be used as food formulation material for infants and young children to prevent micronutrients deficiencies.

Keywords: *biofortification, Phaseolus vulgaris L., nutrients, cooking methods, iron*

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1. Introduction

Micronutrients malnutrition especially iron deficiency anemia affects at least half of the world's population. The World Health Organization (WHO) reported that an estimated 42% of children aged under 59 months are anemic worldwide. The burden is even higher in Africa, reaching 62.3% [1]. In Cameroon by considering iron deficiency anemia, the most affected age groups are children below 59 months (57%) and women of reproductive age (40%) [2]. This is caused by low intake of daily required amount of micronutrients [3]. However, many authors demonstrated that food fortification used as one of the approaches to overcome micronutrient deficiencies is hard for vulnerable and poor people unable to access fortified processed food. Therefore, biofortification of popular food crops like beans was proposed as a better intervention to address micronutrient deficiencies [4,5]. It is a process through which the nutritional value, minerals or vitamins levels of a food crop are enhanced through conventional breeding.

Beans and meat are both important sources of iron and approximately 1-7% of this mineral in dietary sources are absorbed when consumed alone [6]. However, according to Barker [7] and Katharine [8], the consumption of a high quantity of meat increases the risk of cardiovascular diseases and some types of cancer because of the presence of other component. In response, intensive efforts were made to find alternative sources of protein from the underutilized leguminous plants in nutrition and in the formulation of new food products.

Common beans are the most grown and largely consumed legume and are affordable sources of proteins in

Cameroon, making them a good choice for biofortification. Beans are one of the main sources of protein, plant-derived micronutrients, and minerals. Common beans (Phaseolus vulgaris L.) are rich in protein (14-24%) and micronutrients (Fe = 7-8 mg/100g and Zn = 2.5 3.5 mg/100g) [9]. However, the health benefits of beans are associated with their processing methods. Beans should be cooked or processed before intake. These processes increase the bioavailability of nutrients and reduces flatulence (raffinose oligosaccharides) and antinutrients factors [6]. According to many authors, the health benefits of beans are associated with their processing methods. Beans should be cooked or processed before intake [10,11].

Concerning different processing techniques, the studies conducted in Yaounde [12] showed that simple boiling, boiling with sodium bicarbonate, soaking and boiling, boiling with *Echinops giganteus* bark powder reduce significantly (P<0.05) the level of antinutrients, thus improve protein and starch digestibility of bean. Moreover, according to Ranilla et al. [13], cooking imparts desirable sensory properties to grains. Much information is available in the literature on nutrient content and potential contribution of different varieties of raw beans [9,14], but information on biofortified bean is scanty. This study aims to evaluate the effect of different processing methods commonly used by households on the proximate, mineral and antinutrients composition of biofortified bean cultivars (*Phaseolus vulgaris L.).*

2. Materials and Methods

2.1. Sample Collection and Preparation

This was a descriptive study. The study have begun with a survey of bean consumers (traders, households) without distinction of sex in the city of Douala. The purpose of this survey was to identify the different processing methods of beans commonly used in households. The survey took place from October 2020 to December 2020 in Douala (Cameroon). At the end of the survey, three (03) cooking methods most commonly used by householders were identified. Iron biofortified bean variety (FEB 192) and traditional bean variety (PH 201) were purchased from Institute of Agricultural Research for Development (IRAD) of Foumbot station (West region of Cameroon). The two varieties were selected due to consumer appreciation for traditional variety (PH 201) and the availability of iron biofortified bean for FEB 192 (Figure 1).

(a) (b)

(a): FEB 192 (large red seed); (b): PH 201 (small red seed).

Figure 1. Picture of the two bean cultivars (*Phaseolus vulgaris L.*)

