African Journal of Biotechnology Vol. 10(79), pp. 18160-18166, 12 December, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.022

Full Length Research Paper

Effect of boiling on the physicochemical properties of Roselle seeds (*Hibiscus sabdariffa* L.) cultivated in Mali

Fatoumata Tounkara[1,2], Issoufou Amadou[1], Guo-Wei Le[1]* and Yong-Hui Shi[1]

[1]State Key Laboratory of Food Science and Technology, Jiangnan University, No. 1800 Lihu Road, Wuxi, 214122, Jiangsu Province, P. R. China.
[2]Faculté des Sciences et Technique, Université de Bamako, Colline de Badalabougou, Bamako, Mali.

Accepted 15 June, 2011

Effect of boiling on the physicochemical composition of Roselle seeds (*Hibiscus sabdariffa*) grown in Mali was shown. Proximate analysis indicated that boiled whole Roselle seeds (BWRS) are potential high protein source. Moreover, the results of lipid analysis indicated that the seeds were good source of unsaturated fatty acid (74.33%) with that of linoleic (35.55%), oleic (36.64%), palmitic (19.34%) and stearic acid (4.86%) being the major fatty acid constituents. The most predominant inorganic elements were found to be potassium, followed by magnesium and calcium. Aluminum and phosphorus were relatively low in both BWRS and un-boiled whole Roselle seeds (WRS). Roselle seeds exhibited various molecular weight distributions as revealed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Scanning electron microscopic (SEM) analysis revealed that boiling had little influence on the physical particles size. In addition, Roselle seed powder meals maintained excellent protein, lipid and minerals compositions, following boiling with protein quality of powders prepared from whole and boiled seeds being similar.

Key words: Roselle seeds, boiling, physiochemical analysis, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, fatty acid.

INTRODUCTION

Hibiscus is one of the most common flower plants grown worldwide. There are more than 300 species of hibiscus around the world. One of them is *Hibiscus sabdariffa*, Linn, which is a member of the Malvaceae family. The origin of *H. sabdariffa* is not fully known, but it is believed to be native of tropical Africa. It is known by different synonyms and vernacular names such as Roselle (Abu-Tarboush et al., 1997; Chewonarin et al., 1999; Tsai et al., 2002). *H. sabdariffa* is an herbaceous plant also known as karkade, Roselle, graines d'oseille and guinean sorrel (Parkouda et al., 2008). Roselle is a very versatile plant similar to the coconut tree (Quezon, 2005). Roselle can be found in almost all warm countries such as India, Saudi Arabia, Malaysia, Thailand, Philippine, Vietnam, Sudan, Egypt, Mali and Mexico (Quezon, 2005; Amin, 2008). Ombuwajo et al. (2000) reported that Roselle seeds are bigger than the pearl millet varieties having average dimension of 5.21 and 2.81 mm, respectively.

Traditional fermented foods take a significant place in the African nutrition. They improve the population nutritional state for their nutritive values (proteins, minerals, vitamins). Bikalga is a food condiment obtained by a traditional uncontrolled fermentation of *H. sabdariffa* seeds in African countries, including Burkina Faso, Mali Niger, Nigeria, Cameroon and Sudan among others. It is also known as dawadawa botso (Niger), datou (Mali),

*Corresponding author. E-mail: lgw@jiangnan.edu.cn. Tel: +86 51085917789. Fax: +86 51085917789.

Abbreviations: WRS, Whole Roselle seeds; **BWRS,** boiled whole Roselle seeds; **DWRS,** defatted whole Roselle seeds**; DBWRS,** defatted boiled whole Roselle seeds; **MW,** standard molecular weight; **Bikalga,** food condiment obtained by traditional uncontrolled fermentation of *Hibiscus sabdariffa* seeds in African countries; **Dawadawa botso (Niger),** another name for Bikalga; **Datou (Mali),** another name for Bikalga; **Furundu (Sudan),** another name for Bikalga; **Mbuja (Cameroon),** another name for Bikalga; **DHL®,** posting company; **SEM,** scanning electron microscopic; **SDS-PAGE,** sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Furundu (Sudan), Mbuja (Cameroon) (Parkouda et al., 2008; Daramola and Asunni, 2006).

