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RESEARCH ARTICLE

PHYSICAL PROPERTIES AND NUTRITIONAL POTENTIALS OF INDIAN ROSELLE (*HIBISCUS SABDARIFFA L*) SEEDS

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ABSTRACT

The physical and nutritional properties of Indian Roselle (*Hibiscus sabdariffa L*) seeds where studied. In India the Roselle seeds are not well known as such underutilized, the plants are cultivated and used mostly for their fiber, vegetables and calyces for making local drinks. The vast proportions of the seeds often go to waste as it is used mainly for the purpose of propagation. The seeds have been found to be a good source of lipid (23.80 %), proteins (38.06 %), a rich fiber source (19.87 %) and inorganic minerals such as calcium (Ca) 320.45 mg/100 g, magnesium (Mg) 464.36 mg/100 g, phosphorus (P) 590.14 mg/100 g, potassium (K) 1925.67 mg/100 g, iron (Fe) 11.45 mg/100 g, zinc (Zn) 17.43 mg/100 g and manganese (Mn) 7.57 mg/100 g , also containing significant percentages of bioactive components that could provide massive suitable substitute nutritionally as a cheap nutrients source especially in a vegetarian society as India and other parts of the world.

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INTRODUCTION

Roselle (*Hibiscus sabdariffa L*) is a tropical plant a member of malvaceae family of more than 300 species around the world. The seeds have proven huge potentials reported to be rich in dietary fiber, lipid and proteins (El-Adawy and Khalil 1994; Ismail *et al.* 2008). Physical properties are moisture content dependent (Bamgboye and Adejumo, 2009) and are critical for optimizing equipment design, oil extraction and handling physical and chemical properties of agricultural seeds (Karaj and Müller, 2010). Ismail *et al.* (2008) reported Roselle seeds are bigger than pearl millet varieties having average principle dimensions length, width and thickness 2.98-3.36, 1.86-2.24 and 1.70-2.01 mm respectively (Jain and Bal, 1997). Omobuwajo *et al.* (2000) found Roselle seeds dimensions as 5.58, 5.21 and 2.81mm. The lipid profile of the seeds extract suggests beneficial use nutritionally and medically in lowering blood pressure and serum cholesterol (Savage, 2001; Enujiugba and Akanbi, 2008; and Tounkara, *et al.* 2011). Many researchers have found that the plant polyphenolic compounds scavenge free radicals, thereby can protect humans against oxidative damage and diseases (Beecher, 1999; Cicerale *et al.,* 2009; Okawa *et al.,* 2011).

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Roselle seeds are rich source of proteins with high biological values, albumin and globulins (Tounkara, *et al.,* 2013). Ismail *et al.* (2008) reported Roselle to be highest protein containing seeds when compared with other seeds like passion fruits (*Passiflora edulis*), amaranthus, black seeds (*Nigelle sativa* L.) and Pea (*Pisum sativum*) seeds. Roselle seed is considered to have a good ratio of soluble to insoluble fibre fraction (Emmy Hainida *et al.,* 2008). Histopathological studies on broiler performance (Ismail *et al.,* 2008) demonstrated no damage in the liver due to Roselle seeds in the diet. An *in-vivo* study indicated that Roselle seeds can be included in levels up to 30% without adverse effect on weight gain or liver function (Perry, 1980). Nyam *et al.* (2014) included Roselle seed powder into wheat flour for cookie production; hedonic scale sensory evaluation score adjudged 20% as optimum. However, the percentage may vary due to consumer preference and overall acceptability. In Africa it is ground into meal for human food due to their high protein content. They are also roasted to use as a substitute for coffee (Morton, 1987) and with high amount of healthy oil resembling that of cotton seed (Mohammed *et al.,* 2007). Abu-Tarboush and Ahmed (1996) reported defatted flour and protein isolate of Roselle seeds contained protease inhibitors, phytic acid and gossypol, but they would pose no problem if the seeds are properly processed. Emmy Hainida *et al.* (2008); Ismail *et al.* (2008) and Rao (1996) reported Roselle seed contain good amount of inorganic minerals Calcium (Ca), Magnesium (Mg), Potassium (K), Phosphorus (P). Zhang *et al.* (2010) reported that there is a strong positive correlation between mineral concentration and protein contents. These minerals are essential in preventing deficiency diseases (Cissouma *et al.,* 2013) and they play vital roles in sustaining our health (Sleelig, 1980; Durlach, 1988). Hence the aim of this research was to evaluate the physical and nutritional potentials of Indian Roselle seeds and the value it could offer for industrial growth and the overall wellbeing of humanity.