The two varieties of beans FEB 192 and PH 201 were transported to the laboratory where they were sorted by removing dirt or broken beans. Then, they were washed thoroughly, to remove soil and all foreign particles. The seeds obtained were divided into 4 batches. The first batch was used for raw sample analysis, the second batch was boiled, the third batch was soaked (12h) and boiled and the fourth batch was boiled with 15g of limestone. For the first batch, 500g of each raw seeds were finely ground to a fine powder with a Kenwood blender after washing and drying to a constant weight at 50°C for 72 hours. The resultant flour was packaged in an airtight vessel in readiness for analysis. The vessel was appropriately labelled. For the second batch, 500g of bean seeds of each variety were boiled at 100°C for 1h30mn in 2.5L of distilled water in the proportion of 1:5 (w/v). The cooked seeds were dried to a constant weight at 50°C for 72 hours with frequent turning and mill. The resultant flour was packaged in an airtight appropriately labelled vessel in readiness for analysis. For the third batch, 500g of bean seeds of each variety were firstly soaked in distilled water in a 1:5 proportion (w/v) for 12 hours at room temperature 28°C. Subsequently, the soaking water was not removed. Secondly, the soaked bean seeds were boiled at 100°C for 1h30mn in 2.5L of distilled water in the proportion of 1:5 (w/v). The cooked seeds were dried to a constant weight at 50°C for 72 hours with frequent turning and mill. The resultant flour was packaged in an airtight appropriately labelled vessel in readiness for analysis. For the fourth batch, 500g of bean seeds of each variety were boiled at 100°C during the boiling, 15g of limestone were added and the all were left boiling 45mn of distilled water in the proportion of 1:5 (w/v). The cooked seeds were dried to a constant weight at 50°C for 72 hours with frequent turning. The resultant flour was packaged in an airtight appropriately labelled vessel in readiness for analysis.

2.2. Analysis

Moisture, proteins, lipid, crude fibres, ashes and carbohydrates content were determined using standard methods of Association of Official Analytical Chemists [15]. The samples were analyzed in triplicate.

2.3. Proximate Composition

The moisture was determined in an oven set at 105°C, according to standard procedures detailed by AOAC [15] during 72h (to the constent weight). The total nitrogen contents were determined by the Kjeldahl method, as described by AOAC, and the protein content was calculated by multiplying result by 6,25. Lipids content were evaluated by Soxhlet extraction according to the method described by AOAC [15], using hexane as the extractor. The ashes content were determined by calcination in a furnace at 550°C. The total fiber content was determined gravimetrically after delipidation of bean powder using the method described by AOAC [15].

2.4. Mineral Analysis

The mineral contents (iron, zinc, magnesium, phosphorus and calcium) of different samples were determined by

AOAC method N°968.08 [16]. About 100 g of powder for each raw and cooked bean seeds cultivars were oven dried at 105°C for 72 hours. After drying, 5g of beans were separately weighed into crucibles and maintained at 550°C for 24 hr. The ashes were cooled in desicators and then weighed. After weighing, the ashes were dissolved in a solution of 1:1 ratio of H2O: HCl, in which the concentration of the final mixture was 6N HCl. The contents of iron, zinc, magnesium, phosphorus and calcium were determined by atomic absorption spectrophotometer (Shimadzu UNICAM 919, England), while total phosphorus concentration was measured by colorimetric spectrophotometer after incubation with Molybdo-vanadate solution. Potassium and sodium levels were determined by digesting the ashes of the samples with perchloric acid and nitric acid, and then taking the readings on Jenway digital flame photometer/spectronic 20 [15].

2.5. Antinutrients Analysis

Tannins content were determined using ferric reagent in an acidic alcoholic medium using gallic acid as standard [17]. The absorbance was readed at 550 nm. The total oxalate content was assessed by titration with KMnO4 after acid digestion [18]. The phytate content was determined by titration with iron III solution after acid digestion [19]. Saponin contents were determined by weight difference after extraction in solvent as described by Obadoni and Ochuko [20].

2.6. Statistical Analysis

Data were expressed as mean ± standard deviation of triplicate samples and measurements. Analysis of variance (ANOVA) and the comparison of means (Tukey's test, P<0.05) were applied using IBM/SPSS 20.0 software (Statistical Package of Social Science) for Windows. The sodium to potassium (Na/K) and calcium to phosphorus (Ca/P) ratio were calculated in the samples. The energy value (E) per 100g of bean cultivars was obtained using Atwater [21] conversion [21] factors as follows:

$$E(Kcal) = Proteins(\%)x4\ carbohydrate(\%)x4 + lipids(\%)x9.$$

Determination of molar ratio of antinutrients to minerals was predicted by dividing the mole of antinutrient by the mole of minerals [22].

3. Results

The results obtained in the study are summarised in Table 1 - Table 4 and comprising macronutrients contents and the energy value, mineral contents, antinutrient contents and the molar ratio of antinutrients to mineral of biofortified bean seeds according to the various treatments.