Plant protein is potentially important in developing countries. In many countries in Africa such as Mali, Niger, Sudan, etc, animal source foods are mainly consumed by households of higher socio-economic status and majority of the population do not access these foods due to poverty (Al-Wandawi et al., 1983; Koumi et al., 2009; Amadou et al., 2010). The physicochemical and nutritional benefits of boiled Roselle seeds prior to Bikalga making has not been documented as compared with the whole seeds. Literature indicated that Roselle whole seeds powder from other countries contained high amounts of protein, oil and carbohydrate (Abu-Tarboush et al., 1997; Parkouda et al., 2008; Emmy Hainida et al., 2008a). However, no physicochemical and nutritional data has been reported on boiled Roselle seeds grown from Mali. Therefore, the objectives of this study were to investigate the effects of boiling on the physicochemical properties that include proximate composition, protein molecular weight distribution, scanning electron microscopy, and minerals, fatty acids and color changes.

MATERIALS AND METHODS

Seeds of *H. sabdariffa* were obtained from Koutiala, southern region of Republic of Mali and the seeds were transported to Wuxi, China through DHL®. Sodium dodecyl Sulfate (SDS) and Coomassie Brillant Blue R-250 were purchased from Wako Pure Chemical Industries, Ltd (Tokyo, Japan). All other chemicals were obtained from the Chemical Reagent Co., China and were of analytical grade quality.

Samples preparation

Whole Roselle and Roselle seeds (WRS)

Roselle seeds were cleaned by removing dust, stones and plant debris. The seeds were milled using a laboratory scale hammer miller and the resulting powder was sieved through a 60 mesh screen until fine powder was obtained.

Boiled Roselle and Roselle seeds (BWRS)

After the initial cleaning process, the Roselle seeds were cooked for 12 h using a controlled electric stove from Midea Industries Ltd (Guangdong, China). Seeds were cooked until soft, and then dried at 50°C for 20 h. The boiled dried seeds were milled using a laboratory scale hammer miller and the resultant powder were sieved through a 60 mesh screen until fine powder was obtained.

Proximate composition

Moisture content of Roselle seeds powders was determined according to the air-oven method. Ash content was determined by incinerating at 550°C until the constant weight was achieved. Total nitrogen and the protein content were determined based on the Kjeldahl method using the conversion factor of 6.25. Lipids were determined by using Soxhlet method, whereas total available carbohydrate was determined by difference, subtracting the sum of percentage of moisture, crude fat, crude protein and ash contents from 100%. Triplicate samples were analyzed for each sample. All these determinations were based on the methods of AOAC (1990).

Minerals composition

Samples were digested in 100 ml micro-Kjeldahl flask with $HNO_3/HClO_4$ until the solution became colourless. The samples were cooled and diluted to volume in a 25 ml volumetric flask with 0.1 M HCl. Sodium, potassium, calcium, magnesium, iron, zinc, manganese and copper were measured by atomic absorption spectrophotometry, (Garcia et al., 1972) using a Varian spectra atomic absorption spectrophotometer (Varian SpectrAA220, Varian, Palo Alto, CA).

Fatty acid analysis

Fatty acids for the Roselle seeds were determined according to the method of Ceirwyn (1995). Fat was extracted with methyl ether that was prepared directly with the treatment of the fat with sodium methoxide. Gas chromatography/mass spectra (GC/MS) system was used to identify and quantify the fatty acids of the product developed on a FINNIGAN TRACE MS gas chromatograph/mass spectra equipped with a 30 m x 0.25 mm Ov-1701 column. Column flow rate was 0.8 ml/min with helium as the carrier gas, split was 64 ml/min and the source temperature was 270°C. The fatty acid methyl esters were identified by comparison with the retention times of NU CHECK Inc. standards (Elysian, 1L) and quantified by internal normalization.

