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ASSESSMENT OF MINERAL CONTENTS AND ANTIOXIDANT ACTIVITIES OF SOME BEAN SEEDS (PHASEOLUS VULGARIS L.) FROM WEST CAMEROON REGION

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Background: The ever-increasing prevalence of malnutrition due to micronutrient deficiency and oxidative stress makes it a permanent public health problem in Cameroon. The purpose of this present study was to investigate the mineral, total phenolic compounds and antioxidant capacities of five raw and cooking bean seeds varieties. Methods: Mineral contents (Ca, Mg, Fe, and Zn, P) and total phenolic contents of methanol (70%) extracts were investigated using standard analytical techniques. Antioxidant capacities were evaluated using Ferric Reducing Antioxidant Power (FRAP) and DPPH free radical scavenging assays. The IC-50 values for the percentage radical scavenging effects for the extracts were determined. Results: The result showed that processing significantly ($p < 0.05$) affected the content of some minerals, phenolic contents and antioxidant activities in all Phaseolus vulgaris L. seeds. Magnesium was the most abundant mineral. Its content ranged from 130.75 mg to 442.26 mg/100 g. Phosphorus, calcium, iron and zinc contents ranged from 126.84 mg; 1.00 mg; 1.88 mg and 3.58 mg/100 g to 266.88 mg; 1.50 mg; 3,13 mg and 6.08 mg/100 g respectively. The total phenolic contents ranged from 234.82 ± 1.93 to 518.71 ± 0.66 mg GAE/g. FRAP and DPPH IC-50 ranged from 6.53 to 33.67 mg Fe II/100 g and 57.91 to 305,73 µg/µL respectively. Conclusion: The results of the study revealed that Phaseolus vulgaris L. from West Cameroon region are a good source of minerals and phenolic compounds. They are able to manage malnutrition due to micronutrient deficiency and oxidative stress.

Keywords: Malnutrition, *Phaseolus vulgaris* L, West region of cameroon beans, Minerals, Phenolic compounds, Antioxidant activities

INTRODUCTION

These last decades, several studies carried out in the nutritional context were more limited to the evaluation of contents in protein-energetic nutrients. However, the deficiency in micronutrients constitutes a significant threat for the world population's health and the development. Currently, it affects approximately two billion individuals throughout the world (UNICEF/MI/GAIN/USAID, 2009). This alarming situation touch many people's and is considered as risk factor for many chronic diseases, raising it as major health problem in the world (Berger and Gracey, 1994). In Cameroun, victims are mostly children less than five years (0 to 59 months more exposed to the risks of death), pregnant and nursing women. This deficiency is associated in the reduction of resistance to the infectious diseases (MINSANTE, 2011). To manage this situation, several strategies were prescribed by the ministry of public health: the promotion of the micronutrients food

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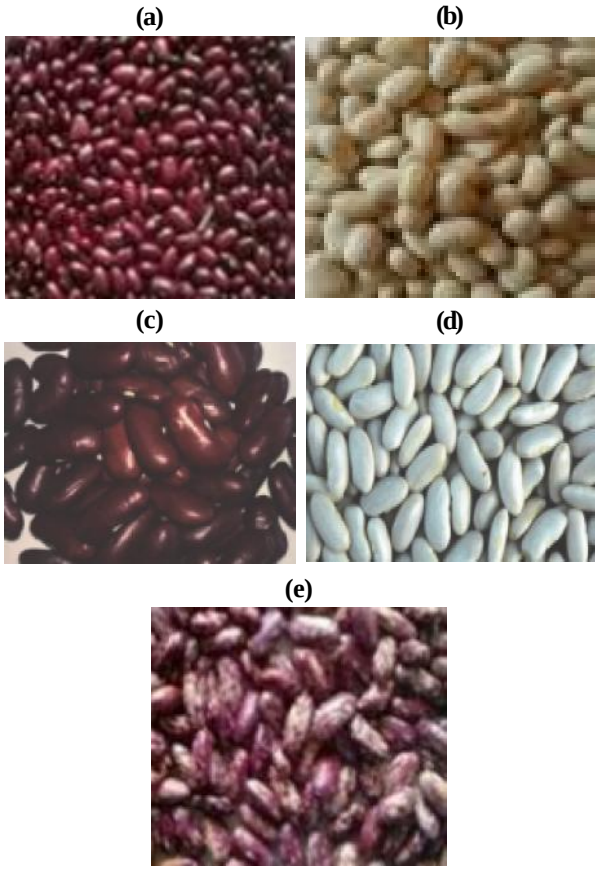
fortification; the promotion of the micronutrients supplemented program to groups of risk peoples (MINSANTE, 2006). We also assist to the marketing of some tablets containing calcium, magnesium and of iron. One limit to that politic is the cost of these tablets. For illustration, within the period from 2013 to 2014, cameroonians spent approximately CFA 255 millions for minerals such as Ca-Mg-Iron (CENAME, 2015). Moreover, these products might create some side effects to consumers as they are purely chemical origin. In addition, since few years, the world has been invaded by a new concept, known as "oxidative stress". Currently, it is admitted that it is potentially at the origin or associated to the complications of chronic diseases during their evolution. However, Cameroon is not saved of the recrudescence of these pathologies. It is the case of coronary heart diseases (Anderson *et al*., 1999; and Bazzano *et al*., 2001); diabetes, cancer (Hangen and Bennink, 2002); sickle cell disease (Fasola *et al*., 2007). Despite the strategies of prevention and care, these problems of health always remain of topicality. In fact, minerals and more secondary metabolites such as the phenolic compounds found in food showed the capacity to solve upstream these problems. As illustration, bean seeds (*Phaseolus vulgaris* L.) with a significant source of natural minerals and phenolic compounds (Wortmann *et al.,* 1998). The objective of the present work is to determine the minerals content, total phenolic compounds, and antioxidant activities of five varieties bean seeds (*Phaseolus vulgaris* L.) from West Cameroon region before and after the cooking in order to contribute managing malnutrition due to micronutrient deficiency and oxidative stress.