Table 1 shows the moisture, proteins, lipids, carbohydrates, crude fibre, ashes contents of biofortified and traditional beans according to different treatments. The two cultivars of beans were high in proteins and carbohydrates, moderate in ashes and crude fibres but low in fats and moisture contents after all the treatments. The proteins and carbohydrates content of biofortified beans were higher (P<0.05) than that of traditional beans. It appeared that the soaked and boiled seeds had the highest protein contents (29.28±4.87g/100g DW; 27.66±1.63g/100g DW respectively for the PH 201 and FEB 192). The soaked and boiled seeds had the highest energy value 356.79 Kcal/100g DW (PH 201) and 355 Kcal/100g DW (FEB 192).

Table 1. Proximate composition and energy of raw and processed biofortified and traditional beans cultivars (*Phaseolus vulgaris* L.)

Cultivars	PH 201				FEB 192			
Parameter (%)	Raw	Boiled	SoB	BSb	Raw	Boiled	SoB	BSb
Moisture	8.17±0.56^{a}	8.26±1.35^{a}	8.61±0.53^{a}	8.31±0.60^{a}	3.97±2.02^{a}	5.88±2.59^{ab}	4.55±0.51^{a}	7.87±0.60^{b}
Protein	18.72±0.82^{a}	19.05±0.43^{a}	29.28±4.87^{c}	24.41±1.63^{c}	19.53±00a	26.04±00b	27.66±1.63^{b}	21.96±0.82^{d}
Lipids	5.04±1.05^{a}	3.09±0.80^{ab}	2.51±1.05^{b}	3.44±0.69^{ab}	5.48±1.66^{a}	3.57±0.39^{ab}	3.32±0.66^{b}	4.79±1.37^{ab}
Carbohydrates	57.79±1.81^{a}	59.62±0.04^{a}	49.38±2.13^{ac}	55.63±4.18^{c}	62.23±1.94^{a}	53.28±0.35^{b}	53.79±2.19^{b}	55.68±0.42^{b}
Ashes	3.23±0.02^{a}	3.68±0.14^{b}	2.57±0.24^{c}	3.09±0.04^{a}	4.33±0.13^{a}	3.24±0.17^{b}	3.09±0.03^{b}	3.08±0.02^{b}
Crude fibre	7.05±0.07^{a}	6.30±0.19^{b}	7.65±0.19^{c}	5.12±0.09^{d}	4.46±0.13^{a}	7.99±0.05^{b}	7.59±0.11^{c}	6.62±0.25^{d}
Energy (Kcal)	351.40	342.49	337.23	351.12	376.36	349.41	355.68	353.67

Values are means ± SD of triplicate determinations, Means within the same line (for the same cultivar) with different superscripts significantly different at p<0.05. SoB = Soaked and boiled; BSb = Boiled with limestone.

Table 2. Mineral contents of raw and processed biofortified and traditional beans cultivars (*Phaseolus vulgaris* L.)

Cultivars	PH 201				FEB 192			
Mineral (mg/100g DW)	Raw	Boiled	SoB	BSb	Raw	Boiled	SoB	BSb
Fe	7.75±0.64^{a}	8.72±0.64^{b}	8.45±1.71^{b}	4.93±0.00^{d}	8.73±0.64^{a}	8.97±1.28^{ab}	7.28±1.50^{c}	5.46±0.86^{d}
Zn	2.23±0.00^{a}	2.73±0.00^{b}	2.64±0.00^{c}	1.90±0.00^{d}	3.10±0.00^{a}	3.38±0.00^{b}	3.70±0.00^{c}	3.14±0.00^{d}
Na	2.81±0.02^{a}	2.76±0.04^{b}	2.32±0.01^{c}	27.26±0.00^{d}	2.83±0.00^{a}	1.88±0.02^{b}	1.86±0.00^{b}	42.29±0.04^{d}
Ca	63.12±0.08^{a}	70.8±0.08^{b}	93.96±0.04^{c}	97.26±0.02^{d}	86.20±0.02^{a}	93.14±0.11^{b}	86.84±0.04^{c}	106.89±0.04^{c}
Mg	242.85±0.06^{a}	197.4±0.04^{b}	179.25±0.08^{c}	188.7±0.02^{d}	234.9±0.04^{b}	187.5±0.00^{b}	168.15±0.03^{c}	192.3±1.80^{d}
P	67.23±5.25^{a}	71.43±2.10^{a}	58.30±11.03^{a}	96.11±7.90^{d}	67.33±4.05^{a}	63.02±13.65^{a}	57.77±12.60^{a}	59.87±15.76^{a}
K	20.69±0.05^{a}	22.25±0.01^{b}	20.24±0.00^{c}	19.10±0.08^{d}	23.33±0.00^{a}	28.80±0.02^{b}	22.86±0.00^{c}	25.90±0.04^{d}
Na/K	0.14	0.12	0.11	1.43	0.12	0.06	0.08	1.63
Ca/P	0.94	0.99	1.61	1.01	1.28	1.48	1.50	1.79