Scanning electron microscopy

Scanning electron microscopic (SEM) studies of Roselle seeds were carried out using scanning electron microscope (Quanta-200 FEI, Netherland). The samples were coated before being loaded to the scanning electron microscopy. The coated samples were loaded into the system and the image was viewed under 5.0 KV potential using secondary electron image. The image was captured using 11.1 mm Ricoh Camera of 600x magnification.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE of Roselle seeds samples were carried out according to the method described by Laemmli (1970) with 4% stacking and 12% separating gel. Separating gel was run at a constant current of 20 mA for about 3 h. The gel was stained in Coomassie Brilliant Blue R-250. Subunit molecular weight (MW) was estimated using low MW calibration kit (Shanghai Institute of Biochemistry, Shanghai, China) consisting of the following proteins: phosphorylase (97.4), bovine serum albumin (66.2), rabbit actin (43.0), bovine carbonic anhydrase (31.0), trypsin inhibitor (20.1) and hen egg white lysozyme (14.4) (kDa).

Color measurements

Sample color was evaluated using the Hunter Lab colorimeter (WSC-S Color Difference Meter, USA) and reported as L*, a* and b* values, in which L* is a measure of lightness, a* represents the chromatic scale from green to red and b* represents the chromatic scale from blue to yellow. All the experiments were performed in triplicate and the results were average of three values.

Table 1. Proximate composition of Roselle seeds powders.

Composition	WRS	BWRS	DWRS	DBWRS
Moisture	8.15±0.18[a]	7.61±0.36[a]	9.85±0.15[c]	8.80±0.20[b]
Ash	4.47±0.11[a]	4.63±0.08[a]	5.59±0.13[b]	5.48±0.21[b]
Protein	27.32±0.39[b]	25.75±0.59[a]	36.00±0.69[c]	36.40±0.10[c]
Lipid	20.83±0.55[a]	21.30±0.74[a]	2.04±0.16[b]	1.99±0.11[b]
CHO	39.23±0.60[a]	40.70±1.06[a]	46.52±0.97[b]	47.33±0.41[b]

WRS, Whole Roselle seeds; BWRS, boiled whole Roselle seeds; DWRS, defatted whole Roselle seeds; DBWRS, defatted boiled whole Roselle seeds; CHO, carbohydrate. Mean values in rows with different letters are significantly different ($P < 0.05$).

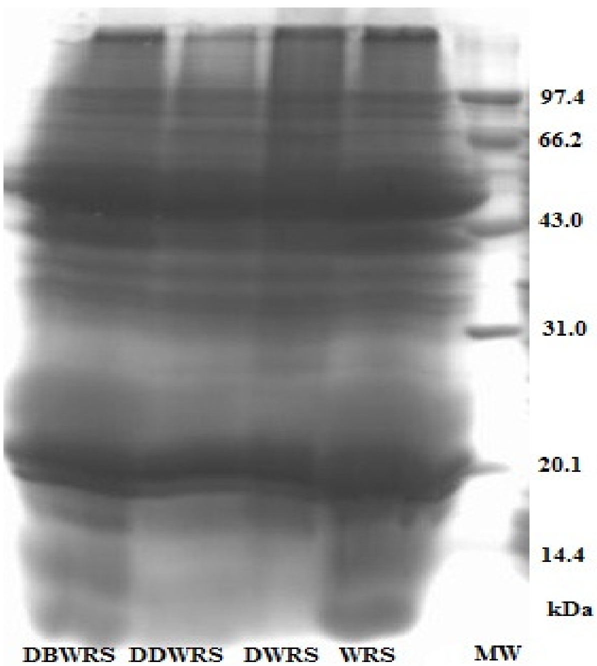

Figure 1. SDS-page of Roselle seed powders. WRS, Whole Roselle seeds; BWRS, boiled whole Roselle seeds; DWRS, defatted whole Roselle seeds; DBWRS, defatted boiled whole Roselle seeds; MW, standard molecular weight (kDa).

Statistical analysis

Data analysis was carried out with SPSS Inc. software (version 16.0). Data was expressed as Mean ± standard deviation of three experiments. One-way analysis of variance (ANOVA) was used to determine significant differences between means, with the significance level taken at $P < 0.05$. Tukey's HSD test was used to perform multiple comparisons.

