ASEAN Food Journal 14 (2): 131-140 (2007)

Short Communication

Protein Quality of Roselle (*Hibiscus sabdariffa* L.) Seeds

Halimatul, S.M.N., *Amin, I., Mohd.-Esa, N.,
Nawalyah, A.G. and Siti Muskinah, M.

*Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences,
Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia*

Abstract. This study investigated the protein quality of two sets of Roselle seeds processed differently (dried and boiled). Twenty weanling *Sprague Dawley* rats were used to conduct the growth and nitrogen balance studies. Rats were fed with 10% (w/w) protein from dried (DS) and boiled (BS) Roselle seeds powder for 4 weeks. Casein was used in this study as a standard reference protein. There was a significantly higher ($p < 0.05$) food intake and weight gain by rats fed with BS compared with DS. In the growth study, there was no significant difference ($p < 0.05$) in protein efficiency ratio (PER) and net protein ratio (NPR) of BS compared to DS, but it was significantly different with casein (CD). PER value of rats fed with DS was significantly lower ($p < 0.05$) than casein. In the nitrogen balance study, true nitrogen absorption (TNA) and nitrogen balance (NB) of BS group was significantly higher ($p < 0.05$) than DS group. However, apparent digestibility (AD), true digestibility (TD) and biological value (BV) for both diets was not significantly different. This study showed that the protein quality of dried Roselle seeds was similar to the Roselle seeds boiled at 100^{o}C for 30 minutes.

Keywords: Growth study, nitrogen balance study, Roselle seeds powder

INTRODUCTION

Hibiscus sabdariffa L. (belonging to the family of Malvaceae) also known as Roselle, sorrel, mesta and karkade, is a popular plant in Middle Eastern countries (Morton, 1987; Abu-Tarboush *et al.,* 1997). Roselle can be found in almost all tropical countries such as Malaysia, South East Asia, Indonesia, Thailand and Philippines (Chewonarin *et al.,* 1999; Rao, 1996). It is a new commercial crop of Malaysia, where it is reported to have been brought in from India. The calyces or petals of the flower are widely used to prepare herbal drink, cold and warm beverages, and for making jams and jellies (Abu-Tarboush *et al.,* 1997; Rao, 1996; Tsai *et al.,* 2002). The brilliant red colour and unique flavour make them valuable food products (Tsai *et al.,* 2002). The current production of this flower in Malaysia is about 240 tonnes annually. In the process of removing the calyces, out of 3 tonnes of raw materials, about 1.5 tonnes of the velvety capsules containing the seeds are being disposed as by-products and unexploited (unpublished results, Agricultural Department Negeri Terengganu, Malaysia). In some parts of Africa, the seeds are reported to be used for its oil. The seeds are reported to be rich in proteins, dietary fiber, carbohydrates and fats (Abu-Tarboush *et al.,* 1997; Rao, 1996; El-Adawy and Khalil, 1994).

Plant proteins are widely recognized as an important source of affordable protein. The use of different sources of protein can vary widely from one country to another depending on food habits and traditions of the community. Nowadays, the rapid rate of population growth

* Corresponding Author
E-mail: *amin@medic.upm.edu.my*

in underdeveloped and third world countries has increased the demand for food supply and food production. This has led to an increase in the incidence of malnutrition problems like marasmus and kwashiorkor or protein-energy malnutrition (PEM) especially in young children.

Roselle plants are abundant in Africa and India. Previous studies showed that the seeds can be used as a potential source of proteins (El-Adawy and Khalil, 1994; Al-Wandawi *et al.*, 1984). Instead of using protein from conventional sources, protein isolates or concentrates from Roselle seeds might be useful as low cost source of protein substitute in dietary supplement or food ingredient in food industry, thus alleviating the problem of protein scarcity. Beside that, in recognition of the worldwide need for cheaper protein sources by low-income groups in developing countries, there have been efforts to develop low-cost protein foods of plant origin (Deshpande *et al.,* 2000).