MATERIALS AND METHODS

Materials

Seeds of Roselle (*Hibiscus sabdariffa* L.) were obtained from the vegetable markets in Ahmednagar District, Maharashtra State, India. All chemicals used were of analytical grade (AR) purchased from M/S. Qualigens Fine Chemicals, Mumbai, M/s. Sarabha M. Chemicals, M/s. Baroda Merck (India) and M/s, S.d. Fine Chemicals Ltd., Mumbai, India.

Sample preparation

Roselle seeds were cleaned by washing to separate poor quality seeds, adhering dust particles, stones, plant debris and were dried carefully at ambient conditions to preserve its nutritive value, packed in a HDPE bag and stored in a cool dry place until used. The mathematical expression given below as reported by Bamgboye and Adejumo (2009) was adopted to adjust the moisture content of seeds.

$$\text{ml of water added} = \left[\frac{100 - M_P}{100 - M_R} - 1\right] \times W_S$$

$$M_P = \text{present moisture content, \%db}$$
$$M_R = \text{required moisture content, \%db}$$
$$W_S = \text{weight of sample, g}$$

Methods

Physical properties

Length, Width and Thickness

The three most important dimensions *viz.* length, width and thickness were measured with a digital micrometer Vernier Caliper manufactured by Mitutoyo Corporation, Japan to an accuracy of 0.001mm.

The thousand seed weight

The thousand seed weight was measured to obtain the average weight of single seed using Sartorius AG Germany (Model: CPA323S-0CE) digital weighing balance of 0.001g accuracy.

Percentage of bran and cotyledon

Percentage of bran and cotyledon was determined by soaking the seeds in distilled water for a period of 6 hrs and drained. The seed was pressed between hand fingers to neatly separate out the bran (seed coat) from the cotyledons. The bran and cotyledons were dried at 105 ^{o}C for 3hrs in an oven. The dry weight was then obtained using Sartorius AG Germany (Model: CPA323S-0CE) digital weighing balance of 0.001g accuracy, and the percentage was calculated and recorded.

Angle of repose

Angle of repose was determined using the method as described by Schlumberger (2008).

The Bulk density

The bulk density (ρ_b) was determined using the method described by Bamgboye and Adejumo (2009).

True density

True density (ρ_t) of the seeds was determined by Toluene displacement, a slight adjustment to the method described by Mohsenin (1986).

Porosity

The Porosity (P_f) of the sample seeds were computed from the values of true density and bulk density of the seeds using the relationship reported by Bamgboye and Adejumo (2009).

$$P_f = \left[1 - \frac{\rho_b}{\rho_t}\right] \times 100$$

Coefficient of friction

Coefficient of friction was determined using the method described by Karaj and Möller (2010).

Proximate analysis

The proximate contents of Roselle seeds were determined according to the Official Method as described by AACC (2000). Approved methods of the American Association of Cereal Chemists (10th ed.), St. Paul, MN: Author (methods 08-01, 30-25, 44-15A, 46-13, and 54-21)

Mineral analysis

Macro elements: Calcium (Ca) and magnesium (Mg) were both determined using complexometric titration with potenciometric indicator method as described by El Mahi *et al.* (1987); potassium (K) was determined by using method described by Knudsen and Peterson (1982); and phosphorus (P) was determined by the method described by Jackson (1973). Iron, Manganese and Zinc were determined from sample diacid digest with the help of the Atomic Absorption Spectrophotometer (AAS) as described by Baker and Suhr (1982).

Fatty acid analysis: The fatty acids content in Roselle seeds were determined according to the method of Ceirwyn (1995).

Total Phenol Content (TPC)

Determined calorimetrically as described by Bray and Thorpe (1954).

Antioxidant activity: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity was determined according to the method of Liu and Yao (2007).

Statistical Analysis

Statistical analysis were conducted using excel 2010 software (Panse and Sukatme, 1985) the results obtained from the present study are represented as the mean values of three individual replication ± standard deviation. One way analysis of variance (ANOVA) was used to determine significant difference between means with the significance level taken at $p<0.05$.