RESULTS AND DISCUSSION

Proximate chemical composition

Proximate composition of whole, boiled and defatted Roselle seeds powders is presented in Table 1. The seeds are regarded as by-product of Roselle leaves processing, and seeds are used in West Africa as raw material for making Bikalga known as datou in Mali a kind of fermented condiment (Parkouda et al., 2008). The protein content of the samples increased significantly ($P < 0.05$) after defatting (Table 1). The defatting process altered the protein content of the WRS and BWRS powder (27.32 to 30.00% and 25.75 to 36.40, respectively). The moisture content of Roselle seed was similar to those of several legume proteins (Aremu et al., 2006; Amza et al., 2010; Fagbenro et al., 2010). The fat content of Roselle seeds cultivated in savanna region of Mali was compared to that of tropical environment of Malaysia and they were relatively similar (Emmy et al., 2008). However boiling had insignificant or little effect on the lipid content (20.8 and 21.30%, respectively), for the WRS and BWRS (Table 1). There were significant differences ($P < 0.05$) between the Roselle seeds with respect to ash content. This can be explained by the fact that boiling does not cause any important mineral lost. This observation corroborated with the work of Emmy et al. (2008a, b) and Abu-Tarboush et al. (1997).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Figure 1 shows the SDS-PAGE profiles of Roselle seed samples under reducing conditions. Figure 1 illustrates that DBWRS, DDWRS, DWRS and WRS indicate polypeptides of a wide range of molecular weights. Defatting has slight influence on band patterns. Larger and thicker bands were located inbetween 43.0 and 66.2 kDa molecular weight distributions and 20.1 kDa which indicated higher protein concentration at these two locations and this may be attributed to higher protein content observed in this study. However, lesser thicker bands were found in the range of 31.0 to 45.0 kDa and between 66.22 and 97.4 kDa polypeptides distribution (Figure 1). Boiling did not show any significant influence on protein distribution which implied similarities in the band width and intensity in the four samples tested (Figure 1). These results correlated with the illustration of scanning electron microscopy shown in Figure 2 (Amza et al., 2010). Halimatul et al. (2007) found that the protein quality of the studied whole Roselle seeds was similar to