Figure 1: Photos of Five Varieties of Beans (*Phaseolus vulgaris* L.) Analyzed, (a) PH201: Small Red Seed; (b) PB: Small White Seed; (c) GGR: Big Red Seed; (d) Mex 142: Big White Seed; (e) GLP 190C: Red Spotted White Seed



MATERIALS AND METHODS

Collection and Preparation of Sample

The five varieties of been seeds were purchased at local market B of Bafoussam and authenticated under PH201(small red seed); PB (small white seed); GGR (big red seed); Mex 142 (big white seed); GLP 190C (red spotted white seed) (Figure 1) at the Agricultural Institute of Research for the Development of Foumbot station, West region of Cameroon. The seeds were cleaned and extraneous materials carefully removed by hand sorting. Sample was divided into two parts in the ratio 1:2.

Processing

Raw Sample: Five hundred g of the each raw seeds sample were finely ground to a fine powder with a Kenwood blender after washing and drying in an oven at 45 °C during 12 hours. Cooked seeds: Five hundred g of the each raw seeds sample were soaked in distilled water for 8 hours at room temperature and then cooked at 100 °C on a hot plate until they became soft to touch. At the end of cooking time, the water was drained and the seeds were oven dried and were finely ground to a fine powder with a Kenwood blender. All samples powder where then stored at 4 °C for further manipulation.

Mineral Analysis

Minerals (Calcium, Phosphorus, Magnesium, iron, and Zinc) Minerals content of the samples was carried out by AOAC method No 968.08 (AOAC, 1995). About 100 g of powders for each raw and cooking bean seeds varieties were oven dried at 105 °C for 24 hours. After drying 5 g were separately

weighed into crucibles and dry ashed in muffle furnace maintained at 550 °C for 24 hr. The ash was cooled in desiccators and then weighed. After weighing, the ash was dissolved in a solution of 1:1 ratio of H_2O:HCl, in which the concentration of the final mixture was 6NHCl. Calcium, Magnesium, iron, and Zinc were determined by atomic absorption spectrophotometer (Shimadzu UNICAM 919, England) when total Phosphorus concentration was measured by colorimetric spectrophotometer after incubation with Molybdo-vanadate solution.

Extraction of Total Phenolic Compounds

Phenolic compounds were extracted according to the methods of Chen *et al.* (2016). Two g of each sample powder bean seeds was defatted twice with 40 mL hexanes to remove lipid by shaking on a wrist action shaker for 12 hours at room temperature. Then, 30 mL of methanol-water (70:30) were added followed the extraction using magnetic stirrer at room temperature under dark conditions. The residues were removed by filtration using Whatman No 1 filter papers. The residues were collected, re-extracted till the solvent become clear less. The combined extracts were refiltered and evaporated using a rotavapor at 45 °C. The dried extracts were dissolved in 10 mL by the origin solvent and mixed thoroughly. The extracts were stored at 4 °C.