Values are means ± SD of triplicate determinations, Means within the same line (for the same cultivar) with different superscripts significantly different at p<0.05. SoB = Soaked and boiled; BSb = Boiled with limestone. Na/K= sodium to potassium ratio; Ca/P = calcium to phosphorus ratio.

Table 3. Antinutrient contents of raw and processed biofortified and traditional beans cultivars (*Phaseolus vulgaris* L.)

Cultivars	PH 201				FEB 192			
Parameter (mg/100g DW)	Raw	Boiling	SoB	BSb	Raw	Boiling	SoB	BSb
Phytates	0.15±0.09[a]	0.10±0.00[b]	0.06±0.00[c]	0.06±0.01[b]	1.89±0.00[a]	0.06±0.00[a]	0.08±0.01[a]	0.04±0.01[a]
Tanninss	0.61±0.06[a]	0.02±0.00[b]	0.09±0.00[c]	0.09±0.01[c]	0.28±0.03[a]	0.07±0.01[b]	0.24±0.01[c]	0.16±0.01[d]
Oxalates	0.70±0.04[a]	0.31±0.00[b]	0.22±0.01[c]	0.31±0.03[b]	0.58±0.04[a]	0.25±0.02[b]	0.31±0.03[bc]	0.40±0.08[c]
Saponins	5.10±0.05[a]	4.83±0.23[a]	4.65±0.15[a]	4.63±0.48[a]	5.33±0.18[a]	4.85±0.00[b]	4.40±0.00[c]	4.40±0.10[c]

Values are means ± SD of triplicate determinations, Means within the same line (for the same cultivar) with different superscripts significantly different at p<0.05. SoB = Soaked and boiled; BSb = Boiled with limestone.

Table 4. Calculated molar ratios of raw and processed biofortified and traditional beans cultivars (*Phaseolus vulgaris* L.)

Cultivars	PH 201				FEB 192				Standard value
Parameters	Raw	Boiling	SoB	BSb	Raw	Boiling	SoB	BSb	
(Phytates/Ca)[a]	$1.443x10^{-4}$	$8.58x\ 10^{-5}$	$3.97x10^{-5}$	$3.74x10^{-5}$	$1.33x10^{-3}$	$3.91x10^{-5}$	$5.59x10^{-5}$	$2.27x10^{-5}$	< 0.24
(Phytates/Fe)[b]	$1.64x10^{-3}$	$9.70x10^{-4}$	$6.01x10^{-4}$	$1.01x10^{-3}$	$1.83x\ 10^{-2}$	$5.66x10^{-4}$	$9.30x10^{-4}$	$6.12x10^{-4}$	< 0.15
(Phytates/Zn)[c]	$6.66x10^{-3}$	$3.63x10^{-3}$	$2.25x10^{-3}$	$3.13x10^{-3}$	$6.04x10^{-2}$	$1.76x10^{-3}$	$2.14x10^{-3}$	$1.26x10^{-3}$	< 10
(Oxalates/Ca)[d]	$3.47x10^{-3}$	$1.37x10^{-3}$	$7.33x10^{-4}$	$9.97x10^{-4}$	$2.10x10^{-3}$	$8.40x10^{-4}$	$1.12x10^{-3}$	$1.17x10^{-3}$	< 1
(Phytates*Ca/Zn)[e]	$1.05x10^{-2}$	$6.41x10^{-3}$	$5.28x10^{-3}$	$7.59x10^{-3}$	$1.30x10^{-1}$	$4.09x10^{-3}$	$4.64x10^{-3}$	$3.37x10^{-3}$	0.5

SoB = Soaked and boiled; BSb = Boiled with limestone; [a]mg of phytate/molecular weight of phytate: mg of calcium/molecular weight of calcium. [b]mg of phytate/molecular weight of phytate: mg of iron/molecular weight of iron. [c]mg of phytate/molecular weight of phytate: mg of zinc/molecular weight of zinc. [d]mg of oxalate/molecular weight of oxalate: mg of calcium/molecular weight of calcium. [e](mg of calcium/molecular weight of calcium) (mg of phytate/molecular weight of phytate)/(mg of zinc/molecular weight of zinc).