Protein quality, also known as the nutritional or nutritive value of a food, depends on its amino acid content and on the physiological utilization of specific amino acids after digestion, absorption and minimal obligatory oxidation rates (Friedman, 1996). Protein quality tests predict the nutritional quality of food proteins and how available are these proteins for growth and cell maintenance. Protein quality tests are designed to directly measure or estimate the dietary essential amino acid content of the test protein or protein-containing food, and how well the protein is digested, absorbed and utilized in human growth and maintenance.

In order to use the seed proteins as a functional ingredient, the protein quality such as protein efficiency ratio (PER), net protein ratio (NPR), true digestibility and biological value should be determined. Thus, this study was conducted to investigate the bioavailability and digestibility of the Roselle seeds proteins before it can be utilized and applied for human consumption or industrial uses. In addition, the effects of thermal treatments (drying and boiling) on the protein quality of the seeds were also studied.

MATERIALS AND METHODS

Plant Materials

Raw Roselle capsules (60 kg) were taken from a Roselle farm in Sungkai, Perak, Malaysia. The velvety capsules containing the seeds were collected after removing its calyxes. The capsules were washed under tap water to remove any foreign matter or dirt. Dried seeds were prepared by direct sun-drying until a constant weight (% moisture content: 9.93 ± 0.17). For boiled seeds, they were boiled at 100°C for 30 min until the capsules became soft. The capsules were then sun-dried until a constant weight was reached (% moisture content: 9.82 ± 0.13). Finally, the seeds were ground using a Waring Commercial Blender (Model 24CB10, Connecticut, USA) into fine particle size (20μm) and kept at 4°C until use.

Protein Quality Evaluation

Twenty male weanling *Sprague-Dawley* rats, body weight of 20-30 g (20-23 days old) were purchased from the Animal Resource Centre, Faculty of Veterinary Medicine, Universiti Putra Malaysia. This study was approved by the Animal Care and Use Committee (ACUC), Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. Rats were housed individually in wire-bottom galvanized steel cages that allow for easy faeces and urine collection and the measurement of food intake. The animal room was well ventilated with alternate periods of 12 hr light and dark and temperature of 32 ± 2°C. Rats were acclimatized for a week, and fed with standard diet. Each animal was supplied with 20 g diet daily and water supplied *ad libitum.* After a week, rats were randomly divided into four different dietary groups of five rats per group. The experimental details are as follows:

Group I – Protein-free diet (PF)
Group II – Casein control diet (CD)
Group III – Dried roselle seed diet (DS)
Group IV – Boiled roselle seed diet (BS)

Table 1: Composition of experimental diets

Ingredient(g/100g)	Type of diet (g/100g)			
	Protein-free	Casein[a]	Dried Roselle seed	Boiled Roselle Seed
Protein source	-	10.0	29.9	32.7
Corn oil	10.0	10.0	10.0	10.0
Corn starch	84.0	74.0	54.1	51.3
Vitamin mix[b]	1.0	1.0	1.0	1.0
Mineral mix[c]	4.0	4.0	4.0	4.0
Wheat bran[d]	1.0	1.0	1.0	1.0
Total	100	100	100	100

[a]Casein low trace metals, MP Biomedicals, LLC. (ICN Biomedicals Inc., Germany)
[b]Vitamin mix, Ain 93-Vx. (ICN Biomedicals Inc., Germany)
[c]Mineral mix, Ain 93 M. (ICN Biomedicals Inc., Germany)
[d]Bran MP Biomedicals, LLC. (ICN Biomedicals Inc., Germany)
Source: (Miller, 1967)

The composition of experimental purified diets is shown in Table 1. All diets contained about 10% proteins, except for group I. Protein content in dried and boiled roselle seeds and all of the diets were determined using the Kjeldahl method (AOAC, 1990). The experiment was carried out for five weeks. Food intake and body weights were recorded daily and weekly, respectively. Faeces and urines were collected every day on the third week during treatment period, and the nitrogen content was determined using a Kjeldahl method. Based on the nitrogen content of the feeds, faeces and urines, the protein efficiency ratio (PER) (National Academy of Sciences/National Research Council (NAS/NRC), 1963), net protein ratio (NPR) (Bender and Doell, 1957), apparent digestibility (AD) and true digestibility (TD) (Dreyer, 1968), true nitrogen absorption (TNA), nitrogen balance (NB) and biological value (BV) (Philips *et al.,* 1981) of CD, BS and DS groups were calculated.