Determination of Total Phenolic Content (TPC)

Total phenolic content The TPC was determined by the Folin-Ciocalteau method using Gallic acid as a standard (Vinson *et al.*, 1998). Firstly, 2 mL aliquot of each of sample extract was mixed with 2 mL of Folin-Ciocalteu reagent 2 N and the mixture was allowed to stand for 5 min at room temperature under dark conditions. Then, 2 mL of 10% sodium carbonate solution was added to the mixture, vortexed vigorously and kept at room temperature in dark for 15 min. The absorbance was measured at 760 nm using a Bioteck spectrophotometer against a blank. A calibration curve was performed with the standard Gallic acid (concentrations ranging from 20 to 200 µg/mL) which is a common reference phenolic compound and the results expressed as mg Gallic acid equivalents/g dry mass.

Evaluation of Antioxidant Activities

Radical DPPH Scavenging Activity

The DPPH scavenging activity of different extracts was evaluated according to the method of evaluated Lopes-Lutz *et al.* (2008). In the tests tube 150 µl of samples were added to different concentration 1.5%; 1.25%; 1%; 0.75% and 0.5%. Then 3 mL of DPPH 0.04% (w/v) were added. Corresponding blank sample were prepared and Gallic acid was used as reference standard. Mixer of 1 mL methanol and 1 mL DPPH solution was used as control. The mixture was shaken well and incubated for 30 min in dark at room temperature. The absorbance was measured at 517 nm after incubation. The result was expressed using the formula:

$$\% \text{ Inhibition} = (Ao-As/Ao) \times 100$$

with As = Absorbance of sample,

Ao = Absorbance of control

Afterwards, a curve of % DPPH bleaching activity versus concentration was plotted using OriginPro 8 Software and IC_{50} values were obtained. The scavenging activity was expressed as IC_{50} (µg/µL).

Ferric-Reducing Antioxidant Power (FRAP)

The FRAP assay was performed as previously reported Benzie and Strain (1996). 0.1 mL was mixed with 3 mL of FRAP reagent and incubated at room temperature for 5 min. The absorbance of solution was measured at 593 nm. the FRAP value was expressed as milligram of Fe^{2+} equivalent per 100 g of dry bean (mg Fe^{2+}/100 g) using the calibration curve of Fe^{2+}.

Statistical Analysis

The data were expressed as mean ± standard deviation of triplicate measurements. Statistical analysis was performed using the software Statistical Package for the Social Science (SPSS) version 20.0 for Windows. One-way ANOVA was used to compare results and significance was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

Minerals Analysis

Minerals (Ca, P, Mg, Fe, and Zn) content in raw and cooked bean seeds are presented in Tables 1 and 2. As with legumes, some undesirable constituents of *Phaseolus vulgaris* L. include antinutrients (trypsin inhibitors, phytic acid, saponins, haematoglutinns and tannins) that interfere with absorption and utilization of important minerals such as calcium, iron, zinc and magnesium. It is known that processing methods such as soaking and cooking process inactivate the antinutrients and can be used to improve the nutritional quality of the beans (Deshpande and Deshpande, 1991; and Qayyum *et al.*, 2012). Of all the minerals

Table 1: Mineral Compositions (Ca, P, Mg) of Five Varieties Bean Seeds Raw and Cooked Accessions (mg 100 g^{-1} Dry Weight Basis) and their Percentage of Reduction

Varieties of Beans	Ca		% Reduction	P		% Reduction	Mg		% Reduction
	Raw	Cooked		Raw	Cooked		Raw	Cooked	
PB	1.60±0.00	1.00±0.00	37	354.41±0.00*	144.35±0.00*	59	714.42±0.00*	442.26±0.00*	38
GLP190-C	1.80±0.00	1.50±0.00	16	249.38±0.00*	196.86±0.00*	21	223.56±0.00*	199.26±0.00*	10
PH201	1.60±0.00	1.40±0.00	12	284.39±0.00*	249.38±0.00*	12	184.68±0.00*	136.08±0.00*	26
MEX142	1.70±0.00	1.10±0.00	35	354.41±0.00*	266.88±0.00*	24	250.72±0.00*	139.08±0.00*	44
GGR	1.60±0.00	1.30±0.00	18	214.37±0.00*	126.84±0.00*	40	260.72±0.00*	130.75±0.00*	49

Note: * p<0.05; Significant different compared to corresponding raw and cooked, and bean seeds varieties.