The mineral contents of biofortified and traditional beans seeds (*Phaseolus vulgaris* L.) according to the different treatments, expressed in mg/100g dry weight (DW), are shown in Table 2. The iron contents of biofortified bean were higher 8.73 (raw) than 7.75 (raw) of traditional bean seeds (P<0.05). The two varieties were high in magnesium, iron and potassium but low in zinc (P<0.05). The Ca/P values ranged from 1.28 to 1.79 for the biofortified bean cultivar and from 0.94 to1.61 for the traditional bean. The Na/K values were in the range of 0.06 to 1.63 for the biofortified variety. The control sample had the highest value of Na/K (1.43) in sample boiled with limestone while the least value (0.11) was held by sample soaked for 12h and boiled.

Table 3 shows the antinutrient contents of raw and processed biofortified and traditional beans cultivars (*Phaseolus vulgaris* L.) expressed in mg/100g DW. The two cultivars (raw) had the same rate of saponins. All processes led to significant reduction in the levels of all the antinutrients (P<0.05).

Table 4 shows the calculated molar ratios of raw and processed biofortified and traditional beans cultivars (*Phaseolus vulgaris* L.). The molar ratios of phytate to calcium (Phy/ca) ranged from $3.74x10^{-5}$ to $1.443x10^{-4}$ for the traditional bean and $2.27x10^{-5}$ to $1.33x10^{-3}$ for the biofortified beans. The ratios of phytate to iron (Phy/Fe) of the two cultivars varied from $5.66x10^{-4}$ to $1.83x\ 10^{-2}$.

4. Discussion

In order to improve the nutritional quality of beans, many cooking methods (such as boiling, soaking, boiling with limestone, boiling with sodium bicarbonate, soaking with sodium bicarbonate) are commonly used by people for preparing beans [12]. All of these processes reduce antinutritional factors but other phenomena may also occur, such as losses of the macronutrients and micronutrients, particularly minerals, during the processes of soaking and cooking [6].

All the bean samples are low in moisture and fell within the recommended range of 0-13.5% [24]. The value of 8.17% (traditional raw seeds) is higher than 3.97 % of biofortified beans (raw). The 3.97% moisture content of the biofortified bean obtained for the raw seed was low when compared to those values obtained and reported by Alayande et al. [25] for brown (5.08) and white (3.56) beans and the one reported by Brigide et al. [6] for Porto real (15.24) and Pirata (13.89) biofortified bean cultivars. It can be seen that all processes (boiling, roasting and boiling, boiling with limestone) increases the water content. This phenomenon can be explained by the fact that during these treatments the cells absorb water. The low moisture content of PH 201 and FEB 192 remains an asset in storage and preservation of the nutrients.

The proteins content ranged from 19.53% -27.66% for biofortified beans and 18.72%-29.28% for the traditional beans. The values lay within the range (18-23%) recorded by Faldu et al. [26] for raw and processed kidney bean seeds. The flour sample from the seed of biofortified bean soaked for 12h had the highest value (27.66%) while the traditional sample boiled with limestone had the highest value (29.28%). A significant difference (P<0.05) is observed between the protein contens of the raw and the processed seeds. The boiled seeds (26%), soaked and boiled (27.66%) and boiled with limestone (21.96%) had the highest content compared to the raw seeds (19.53%). The different processing methods enhaced crude protein content of the traditional and biofortified bean. This could be justified by changes in the association and dissociation properties of proteins caused by heating. 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 antinutrients, thus increasing the digestibility and the biological values of the protein of the bean [27]. This observation in agreement with the findings of Audu et al. [28] for sample processed by roasted, fermented and boiled. We observed an increase in nutrients particularly protein. Processed biofortified bean cultivars can be a good source of protein for the formulation of infants foods.