RESULTS AND DISCUSSION

Physical properties of Roselle seed

Physical properties are properties that are measurable, whose values describes a state of a physical quality and are dependent on moisture contents (Bamgboye and Adejumo, 2009; Sanchez-Medoza *et al.,* 2008; and Omobuwajo *et al*., 2000) and are critical for optimizing equipment design, oil extraction and handling physical and chemical properties of agricultural seeds (Karaj and Müller, 2010). The results for physical properties of Roselle seed estimated at 9.5 % moisture content agrees with reported literature values at moisture content 8.8 to 19 % (Bamgboye and Adejumo, 2009) (Table 1) with the exception of percent bran 42.61 % and cotyledon 57.39 % not reported. Omobuwajo *et al*. (2000) reported higher values of 5.58 x 5.21 x 2.81 mm for L x W x T at 7.7 % moisture content, aside the factor of moisture content this disparity could also be as a result of locality of harvest. Roselle seed is a dicotyledonous seed with convoluted and shriveled thick bran (seed coat) as evident in the percentage ratio Table 1. The results obtained from this study corroborate literature reviews and provides the base information for handling and industrial utilization of Roselle seeds.

Table 1. Physical properties of Roselle seeds

Properties		Estimated Value at moisture content 9.5 % wb	Reported Values at moisture content 8.8 to 19 % wb
Dimension, mm:			
Length		4.84 ± 0.26	4.75 – 4.85
Width		4.16 ± 0.23	4.15 – 4.26
Thickness		2.69 ± 0.15	2.62 – 2.67
Seed weight, g		0.0355 ± 0.045	0.0345 – 0.0411
Seed weight, g		0.0355 ± 0.045	0.0345 – 0.0411
Bran, %		42.61 ± 0.62	nr
Cotyledon, %		57.39 ± 0.61	nr
Angle of Repose, ^{0}		24.47± 0.59	20.13 – 24.85
Bulk density, Kg/m^3		645.93 ± 7.92	619.14 - 648.31
True density, Kg/m^3		1375.82 ± 33. 17	1367.0 – 1487.4
Porosity		53.59 ± 1.93	52.6 – 58.3
Coefficient of static friction	Glass	0.27 ± 0.05	0.22 – 0.30
	Steel plate	0.31 ± 0.075	0.23 – 0.32
	Plywood	0.41 ± 0.07	0.38 – 0.45

Mean values in rows are significantly different ($p<0.05$); nr – not reported

Proximate composition of Roselle seeds: Estimated on dry weight basis (dw) and the outcome of the analysis showed sample seeds contained 5.66 % moisture, 23.80 % lipid, 38.06 % crude protein, 4.40 % ash, 19.87 % crude fiber and 13.87 % total Carbohydrate (Table 2). The seed is rich in protein and lipids. The results obtained are in harmony with the findings reported by Al-Wandawi *et al.* (1984) for moisture and ash contents; Emmy Hainida *et al*. (2008) and Tounkara *et al.* (2013) for protein, lipid contents, crude fiber and total carbohydrate contents. The overall proximate composition showed that Roselle seed holds a huge potential for nutrition and industries.

Table 2. Proximate composition of Roselle (*Hibiscus sabdariffa L.*) seeds

Parameter %	Mean ±S.D.
Moisture	5.66±0.31
Lipid	23.80±0.14
Crude Protein	38.06±0.21
Ash	4.40±0.18
Crude fiber	19.87±0.75
Carbohydrate	13.87±0.11

Mean values in rows are significantly different ($p<0.05$)

Inorganic minerals: Results in (Table 3) showed that Roselle seed as a dense mineral source. The outcomes for calcium (Ca) 320.45 mg/100 g, magnesium (Mg) 464.36 mg/100 g, phosphorus (P) 590.14 mg/100 g, potassium (K) 1925.67 mg/100 g, iron (Fe) 11.45 mg/100 g; zinc (Zn) 17.43 mg/100 g; and manganese (Mn) 7.57 mg/100 g corroborate the findings reported by previous studies (Rao, 1996; Nzikou *et al.,* 2011; and Cissouma *et al.,* 2013).