Statistical Analysis

Data were expressed as means ± standard errors (S.E.M). Data were statistically analysed using the comprehensive statistical software, SPSS version 12.0.1 for windows (SPSS Inc, Chicago, Illinois, USA). One-way ANOVA was applied to find the difference between the groups. Tukey's Bonferroni test was used to find the significant difference among means at the probability level < 0.05.

RESULTS

Average changes in body weight and food intake of rats during 28 days of growth are shown in Figures 1 and 2, respectively. There were significant differences ($p < 0.05$) in body weights among groups, except for rats fed with protein-free diet (PF). The weight gain of rats fed with casein (CD) and dried Roselle seed (DS) diets were higher than that for PF and boiled Roselle seed diets (BS). This result reflects the food intake of the rats (Figure 2). The food intake of rats fed with CD and DS were significantly higher ($p < 0.05$) compared to PF and BS.

Protein content in all test diets of PF, CD, DS and BS were 0.4%, 10.1%, 10.7% and 10.6%, respectively. From these values, the total protein consumed by each group during the treatment was calculated. Figure 3 shows the average total protein consumed by different dietary groups during the growth study. These

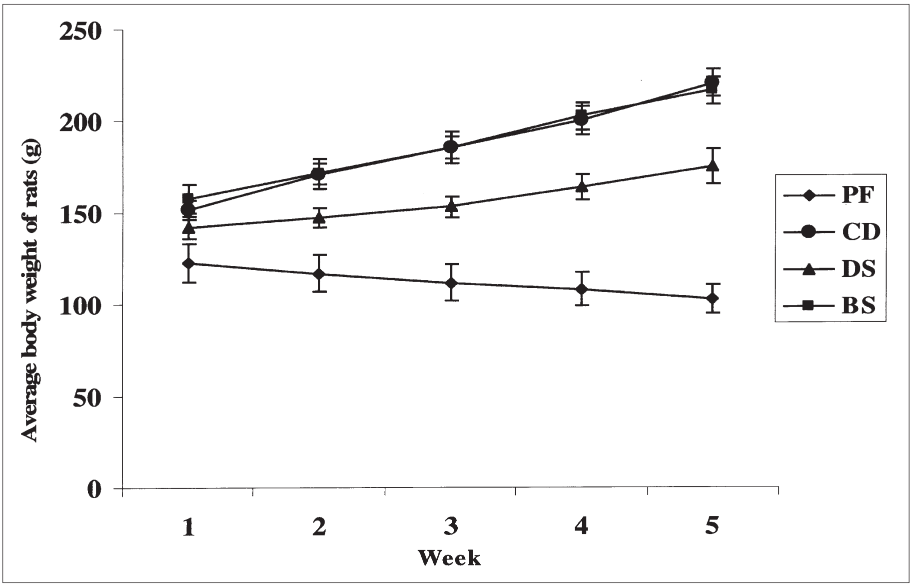

Figure 1: Changes in body weight of rats of different dietary groups during the growth study. PF, protein-free; CD, casein; DS, dried Roselle seed; BS, boiled Roselle seed. Values are expressed as mean ± S.D (n = 5). Average body weights gain during the study weeks were significantly different ($p < 0.05$) among groups, except for rats fed with protein-free (PF) diet. All data revealed CV between 3.1 - 8.8 %.