The lipid content were in the range of 3.32-5.48% for FEB 192 and 2.51-5.04% for PH 201. All the samples (raw and processed) biofortified and traditional bean cultivars contained low fat. Lipid content of the biofortified and traditional bean cultivars obtained for raw and processed seed was high when compared those values obtained and reported by Lajide et al. [29] in Ghana and by Farinde et al. [24] for Lima bean flour (1.14%) after cooking. However, the different processing methods reduced fat content of our two bean cultivars. This could be due to loss of total solids during soaking prior to further processing and denaturation of the fat by heat processing and leaching into the processing water [30]. Boiling with limestone resulted in increase the lipid content of the cultivars. This could be explained by the fact that limestone breaks the cells and membrane during boilingand then, lipids are liberated hence making lipids available [30]. 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 [31].

The crude fibre contents in the two cultivars ranged between 4.46% in the raw biofortified bean to 7.99% in the boiled biofortified bean. Processing also increased the crude fibre in the all batches for biofortified beans. However, crude fibre content decreased in boiling (6.30%) and boiling with limestone (5.12%) of traditional beans. The crude fibre content of the biofortified bean flours was 4.46-7.99%. The traditional sample had the highest value (7.65%) while the least value (5.12%) was held by the sample soaked for 12h and boiling. The values were within the range of 4.5%-17.5% crude fibre content for different bean cultivars in America as reported by Messina et al. [32]. The range of values was less than 17.41%-28.20% reported by ENV. [33] for 13 bean cultivars in Paris. The high rate of crude fibre in the processed sample could be explained by the fact that heat treatments can have variable effects on crude fibre 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 fibre of cooked foods compared to that of raw foods [6]. Our findings suggest that the value of crude fibre is within acceptable limit which helps to maintain the health of gastrointestinal tract [34].

The ash content of the biofortified bean cultivars was 3.08- 4.33%. The ash content of traditional bean cultivars was 2.57%-3.68% (Table 1). The values were within the range of 3.94-4.82% ash content for Lima bean (*Phaseolus Lunatus*) flour reported by Oraka et al. [35] and Duru et al. [36] for Velvet beans (3.18%-3.44%). There was a reduction in the ash content in the processed seeds except for the boiling traditional bean cultivar (3.68%). Low ash content in the bean could be due to leaching of salts and minerals into the cooking water [24].

The carbohydrates contents of our study suggest that biofortified bean cultivar could be a good supplement to which could be use for feed formulation. The carbohydrate values were low compare to those reported by Alayande et al. [25] for white and brown beans in Jos North (Nigeria) (56.80%-60.47%) and (55%-65%), (82.90%-87.29%) reported for the common beans (*Phaseolus vulgaris*) respectively by Alayande et al. [25] and Idoko et al. [37]. These values are close (51% to 41%) to those reported for the processed Velvet beans (*Mucuna Pruriens*) [36]. All the processing methods decrease the carbohydrate contents except for the boiling traditional seeds (59.62%). Similar observations were obtained on the seeds in Nigeria [31]. According to fernandes et al. [27], that reduction of the amounts of available carbohydrates could be due by the fact that carbohydrates being the water soluble compounds and then, they have been hydrolyzed and diffused in the soaking and cooking water. This low carbohydrate content in our study suggests that biofortified bean cultivar could be a potential good source of formulation for people suffering from diabete.

The energy content of our seeds depends on some of the products of each major food (proteins lipids and carbohydrates). The soaking and boiled biofortified bean cultivar had the highest value (355.68), followed by boiled with limestone (353.67) and raw seeds (376.36). The energy value of biofortified bean in this study showed that the biofortified bean cultivar has an energy concentration more favourable than extruded biofortified bean flours [38].