Table 2: Mineral Compositions (Fe, Zn) of Five Varieties Bean Seeds Raw and Cooked Accessions (mg 100 g^{-1} Dry Weight Basis) and their Percentage of Reduction

Varieties of Beans	Fe		% Reduction	Zn		% Reduction
	Raw	Cooked		Raw	Cooked	
PB	3.75±0.00	3.13±0.00	16	4.66±0.00*	3.58±0.00*	23
GLP190-C	2.50±0.00*	1.88±0.00*	24	6.67±0.00*	4.96±0.00*	25
PH201	3.13±0.00*	2.50±0.00*	20	6.97±0.00	6.08±0.00	12
MEX142	3.75±0.00*	2.50±0.00*	33	6.38±0.00*	5.59±0.00*	12
GGR	3.13±0.00*	2.50±0.00*	20	5.00±0.00	4.66±0.00	6

Note: * p<0.05; Significant different compared to corresponding raw and cooked, and bean seeds varieties.

determined, magnesium is the most abundant having a value range of 714.42 mg/100 g in the raw to 442.26 mg/100 g in the cooking PB sample. It is followed by phosphorus (266.88 mg/100 g) in the cooking MEX142 sample; zinc (6.08 mg/100 g) in the cooking PH201 sample; iron (3.13 mg/100 g) in the cooking PB sample and calcium (1,5 mg/100 g) in the GLP190-C cooking sample. Nevertheless these results showed that, cooking process caused the greatest loss of phosphorus (59%). However, that difference depends of the varieties of bean seeds. The minerals leached from the bean seeds into the water during cooking treatment. Cooking process caused a loss of Ca, P, Mg: 10.71%; 5.88% and 20.86% respectively (Mubarak, 2005). Haytowitz and Matthews (1983) reported that cooking also caused a great loss of Fe (8%). In this study, the loss of Fe is from 16 to 30%. Generally, the decrease significantly in mineral content ($p<0.05$) may be due to leaching out during cooking processes. This is why the results of cooked seeds had retained more our attention.

Magnesium is required for bone formation which maintains the electrical potential in nerves (Shils and Young, 1998). The calcium and phosphorus levels are reasonably distributed in the sample. Phosphorus is always found with calcium in the body both contributing to the blood formation and supportive structure of the body (Ogunlade *et al*., 2005). 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 (Payne, 1990). Iron helps in the formation of blood and in the transfer of oxygen and carbon dioxide from one tissue to another (Guthrie, 1989). Iron deficiency results in impaired learning ability and behavioral problems in children, and also anaemia (McDonald, 1995).

Total Phenolic Content

Total phenolic contents in the extracts from the raw and cooked bean seeds are shown in Table 3. Results, expressed as mg GAE/g (dw of seeds), showed significant differences ($p\leq0.01$) among samples and within the same group of beans.

Table 3: Total Phenolic (mg EAG/g) Content in Raw and Cooked Bean Seeds with their Percentage of Reduction

Values (mg EAG/g)			
Varieties of Beans	**Rawextract**	**Cookedextract**	**% Reduction**
PB	522.87±1.4^{d}	382.87±1.84^{b}	26.7±1.62
GLP190-C	539.72±8.89^{e}	234.82±1.93^{a}	56.4±5.41
PH201	523.79±0.939^{de}	518.71±0.66^{d}	1±0.79
MEX142	481.76±2.98^{c}	473.89±2.26^{c}	1.6±2.62
GGR	521.39±1.05^{d}	510.00±1.15^{d}	2.18±1.1

Note: Values marked by the same letter within a line are not significantly different ($p < 0,05$).

Maximal quantitative differences in TPC were obtained in the extracts of raw GLP190-C (539.72); followed of PH201 (523.79); PB (522.87) and GGR (521.39). The extracts obtained from the cooked beans showed a lower amount of TPC without respect to the corresponding raw samples because maximal quantitative differences in TPC were obtained in the extracts of PH201 (518.71) and GGR (510.00). The differences in total phenolic contents caused by cooking processing between varieties might be due to the differences in the distributions and compositions of individual phenolic compounds in bean seeds coat and cotyledon (Xu and Chang, 2009). Bressani and Elias (1980) observed that about 30-40% of phenolic could be removed from common beans by cooking and discarding in cooking water. In the present study, it was found that about 1-56.4% of TPC were reduced in different varieties of beans. However Xu and Chang (2009) reported that cooking process caused a decrease of TPC to about 63-77% in two varieties of bean seeds (pinto and black bean). These significant losses might be attributed to those water-soluble phenolic that were leached into soaking and cooking water before and during thermal processing as well as the breakdown of phenolic compounds during processing. The beneficial effects of phenolic compounds on human health are expressed primarily through the reduction of the oxidative stress (Ranilla *et al.*, 2010).