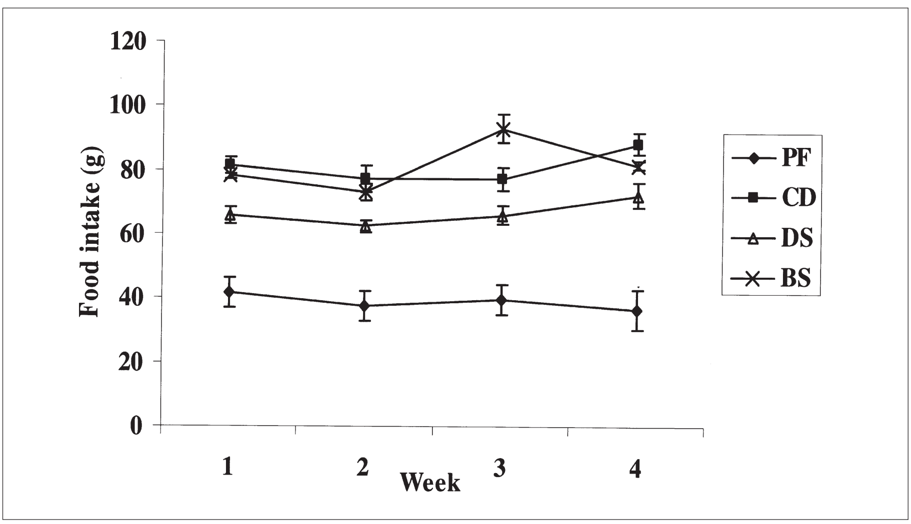

Figure 2: Average of total food intake of rats from different dietary groups during the growth study. PF, protein-free; CD, casein; DS, dried Roselle seed; BS, boiled roselle seed. Values are expressed as mean ± S.D (n = 5). All data revealed CV between 1.0 – 13.0 % except for PF at week 4.

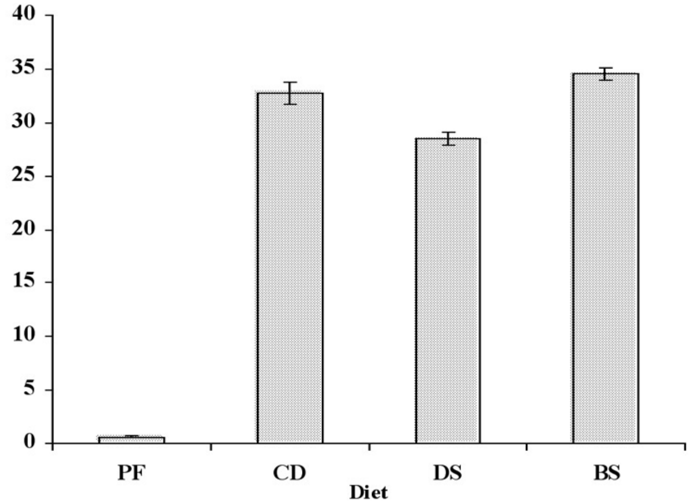

Figure 3: Average of total protein consumed (g) per rat from different dietary groups during the growth study.
PF, protein-free; CD, casein; DS, dried Roselle seed; BS, boiled roselle seed. Values are expressed as mean ± S.D (n = 5). Means with different letters are significantly different ($p < 0.05$).
All data revealed CV between 1.7 – 12.9 %.

findings indicate that the total protein consumed by the different groups paralleled the total food intake. Total protein consumed was found to be highest in BS, followed by CD, DS and PF groups. Total protein consumed by rats fed with BS was not significantly different compared to rat fed with CD, but was significantly different ($p < 0.05$) with those fed with DS and PF.

Protein efficiency ratio (PER) and net protein ratio (NPR) are shown in Table 2. The highest PER value was found for CD, followed by BS and DS with PER values of 2.12, 1.71 and 1.15, respectively. PER for CD was significantly higher ($p < 0.01$) than DS, but was not significantly different compared to BS. However, PER of BS was not significantly higher than DS. NPR value was higher for CD, followed by BS and DS. NPR of CD was significantly higher compared to DS ($p \leq 0.01$), but not to BS.

After seven days of the nitrogen balance study, rats fed with BS showed the highest weight gain followed by rats fed with CD and DS. However, there was a weight loss observed for the PF group. This is reflected in food consumption of the rats studied. Table 3 shows the *in vivo* protein digestibility of rats fed with PF, CD, DS and BS diets. Results showed that the average food intake, protein intake and the nitrogen consumed for 7 days were significantly higher ($p < 0.05$) for rats fed with BS compared to other diets. In addition, total faeces collected and total nitrogen excreted were also significantly higher ($p < 0.05$) in the BS group. Rats fed with CD had the highest apparent digestibility (AD), true nitrogen absorption (TNA), true digestibility (TD), nitrogen balance (NB) and biological value (BV), followed by BS and DS (Table 4). TNA and NB of BS showed significant higher ($p < 0.05$) values compared to the DS diet. However for AD, TD and BV, there were no significant differences between both groups.