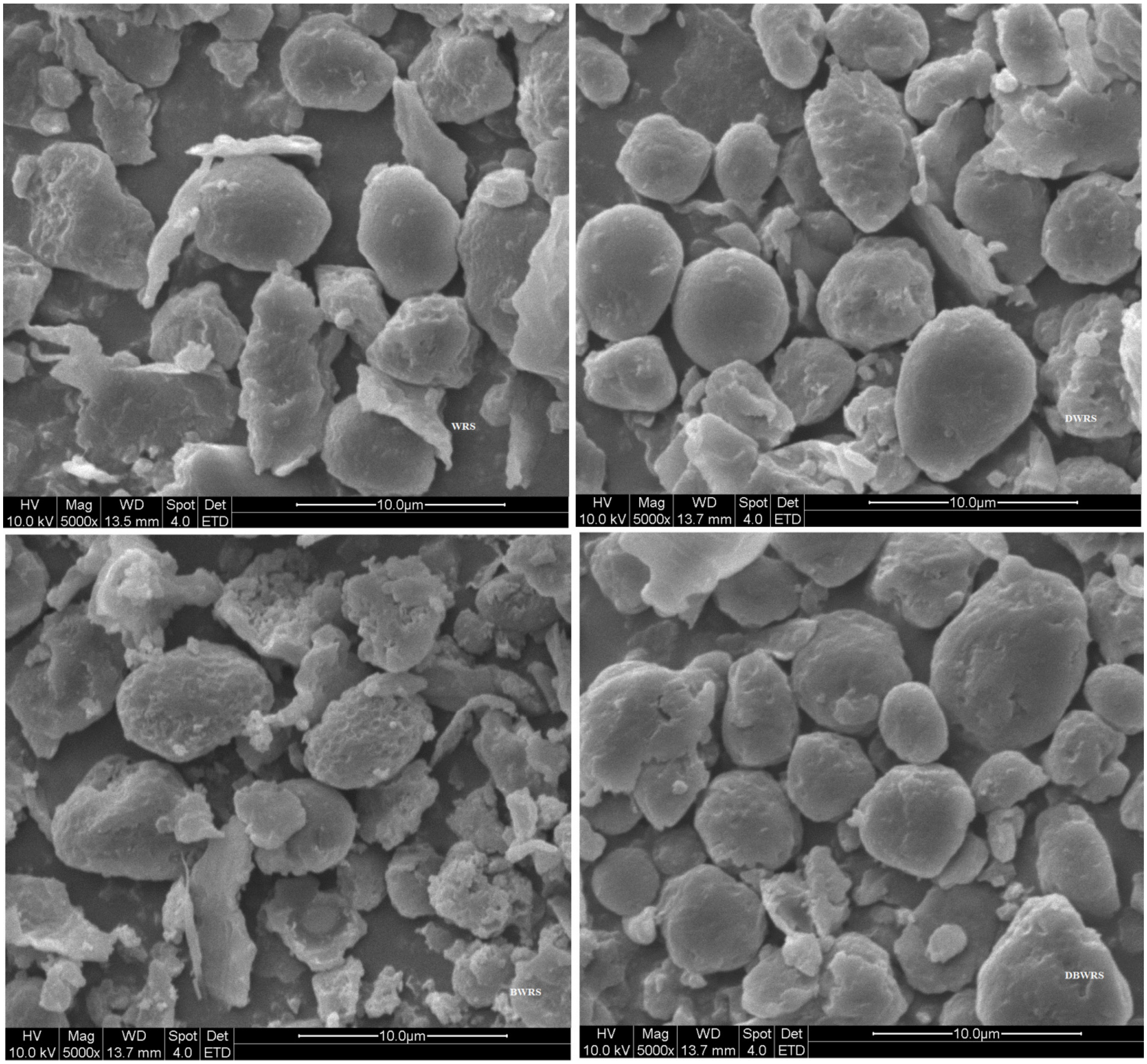

Figure 2. Scanning electron microscopic (SEM) of Roselle seeds powders. WRS, Whole Roselle seeds; BWRS, boiled whole Roselle seeds; DWRS, defatted whole Roselle seeds; DBWRS, defatted boiled whole Roselle seeds.

that of boiled seeds. Therefore, the similarities of bands in the SDS-page were logical.

Scanning electron microscopy

Scanning electron microscopy was used to examine the micro structural changes of proteins after defatting (DWRS and DBWRS). The SEM pictures of boiled and un-boiled Roselle seed (WRS and BWRS, defatted WRS and BWRS Roselle seed powder), respectively, are shown in Figure 2. The results indicated that the two powdered meals had minimally degraded into their fragments after defatting and seemed to have no boiling effect on the dried powdered structural particles. Furthermore, the results showed that the particles were clustered together and a scenario that showed degradation of the protein particles does take place during the defatting process. Our findings agree with the report of Radha et al. (2007) and Adeogun and Adeogun (2009) on the oil seeds.

Fatty acid analysis

Fatty acid composition of the oil extracted from Roselle seeds is given in Table 2. The oil of Roselle seed showed considerable fat content even when compared with oil

Table 2. Fatty acids analysis of Roselle seeds oils (contents are area %).

Fatty acid (%)	Whole Roselle seed oil	Boiled whole Roselle seed oil
Saturated		
Myristoleic acid (C14:0)	0.21	0.23
Palmetic acid (C16:0)	19.21	19.34
Stearic acid (C18:0)	5.13	4.86
Arachidic acid (C20:0)	0.67	0.64
Total	25.22	25.07
Unsaturated		
Palmitoleic acid (C16:1)	0.36	0.36
Oleic acid (C18:1)	36.9	36.64
Linoleic acid (C18:2)	35.02	35.55
Alpha-linolenic acid (C18:3)	1.85	1.78
Total	74.13	74.33