The result of mineral contents of raw and processed biofortified and traditional beans cultivars (*Phaseolus vulgaris* L.) shown in Table 2 indicate that the raw seeds of the two cultivars and the processed seeds contained appreciable amount of minerals (magnesium, iron, calcium, phosphorus and zinc). The most abundant mineral in the raw seed samples was magnesium (242.85 mg/100g and 234.9 mg/100g), calcium (63.12 mg/100g and 86.20 mg/100g) respectively for the traditional and biofortified bean cultivars. The raw seeds of biofortified beans were also rich in iron (8.73 mg/100g), phosphorus (67.33 mg/100g) and potassium (23.33 mg/100g). Processing significantly ($P<0.05$) decreased the mineral content (except for the calcium) in the raw seeds of the two cultivars. Reduction of the mineral content of the processed samples could be due to leaching of the minerals into the cooking water during boiling. However, according to Bamigboye et al. [31], increase of calcium content could be explained by the fact that during cooking the bonds between antinutrients and calcium were broken, which led to release of calcium into the cooking water resulting a higher concentration [31]. The results obtained for the two cultivars in magnesium are agreement with the report of the literature [24,39]. Furthermore, contradicts the reports of Mananga et al. [9] that potassium is the most abundant mineral in ten red bean cultivars. The value for sodium in the raw samples is lower than values reported by Hassan and El Syiad. [40] for white bean seeds.

Several authors found iron levels in 100g varied from 7.4 mg to 11.5 mg [28]. Iron is required for oxygen to travel to tissues and organs. It helps to carry oxygen throughout the body in form of heamoglobin and myoglobin, it is an integral part of many proteins and enzymes and it also helps in energy metabolism [41]. Our findings are within the range of RDA for iron (7-16 mg/100g) [42]. Biofortified bean cultivars was good source of iron and can be used for formulation for infants with iron deficiency anemia. Magnesium is an activator of many enzymes system and maintains the electrical potential in nerves [43]. It values in this study are above the recommended daily magnesium requirements (80-130mg for children) [43].

The very low sodium (except for boiling with limestone samples) and high potassium content of the raw bean cultivars is advantageous and make them suitable for management of metabolic syndrome. Indeed, treatment of high blood pressure requires a low sodium and high potassium for intra and extracellular electrolyte balance [31]. Potassium is nutritionally important for pH regulation and the proper functioning of carbohydrate and protein metabolism [44]. For this reason, bean seeds are an excellent food to cover daily potassium requirements (800 to 1600 mg/100g) for children of 2 to 9 years old [45]. The Na/K ratio in the body is of great concern for the prevention of high blood pressure. The Na/K ratio less than one is required. So, biofortified bean seeds could be used in the prevention of high blood pressure.

The value for zinc in the raw flour samples is higher than values reported by Faldu et al. [26] for chickpea (*Cicer arietnum)* and kidney bean (*Phaseolus vulgaris*). Zinc boosts the health of our hairs, plays a role in the proper functioning of some sense organs such as ability to taste and smell [6]. The zinc contents of our samples are lower than the RDA of 3 mg/day (children) and 11 - 12 mg/day (pregnant women) [43]. FEB 192 are an excellent food to cover daily zinc requirements.

Calcium is known as a macroelement necessary for the development of teeth, bones and the release of hormones [46]. Daily calcium requirements are 700-1300 mg for children [47]. Biofortified bean cultivars should be prepare and consumed with calcium rich foods for a good nutritional balance. The value for phosphorus in the raw flour samples is lower than values reported by Otitoju et al. [48] for the Cowpea (*Vigna Unguiculat*a). Phosphorus enables the fixation of calcium in the bones by decreasing its urinary excretion and takes part in the mechanism of energy storage and release [30]. According to Audu and Aremu. [28], phosphorus is always found with calcium in the body both contributing to the blood formation and supportive structure of the body. This led to the concept of calcium phosphorus ratio. If the Ca/P ratio is low, calcium will be low and there will be high phosphorus intake which leads to calcium loss in the urine more than normal. If the Ca/P ratio of any food is above one that food is considered “good” and “poor” if the ratio is less that 0.5, while a Ca/P ratio above two helps to increase the absorption of calcium in the small intestine. The Ca/P ratio in the biofortified bean cultivars particularly processed samples were above one. This suggest that biofortified bean could be considered good food.

Variations in the micronutrient contents of cultivars can be attributed to a number of factors: plant characteristics, such as plant age, maturity, species, variety, cultivar, environmental features, such as climate, soil, rainfall, season and processing factors, such as storage time, temperature, method of preservation, and preparation of food.