Table 3. Inorganic mineral composition of Roselle (*Hibiscus sabdariffa L.*) seeds

Parameter (mg/100g)	Mean
Calcium (Ca)	320.45±1.58
Magnesium (Mg)	464.36±1.34
Potassium (K)	1925.67±1.71
Phosphorus (P)	590.14±1.20
Iron (Fe)	11.45±1.89
Zinc (Zn)	17.43±1.69
Manganese (Mn)	7.57±1.91

Mean values in rows are significantly different ($p<0.05$)

These minerals are essential in preventing deficiency diseases (Cissouma *et al.,* 2013) and could play a vital role in the normal functions of the body's biochemical processes. For example, Calcium is a mineral that is necessary for life. In addition to building bones and keeping them healthy, calcium helps for preventing blood clot; nerves send messages and muscles contract (Nof, 2016). Magnesium plays a very critical role in energy synthesis and storage, as phosphate and magnesium ion interaction makes magnesium essential to the basic nucleic acid chemistry of all cells of all known living organisms; boosting over 300 enzyme actions including all enzymes using or synthesizing ATP and those that use other nucleotides to synthesize DNA and RNA; as ATP molecules are normally found in a chelate with a magnesium ions (Romani and Andrea, 2013); essential for normal function of the heart, kidney, arteries and bone (Sleelig, 1980) and for neuromuscular system (Durlach, 1988). Symptoms of poor magnesium intake include muscle cramps, facial tics, poor sleep, and chronic pain.

Fatty acid profile composition of Roselle seed oil: The major fatty acid found for raw Roselle seed oil were linoleic acid (41.18 %) followed by oleic acid (31.22 %), palmitic acid

(21.45 %) and stearic acid (3.94%); the results obtained in this research findings (Table 4) are in close harmony with El-Adawy and Khalil (1994); Emmy Hainida *et al.* (2008); and Cissouma *et al.* (2013). Ratio of saturated to unsaturated fatty acids in Roselle seed lipid corroborates with Mohiuddin and Zaidi (1975) of 1:3 (Table 4), in contrast to widely acknowledged 1:2 ratio El-Adawy and Khalil (1994). The Roselle seed oil can be classified as belonging to linoleic-oleic category as the most abundant unsaturated fatty acids present, suggesting it beneficial use in lowering blood pressure and serum cholesterol (Savage, 2001; Enujiugba and Akanbi, 2008; Tounkara *et al.,* 2011 and Cissouma *et al*., 2013)

Table 4. Fatty acid composition of Roselle seed oil

Fatty acids	% profile
Lauric acid (C12:0)	0.01
Myristic acid (C14:0)	0.20
Palmitic acid (C16:0)	21.45
Stearic acid (C18:0)	3.94
Arachidic acid (C20:0)	0.59
Eicosenoic acid (C20:0)	0.01
Behenic acid (C22:0)	0.25
Lignoceric acid (C24:0)	0.14
Total Saturated Fatty Acid	26.2
Palmitoleic acid (C16:1)	0.44
Oleic acid (C18:2n6c)	31.22
Linoleic acid (C18:2n6c)	41.18
Gamma linolenic acid (C18:2n6)	0.01
Alpha linolenic acid (C18:3n3)	0.27
Cis-11,14-eicosadienoic acid (C20:2)	0.01
Total Unsaturated Fatty Acid	73.13
Lipid Dietary Profile Ratio	1: 2.79

Total phenol content (TPC) of Roselle seed

Total phenol content (TFC) in Roselle seed was extracted using 80 % ethanol and expressed as mg Catechol /g of dry sample. The total phenol obtained for the whole Roselle seed was 2.23 mg /g dry weight was in conformity with the results, 1.99 to 1.66 mg GAE/g for 30 % acetone and water extract respectively reported by Cissouma *et al.* (2013). The slight variation may be related to the polarity of solvent type used in the extraction as different solvent will yield different total phenolic contents (Cissouma *et al.*, 2013). The result obtained in this study confirms the high total phenol content in Roselle seeds.

Antioxidant activity potential of Roselle seed

DPPH free radical test is based on the exchange of a proton between the antioxidant and the stable DPPH free radical and shows absorption at 517 nm. In this study the analysis revealed 69.11 % DPPH activity for Indian seeds as against to 44.7 % reported by Nyam *et al.* (2014) for Malaysian seeds. This demonstrates a more positive radical scavenging activity for Indian seeds. The variation could be as a result of difference in varieties.

Conclusion

The outcome of these studies confirms Roselle seed as a source of potential vital nutrients and bioactive compounds responsible for essential biological activities for overall well being. Minerals are essential elements that activate enzymes performing metabolic functions of human body processes; deficiency may develop various chronic diseases. Antioxidant helps the body fight free radicals that make us look old and free radicals responsible for many secondary diseases. Fortification of bakery products with a more economical nutrients source such as Roselle seeds flour could improve the dietary properties, since cereal flours in baking industry are usually deficient in some vital mineral elements.