Table 3. Color difference between Roselle seed powders

Parameter	ΔL	Δa	Δb	ΔL^2	Δa^2	Δb^2	ΔE
I-II	1.823333	-0.07	-0.05667	3.324544	0.0049	0.003211	1.825556
I-III	5.276667	0.793333	2.3	27.84321	0.629378	5.29	5.810558
I-IV	10.74	-0.96333	-2.49	115.3476	0.928011	6.2001	11.06687

WRS (I): Whole Roselle seeds, BWRS (II): boiled whole Roselle seeds, DWRS (II): defatted whole Roselle seeds, DBWRS (IV): defatted boiled whole Roselle seeds. Mean values in column are not significantly different at (P < 0.05).

seeds; with more than 73% of unsaturated fatty acids (Table 2) for this cultivar. Oleic and linoleic acids were the highest fatty acid, and accounted for 36.90 and 35.02% WRS; however, palmitoleic acids had the lowest level (0.36%) of WRS among the unsaturated fatty acids (Table 2). Whereas, palmitic acid (19.21% WRS) was the highest among the saturated fatty acid content as shown in Table 2. The proximate composition of Roselle seed revealed high fat content in about 21% (Table 1) and the fatty acid composition confirmed the high shear in unsaturated fatty acid, especially linoleic acid (35.02% WRS), thus, indicating the nutritional benefit of Roselle seeds. Linoleic acid had beneficial effect on blood lipids, lowering blood pressure and serum cholesterol (Savage, 2001; Enujiugha and Akanbi, 2008). These results are in good agreement with the findings reported by Emmy et al. (2008b) and El-Adawy and Khalil (1994). The test showed no significant difference (P < 0.05) among the sample in terms of fatty acid content and composition.

Color measurement

Color characteristics of boiled and defatted Roselle seeds powders (BWRS, DWRS and DBWRS) in comparison with the whole Roselle seeds powder (WRS), after boiling/defatting are given in Table 3. The color difference (ΔE) of the Roselle powders marked I-II, I-III and I-IV accounted for 1.82, 5.81 and 11.06, respectively; where I, II, III and IV stands for WRS, BWRS, DWRS and DBWRS, respectively. Generally, defatting and boiling had little influence on the color variation (Table 3). There were no significant differences (P < 0.05) among the samples studied; however, DWRS showed higher yellowness which is an indication of color quality. The yellowness of flour color ranges is an important quality and attribute in many cereal/seeds based foods for end-use products, and is therefore one of the many flour specifications required by end-users. Nonetheless, our results corroborated with other plant seed powder studied (Omobuwajo et al., 2000; Emmy et al., 2008a, b; Enujiugha and Akanbi, 2008).

Minerals

The mineral composition of WRS, DWRS, BWRS and DBWRS seeds is presented in Table 4. The mineral compositions of Roselle seeds boiled and un-boiled were found to contain between 2282.83 and 6345.12 µg/g calcium and 20.65 and 129 µg/g zinc (Table 4). In general, the results showed that boiling and defatting increased the concentration of almost all the mineral elements investigated in this study, except magnesium. However, defatting significantly (P < 0.05) decreased manganese when compared with the un-boiled sample

Table 4. Minerals composition of Roselle seeds powders (µg/g).

Element	WRS	BWRS	DWRS	DBWRS
Zinc (Zn)	128.83±1.04^{b}	129.00±0.82^{b}	21.75±0.17^{a}	20.65±0.25^{a}
Potassium (K)	20341.67±77.83^{b}	20058.33±7.63^{a}	29347.61±13.17^{d}	21050.01±50^{c}
Iron (Fe)	93.78±6.50^{c}	59.00±0.81^{a}	80.18±0.40^{b}	80.06±0.81^{b}
Phosphore (P)	8.81±0.10^{a}	8.91±0.04^{a}	14.48±0.08^{d}	13.03±0.06^{c}
Magnesium (Mg)	5433.33±131.23^{c}	2023.35±14.33^{a}	2679.12±0.62^{b}	2473.33±10.27^{b}
Maganese (Mn)	146.67±2.23^{c}	148.67±0.62^{c}	121.35±11.38^{b}	63.75±0.20^{a}
Sodium (Na)	489.33±3.92^{a}	845.33±3.68^{c}	834.00±2.23^{b}	1370.00±0.81^{d}
Cupper (Cu)	46.90±2.20^{b}	37.03±0.36^{a}	180.44±0.84^{d}	111.07±0.06^{c}
Aluminium (Al)	2.71±0.50^{a}	3.04±0.04^{ab}	8.45±0.04^{c}	3.78±0.01^{b}
Calcium (Ca)	2282.83±65.34^{a}	5250.00±8.16^{b}	6345.12±0.40^{d}	5969.67±0.84^{c}