Antinutrients are found at some level in almost all foods. However, their levels are reduced through various traditional methods [49]. The findings of this study revealed that all the processing methods significantly ($p<0.05$) reduced antinutrients content in PH 201 and FEB 192 bean seeds. The amount of oxalates ranged from 0.70% (raw) to 0.22% (soaked and boiled) in traditional bean and 0.58% (raw) to 0.25% (boiled) in biofortified bean. The similar results have been reported by Farinde et al. [24] for processing Lima beans (*Phaseolus Lunatus*). In the literature, it was reported that oxalates reduce the availability of essentials nutrients [50]. Diet high in oxalate has been reported to increase the risk of development of kidney stone [51]. The oxalate to calcium (Oxalates/Ca) ratio of the biofortified beans varied from 2.10×10^{-3} to 8.40×10^{-4} (Table 4). This result implies that oxalate cannot have any adverse effects on bioavailability of dietary calcium in these seeds.

Tannin contents ranged between 0.61% (raw) to 0.09% (soaked and boiled and boiled with limestone) and 0.28% (raw) to 0.07% (boiled) in PH 201 and FEB 192 respectively. Processing significantly reduced the tannin contents in traditional and biofortified beans (more than 90%). The low tannin contents in the processed biofortified beans are similar to those values obtained and reported by Silva et al. [52] for the biofortified carioca bean (2.15% to 0.02) and the common bean (0.42 to 0.03%), and one reported by Mugabo et al. [53] for bean flours (0.90%-0.19%). According to Toledo et al. [54] tannins can interact with protein and interfere with digestibility of beans, decreasing the hydrolysis of phaseolin. Soaking and cooking are processing that can influence the amount of tannins.

Concerning to the determination of phytates, the concentration varied from 0.15% (raw) to 0.06 (soaked and boiled and boiled with limestone) in the traditional and 1.89% (raw) to 0.04% (boiled with limestone) in biofortified beans. These values are less than that of processed bean flours in Rwanda (2.42% -1.09%) [53]. According to Fabbri and Crosby. [55], boiling reduces phytates content of vegetables. However, a longer cooking time often results in greater reduction of phytates. Boiling with limestone and simple boiling were found to significantly reduced ($p<0.05$) all the phytates evaluated in the biofortified bean seeds. The molar ratio of phytate to iron (Phy/Fe) of biofortified bean ranged from 6.12×10^{-4} to 1.83×10^{-2}. According to Fekadu et al. [56], the phytate/iron molar ratios <0.15 is indicative of good iron bioavailability. The result indicated that the biofortified bean contain the phytate/iron molar ratios of less than the critical value this implies the good availability of iron. The phytate/calcium molar ratio varied from 2.27×10^{-5} to 1.33×10^{-3} (Table 4). The values are below the standard value (< 0.24), this implies good calcium bioavailability of biofortified bean [56]. The molar ratio of phytate to zinc (Phy/Zn) ranged from 6.04×10^{-2} to 1.26×10^{-3}, was less than 10, which is the critical molar ratio of Phy/Zn [56]. This indicates that biofortified bean have adequate availability of zinc.

Saponin contents varied from 5.10% (raw) to 4.63% (boiled with limestone) and 5.33% (raw) to 4.40% (soaked and boiled, boiled with limestone) in traditional and biofortified beans respectively. These values was higher than the value (0.05%) reported for boiling and roasting kidney bean seeds flour in Nigeria [58]. Boiling with limestone were found to significantly reduced ($p<0.05$) saponins evaluated in the samples. Saponins are known for their foaming properties in aqueous solution astringent taste and haemolytic activity on red blood cells [14]. Saponins are secondary metabolites with antibacterial and antithelmintic activities [59]. In conclusion, the differents processes (boiling with limestone, boiling, soaking and boiling) applied reduce significantly the antinutrient contents and make the minerals bioavailable.

5. Conclusion

Biofortified bean cultivars (FEB 192) were good sources of proteins, carbohydrates, crude fiber, iron, zinc, magnesium and potassium. The processing methods used by households improve the protein contents and reduce significantly the values ($P<0.05$) of antinutrients (oxalates, phytates, saponines and tannins). However, boiling, soaking and boiling, boiling with limestone decrease mineral contents (except calcium) slightly but remain high enough to cover the recommended daily needs for Fe, Zn, Ca, Mg in children. The processing method have improved the bioavailability and absorption of iron, zinc and calcium. Biofortified bean cultivars may be exploited in food formulation to prevent malnutrition and micronutrient deficiencies.

Conflict of Interests

The authors have not declared any conflict of interests.

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