Antioxidant Activities of Bean Seeds

The antioxidant effects in bean extracts were investigated by DPPH and FRAP tests. Overall, the samples exhibited relevant antioxidant qualities and few µg of the extracts were sufficient to inhibit at 50% of the activity of 1 µL of the free radical DPPH (Table 4). Results showed significant

Table 4: Inhibition Concentration (IC_{50} µg /µL) of Raw and Cooked Bean Seeds with their Percentage of Reduction

IC_{50} (µg / µL)			
Varieties of Beans	**Raw Extract**	**Cooked Extract**	**% Reduction**
PB	294,03±3,46^{e}	183,3±0,00^{d}	37,65±1,73
GLP190-C	169,73±15,52^{cd}	113,3±1,84^{b}	33,2±8,68
PH201	146,26±3,1^{c}	57,91±0,38^{a}	60,4±1,74
MEX142	345,5±4,5^{f}	305,73±1,96^{e}	11,5±3,23
GGR	150±1,73^{c}	94,9±1,49^{b}	36,7±1,61
Gallicacid (1 µg/µL)	21,1±0,37	21,1±0,37	

Note: Values marked by the same letter within a line are not significantly different ($p < 0,05$).

differences ($p \leq 0.01$) among raw and cooked samples; and on the same group of bean seeds. Maximal quantitative differences in IC_{50} (µg/µL) were obtained between the extracts of MEX142 (ranging from 345.5 to 305.73) and PB (ranging from 294.03 to 183.3) but very lowest than IC_{50} of Gallic acid (the standard antioxidant). FRAP was expressed as Fe II/100 g (Table 5). FRAP of MEX142 and PH201 were evaluated as 142 and 48.1 for raw samples; 57.13 13 and 33.67 for cooked samples. In comparison with the raw bean seeds, cooking treatment caused significant ($p<0,05$) decreases of DPPH and FRAP values. Boiling is generally considered as destructive of antioxidant compounds. This was verified by our antioxidant assays. These results showed that, cooking processes decreased about 11.5-60.4%

Table 5: FRAP (mg Fe II/100 g) Values of Raw and Cooked Beans with their Percentage of Reduction

Values (mg Fe II / 100 g)			
Varieties of Beans	**Raw Extract**	**Cooked Extract**	**% Reduction**
PB	36,73±0,54^{d}	10,96±1,33^{a}	70±0,9
GLP190-C	38,43±1,07^{d}	6,53±0,14^{a}	83±0,6
PH201	48,1±0,36^{e}	33,67±1,12^{cd}	37,8±0,74
MEX142	57,13±0,22^{e}	26,33±5,3^{bc}	53,9±2,76
GGR	48,1±0,36^{e}	21,26±1,11^{b}	55,8±0,7

Note: Values marked by the same letter within a line are not significantly different ($p < 0,05$).

of DPPH values, and 37.8-83% of FRAP values. These values was similar to the results obtain by Xu and Chang (2009) who had obtained 61.1-66.4% of DPPH values and 69.5-74.3% of FRAP values.

Phenolic compounds are able to act as antioxidants in a number of ways. Phenolic hydroxyl groups are good hydrogen donors: hydrogen-donating antioxidants can react with reactive oxygen and reactive nitrogen species (Choi *et al*., 2002). Several varieties of beans seeds (*Phaseolus vulgaris* L.) are consumed throughout the world primarily as an important source of plant proteins. However, in recent years bean seeds phenolic compounds have received considerable attention mainly due to their health promoting properties (Cardador-Martinez *et al*., 2002).

CONCLUSION

From the result of this study, it has been revealed that these five varieties of *phaseolus vulgaris* L. from West region of Cameroon have both minerals and phenolic compounds values. Small white grain (PB) variety contained a high value of magnesium and iron; Big white grain (MEX 142) was a high of phosphorus; Red spotted white (GLP 190C) had a high value of calcium; Small red grain (PH201) variety contained a high value of zinc and the high antioxidant activity has showed in small red grain (PH201). They can contribute for reduction of mineral malnutrition and oxidative stress.

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