DISCUSSION

Based on the findings, boiling has been shown to improve the protein quality of the Roselle seeds. The increment in body weights, PER and

Table 2: Protein efficiency ratio (PER) and net protein ratio (NPR) of casein (CD), dried (DS) and boiled (BS) Roselle seeds

Diet	Weight gain (g)	Total food intake (g/rat/28 days)	Protein in food (%)	Protein consumed (g/rat/28 days)	PER	NPR
PF	-20.25 ±11.35^{a} (56%)	155.63 ± 40.28^{a} (25%)	0.40 ± 0.05	0.62 ± 0.16^{a} (25%)	NA	NA
CD	69.00 ± 5.83^{c}	324.34 ± 21.95^{c}	10.10 ± 0.46	32.76 ± 2.22^{c}	2.11 ± 0.20^{a**}	1.49 ±0.18^{a**}
DS	32.80 ±16.50^{b} (50%)	266.19 ± 12.16^{b}	10.73 ± 0.20	28.48 ± 1.30^{b} (52%)	1.15± 0.60^{b}	0.44 ± 0.60^{b} (130%)
BS	59.00 ±11.77^{c} (19%)	325.55 ± 12.82^{c}	10.61 ± 0.31	34.51 ± 1.36^{c}	1.71 ±0.34^{ab} (19%)	1.12 ±0.33^{ab} (29%)

Values are expressed as mean ± S.D (n = 5).
In the same column, values with different superscript letters are significantly different at $p < 0.05$ or $p < 0.01$ indicated by (**).
PF: Protein-free; NA: (results are not available);
All data revealed CV between 1-15%, except for values in parentheses.

Table 3. Protein digestibility of casein (CD), dried (DS) and boiled (BS) Roselle seeds

Diet	Total food intake (g/rat/ 7 days)	Protein intake (g/rat/ 7 days)	Nitrogen in food (%)	Nitrogen consumed (g/rat/ 7 days)	Weight of feces collected (g/rat/ 7 days)	Nitrogen in dried feces (%)	Total nitrogen in dried (g/rat/ 7 days)
PF	39.58 ± 9.28 (23%)	0.16± 0.04^{a} (25%)	0.06 ± 0.01 (16%)	0.03 ± 0.01^{a} (33%)	1.63 ± 0.01^{a}	1.64 ± 0.12	0.03 ± 0.01^{a} (33%)
CD	77.38± 7.98	7.82 ± 0.81^{b}	1.61 ± 0.07	1.25 ± 0.13^{b}	4.43 ± 0.12^{b}	4.64 ± 0.15	0.21 ± 0.12^{b} (57%)
DS	65.85 ± 6.27	7.05 ± 0.67^{b}	1.72 ± 0.03	1.13 ± 0.11^{b}	10.99 ± 0.03^{c}	3.42 ± 0.06	0.38 ± 0.03^{c}
BS	93.00 ± 9.91	9.86 ± 1.05^{c}	1.70 ± 0.02	1.58 ± 0.17^{c}	15.65 ± 0.04^{d} (25%)	3.57 ± 0.10	0.56 ± 0.04^{d}

Values are expressed as mean ± S.D (n = 5);
In the same column, values with different superscript letters are significantly different at $p < 0.05$;
PF: Protein-free; NA: (results are not available);
All data revealed CV between 0.2 -15%, except for values in parentheses.