REFERENCES

AACC. 2000. Approved methods of the American Association of Cereal Chemists (10th ed.). St. Paul, MN: Author (methods 08–01, 30–25, 44–15A, 46–13, 54–21, 10-50D).

Abu-Tarboush, H.M. and Ahmed, S.A.B 1996. Studies on karkade (*Hibiscus sabdariffa*): protease inhibitors, phytate, *in-vitro* digestibility and gossypol content. *Food Chemistry,* 56, 15-19

Al-Wandawi, H., Al-Shaikhly, K. and Abdul-Rahman, M. 1984. Roselle seed a new protein source. *Journal of Agricultural and Food Chemistry* 32, 510-512.

Baker, D.L. and Suhr, N.H. 1982. Atomic absorption and flame emission spectrometry, pp. 13-27. In: A.L. Page (Ed.), *Method of Soil Analysis.* Part 2, Chemical and microbiological properties. Agronomy monograph No. 9. American Society of Agronomy, Madison, WI.

Bambgoye, A.I. and Adejumo, O.I. 2009. Physical Properties of Roselle (*Hibiscus sabdariffa* L.) *Seed. Agricultural Engineering International: the CIGR Ejournal*. Manuscript 1154.Vol. XI.

Beecher, G.R. 1999. Phytonutrients' role in metabolism: Effects n resistance to degenerative processes. *Nutri. Rev.,* 57:53-56

Bray, H.G. and Thorpe, W.V. 1954. *Meth. Biochem. Anal.* 1, 27-52.

Ceirwyn, J.S. 1995. Analytical Chemistry of Foods Blackie Academic and Professional, London, pp: 71-135.

Cicerale, S., Conlan, X.A., Jinclair, S.A. and Keast, R.S. 2009. Chemistry and health of olive oil phenolics. *Crit. Rev. Food Sci.,* 49: 218-236.

Cissouma, A.I., Tounkara, F., Nikoo, Mehdi, Yang, N. and Xu, X. 2013. Phyico Chemical Properties and Antioxidant Activity of Roselle Seed Extracts. *Adv. J. Food Sci. Technol.,* 5(11):1483-1489.

Durlach, J. 1988. Magnesium in Clinical Practice. *Libbey Eurotext, London, Paris*, pp:360.

El Mahi, H.E., Ibrahim, I.S., Abdel, M., Magid, H.M., and Eltilib, A.M.A. 1987. A simple method for the estimation of calcium and magnesium carbonates in soils. *Soil Sci. Soc. Am. J.,* 51:1152-1154.

El-Adawy, T.A. and Khalil, A.H. 1994. Characteristics of roselle seeds as a new source of protein and lipid. *Journal of Agricultural and Food Chemistry,* 42, 1896-1900

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

Enujiugba, V.N. and Akanbi, C.T. 2008. Quality evaluation of canned fermented oil bean seed slices during ambient storage. *Afr. J. Agric. Res*., 5(22): 3096-3101.

Ismail, A., Emmy Hainida, K.I. and Halimatul, S.M.N. 2008. Roselle (*Hibiscus sabdariffa L.*) seeds–Nutritional Composition, Protein Quality and Health Benefits, *Food,* 2(1), 1-16.

Jackson, M.L. 1973. Soil Chemical analysis, Prentice Hall of India, Pvt. Ltd., New Delhi pp. 498

Jain, R.K. and Bal, S. 1997. Properties of pearl millet. *Journal of Agricultural Engineering Research,* 66, 85-91.

Karaj, S. and Möller, J. 2010. Determination of physical, mechanical and chemical properties of seeds and kernels of Jatropha curcas *L. Ind. Crops Prod.*

Knudsen, D. and Peterson, G.A. 1982. Lithium, sodium and potassium. P 225-246. In A.L. Page. (Ed.) Methods of soil analysis. Aronomy monogram No. 9, *Am. Soc. Agron. Inc., Soil Sci. Soc. Am Inc.* Publisher, Madison, Wisconsin, USA.