WRS, Whole Roselle seeds; BWRS, boiled whole Roselle seeds; DWRS, defatted whole Roselle seeds; DBWRS, defatted boiled whole Roselle seeds. Mean values in rows with different letters are significantly different ($P < 0.05$).

(WRS). There was significant ($P < 0.05$) difference between the samples. Potassium was the predominant element in the seeds, followed by magnesium and calcium (Table 4). Aluminum and phosphorus were relatively low in all the samples studied. The increase may be attributed to the long time boiling, though studies reported that incineration may drain or leak some of the minerals (Seena et al., 2004). Mineral elements were reported to be significantly influenced by variety, location and environmental conditions (Emmy et al., 2008a, b). Hence, these factors may be responsible for different variations exhibited by the current and previous values. El-Adawy and Khalil (1994) reported K, Mg, Na and Ca to be the major predominant element in Roselle seeds. Similar work was also reported by Emmy et al. (2008a) in their study on nutritional and amino acid contents of differently treated Roselle seeds.

Conclusion

Physicochemical properties of boiled Roselle seeds (BWRS) for human consumption were comparable with whole raw seeds (WRS). The analysis of whole and boiled seeds revealed that these seeds are promising, with good sources of proteins, fatty acids and minerals. These potential nutritional properties of Roselle seeds powder should be exploited and used as functional ingredients for the development of nutraceuticals or functional food products. Boiling had insignificant influence on the physiochemical properties of Roselle seeds. Since cereal powders in the baking industry are usually deficient in some mineral elements such as calcium, fortification with Roselle seeds powder may improve the dietary properties.

ACKNOWLEDGEMENTS

This research was supported by the National Natural Science Foundation of China (No. 30671525), the National High Technology Research and Development Program ("863" Program) of China (No. 2007AA10Z325) and 111 project-B07029. The authors wish to thank Mr Coulibaly Oumar (Bamako, Mali) for providing Roselle seeds down to Wuxi, People's Republic of China.

REFERENCES

Abu-Tarboush HM, Ahmed SAB, Al-Kahtani HA (1997). Some nutritional properties of Karkade (*H. sabdariffa*) seed products. Cereal Chem. 74: 352-355.

Adeogun AI, Adeogun MB (2009). Effect of irradiation and extractive solvents on the Thevetia seed oil. Afr. J. Biotechnol. 8(5): 841-843.

Al-Wandawi H, Al-Shaikhly K, Abdul-Rahman M (1983). Roselle seeds: a new proteins source. J. Agric. Food Chem. 32: 510-512.

Amadou I, Amza T, Foh MBK, Kamara MT, Le GW (2010). Influence of *Lactobacillus plantarum* Lp6 Fermentation on the functional properties of soybean protein meal. Emi. J. Food Agric. 22(6): 456-465.

Amin I, Emny Haida KI, Mohd-Nazri HS (2008). Roselle (*Hibiscus sabdariffa* L.) seeds-nutritional composition, protein quality and health benefits. Food © Global Sci. Books, 2: 1-16.

Amza T, Amadou I, Kamara MT, Zhu K, Zhou H (2010). Chemical and Nutrient Analysis of Gingerbread Plum (*Neocarya macrophylla*) Seeds. Adv. J. Food Sci. Technol. 2(4): 191-195.

AOAC (1990). Official methods of analysis 16th edition. Association of official Analytical chemists, Washington, DC.

Aremu MO, Olonisakin A, Atolaye BO, Ogbu CF (2006). Some nutritional and functional studies of prosopis Africana. Electron. J. Environ. Agric. Food Chem. 5(6): 1640-1648.