Liu, Q. and Yao, H. 2007. Antioxidant activities of barley seed extracts. *Food Chem.,* 102: 732-737

Mohammed, R., Fernendez, J., Pineda, M. and Aguilar, M. 2007. Roselle (*Hibiscus sabdariffa*) seed oil is a rich source of gamma *tocopherol. J. Food Sci*., Vol. 72, pp. 207-211.

Mohesenin, N.N. 1986. Physical Properties of Plant and Animal Materials. Gordon and Breach Publishers, New York, 51-85

Mohiuddin, M.M. and Zaidi, H.R. 1975. Composition and characteristics of *Hibiscus sabdariffa* seed oil. *Fat, Soaps and Coating Materials,* 77, 488-489.

Morton, J.F. 1987. Roselle, In: *Fruits of Warm Climates,* Florida Flair Books, Maimi, USA, pp 281-286

Nof, 2016. National Osteoporosis Foundation, 251 18th Street S, Suite 630, Arlington, VA, 22202-1 (800) 231-4222. https://www.nof.org/patients/treatment/calciumvitamin_d/

Nyam, Kar-Lin; Leao, Sod-Ying; Tan, Chin-Ping; and Long, Kamariah 2014. *Functional Properties of Roselle (Hibiscus sabdariffa L.) seed and its application as bakery product,* J. Food Sci. Technol 51(12):3830-3837 DOI 10.1007/s13197-012-0902-x

Nzikou, J.M., Bouanga-Kalou, G., Latos, L., Ganongo-Po, F.B., Mboungou-Mboussi, P.S., Moutoula, F.E., Panyoo-Akdowa, E., Silou, T.H., and Desobry, S. 2011. Characteristics and Nutritional Evaluation of Seed oil from Roselle (*Hibiscus sabdariffa* L.) in Congo-Brazzaville, *Current Research Journal of Biological Sciences,* 3(2): 141-146.

Okawa, M., Kinjo, J., Nohara, T. and Ono, M. 2001. DPPH (1,1 – diphenyl-2-picrylhydrazyl) radical scavenging activity of flavonoids obtained from some medicinal plants. *Biol. Pharm. Bull*., 24: 1202-1205.

Omobuwajo, T.O., Sanni, L.A. and Balami, Y.A. 2000. Physical properties of sorrel (*Hibiscus sabdariffa*) seeds. *Food Engineering,* 45, 37-41

Panse, V.G. and Sukhate, P.V. 1985. Statistical methods for Agricultural Workers. 4th ed., New Delhi

Perry, L.M. 1980. *Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses,* MIT Press, Cambridge, UK, pp. 632

Rao, P.U. 1996. Nutrient composition and biological evaluation of mesta (*Hibiscus sabdariffa*) seeds. *Plant Food for Human Nutrition* 49, 27-34.

Romani, Andrea, M.P. 2013. "Chapter 3. Magnesium in Health and Disease". In Astrid Sigel; Helmut Sigel; Roland K.O. Sigel. *Interrelations between Essential Metal Ions and Human Diseases.* Metal Ions in Life Sciences 13. *Springer*. Pp.49-79.

Savage, G.P. 2001. Chemical composition of walnuts (Juglans regia L.) grown in New Zealand. Plants Food Hum. *Nut*. 56: 75-82.

Sclumnberger, S. 2008. Building a Hele-Shaw Cell-Experiment, http://www.see.slb.com/en/scictr/lab/heleshaw/largecell.html (21 August, 2008).

Sleelig, G.F. 1980. Noncatalytic subunits of human bllod plasma coagulation factor XIII. Preparation and partial characterization of modified forms. *J.Biol. Chem.,* 255918: 8881-8886.

Tounkara, F., Amadou, I., Le, G. and Shi, Y. 2011. Effect of boiling on the physicochemical properties of Roselle seeds (*Hibiscus sabdariffa L.*) cultivated in Mali, *African J. of Biotechnology,* Vol. 10(79), pp. 181860-18166.

Tounkara, F., Amza, T., Lagnika, C., Le, G. and Shi, Y. 2013. Extraction, characterization, nutritional and functional properties of Roselle (*Hibiscus sabdariffa Linn*) seed proteins. Songklanakarin *J. Sci. Technol.,* 35(2), 159-166.

Zhang, Y., Song, Q., Yan, J., Tang, J., Zhao, R., He, Z., Zou, C. and Ortiz-Monasterio. 2010. Mineral elements concentrations in grains of Chinese wheat cultivars. *Euphytica*., 174: 303-313.

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