Ceirwyn JS (1995). Analytical Chemistry of Foods. Blackie Academic and Professional, London, pp. 71-135.

Chewonarin T, Kinouchi T, Kataoka K, Arimochi H, Kuwahara T, Vinitketkumnuen U, Ohnishi Y (1999). Effects of Roselle *(Hibiscus sabdariffa* Linn.), a Thai medicinal plant, on the mutagenicity of various known mutagens in *Salmonella typhimurium* and on formation of aberrant crypt foci induced by the colon carcinogens Azoxymethane and 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine in F344 rats. Food Chem. Toxicol. 37(6): 591-601.

Daramola B, Asunni OA (2006). Nutrient composition and storage studies on Roselle extract enriched deep-fat-fried snack food. Afr. J. Biotechnol. 5(19): 1803-1807.

El-Adawy TA, Khalil AH (1994). Characteristics of Roselle Seeds as a New Source of Protein and Lipid. J. Agric. Food Chem. 42: 1896-1900.

Emmy Hainida KI, Amin I, Normah H, Mohd-Esa N (2008a). Nutritional and amino acid contents of differently treated Roselle (*Hibiscus sabdariffa* L.) seeds. Food Chem. 111: 906-911.

Emmy Hainida KI, Amin I, Normah H, Norhaizan ME, Ainul Z (2008b). Effects of defatted dried roselle (*Hibiscus sabdariffa* L.) Seed powder on lipid profiles of hypercholesterolemia rats. J. Sci. Food Agric. 88: 1043-1050.

Enujiugha VN, Akanbi CT (2008). Quality evaluation of canned fermented oil bean seed slices during ambient storage. Afr. J. Food Sci. (2): 054-059.

Fagbenro OA, Adeparusi EO, Jimoh WA (2010). Nutritional evaluation of sunflower and sesame seed meal in Clarias gariepinus: An assessment by growth performance and nutrient utilization. Afr. J. Agric. Res. 5(22): 3096-3101.

Garcia WJ, Blessin CW, Inglett GE (1972). Mineral constitutents in corn and wheat germ by atomic absorption spectroscopy. Cereal Chem. 49: 158-167.

Halimatul SMN, Amin I, Mohd-Esa N, Nawalyah AG, Stti Muskinah M (2007). Protein quality of Roselle (*Hibiscus sabdariffa* L.) seeds. Asean Food J. 14: 131-140.

Koumi AR, Atse BC, Kouame LP (2009). Utilization of soya protein as an alternative protein source in Oreochromis niloticus diet: Growth performance, feed utilization, proximate composition and organoleptic characteristics. Afr. J. Biotechnol. 8(1): 091-097.

Laemmli UK (1970). Cleavage of structural proteins during the assembly of head of bacteriophage T4. Nature, 227(5259): 680-685.

Omobuwajo TO, Sanni LA, Balami YA (2000). Physical properties of sorrel (*Hibiscus sabdari* A) seeds. J. Food Eng. 45: 37-41.

Parkouda C, Diawara B, Ouoba LII (2008). Technology and physico-chemical characteristics of Bikalga, alkaline fermented seeds of *Hibiscus sabdariffa*. Afr. J. Biotechnol. 7(7): 916-922.

Quezon (2005). Roselle: The dawn of a sunrise industry. Avalable online: http:www.quezon.org/Roselle

Radha C, Kumar PR, Prakash V (2007). Preparation and characterization of protein hydrolysates from an oilseed flour mixture. Food Chem. 106(3): 1166-1174.

Savage GP (2001). Chemical composition of wallnuts (*Juglans regia* L.) grown in New Zealand. Plants Foods Hum. Nut. 56: 75-82.

Seena S, Sridhar KR, Jung K (2004). Nutritional and anti-nutritional evaluation of raw and processed seeds of a wild legume, Canavalia cathartica of coastal sand dune of India. Food Chem. 92(3): 465-472.

Tsai PJ, Mc Intosh J, Pearce P, Camden B, Jordan BR (2002). Anthocyanin and antioxidant capacity in Roselle (*H. sabdariffa* L.) extract. Food Res. Int. 35: 351-356.