Table 4: Apparent digestibility (AD), true nitrogen absorption (TNA), true digestibility (TD), nitrogen balance (NB) and biological value of casein (CD), dried (DS) and boiled (BS) Roselle seed based on nitrogen balance study

Diet	AD (%)	TNA (%)	TD (%)	NB	BV (%)
CD	83.41 ± 1.66	1.07 ± 0.13[b]	85.83 ± 1.41[b]	1.04 ± 0.13[b]	97.18 ± 0.33
DS	66.42 ± 3.28	0.78 ± 0.11[a]	69.10 ± 3.01[a]	0.75 ± 0.11[a]	96.10 ± 0.56
BS	64.26 ± 3.76	1.05 ± 0.17[b] (16%)	66.18 ± 3.56[a]	1.02 ± 0.17[b] (16%)	97.08 ± 0.46

Values are expressed as mean ± S.D (n = 5).
In the same column, values with different superscript letters are significantly different at $p < 0.05$ or $p < 0.01$ indicated by (**).
All data revealed CV between 0.2 -15%, except for values in parentheses.

NPR values of BS were higher than that of DS diet. The results were in agreement with Rao (1996), who reported that food intake, gain in body weight, PER and NPU values of rats treated with cooked Roselle (*Hibiscus sabdariffa* grown in Africa) seed diets were significantly higher than raw seed diet. This could be due to the inhibition of anti-nutritional activities. Seeds and legumes commonly contain anti-nutritional components such as phenolics, enzymes inhibitors, lectins, hemagglutinin, alkaloids, tannins and phytic acid which may reduce their nutritional value and protein digestibility (Hernandez-Infante *et al.,* 1979; Antunes and Sgarbieri, 1980; Abu-Tarboush and Ahmed, 1996; Giami, 2005; Agbede and Aletor, 2005). Although anti-nutritional factors are found to largely affect the protein quality of foods, many of them can be eliminated or inactivated, to a large extent, by appropriate heating and processing during foods preparation (Mwanjala *et al.,* 1999). Heat treatment has been shown to improve the nutritive value of legumes and other edible seeds by reducing the levels of anti-nutritional factors and increasing protein digestibility (Siddhuraju and Becker, 2001; Giami *et al.,* 2001; El-Adawy, 2002; Fagbemi *et al.,* 2005; Giami, 2005). Abu-Tarboush and Ahmed (1996) reported that trypsin inhibitor activity decreased about 66% during the first 10 min of heating, and further loss of its activity during the next 30 and 50 min. This finding supports the notion that heat treatment (boiling) of Roselle seeds could lead to inactivation of anti-nutrient or enzyme inhibitors. Thus, it could explain the higher protein quality found in boiled Roselle seeds. PER and NPR values of both BS and DS were lower compared to that of cowpea (Giami, 2005). A study by Giami (2005), reported that boiled cowpea diet showed similar PER, NPR and TD to those of the casein control diet.

The highest food intake was observed in BS group compared to other groups, which could be due to the palatability of the diet. Cooking or heat treatment has a significant effect on the food consumption as shown by these findings. Higher PER and NPR values of BS compared to DS might also be due to the digestibility of the seeds protein in the digestive tract. Boiling of Roselle seeds at 100°C will denature the protein structures that can generally lead to increase in protein digestibility. Denaturation of Roselle seed proteins by heat might be involved in a change in the tertiary structure to a less ordered arrangement of polypeptide chains, rendering it more digestible.

The results of this growth study suggest that Roselle seeds protein may be suitable as a source of proteins in human diet. Agbede and Aletor (2005) and Dabbour and Takruri (2002) reported that jack bean, devil bean and edible

Jordan mushroom did not support rats growth, due to the negative value of PER. They attributed this poor performance to the presence of anti-nutrients, toxic components and lack of palatability of the diets.

Amino acid composition and ratios of essential amino acids also influence the nutritional value of food proteins. Biological value or quality of food proteins is directly influenced by the ratio of indispensable amino acids (Friedman, 1996). Biological value of casein is superior to those of proteins from Roselle seeds since casein contains all essential amino acids in adequate amounts. Lysine content was reported to be high in Roselle seed and similar to that of the FAO reference protein (Rao, 1996; El-Adawy and Khalil, 1994; Al-Wandawi *et al.,* 1984).

AD, TNA, TD, NB and BV values for both DS and BS were lower than that of the casein diet. AD and TD values for Roselle seeds were lower compared to 12 cultivars of sunflower seeds (Canibe *et al.,* 1999), cooked jack bean and roasted devil bean (Agbede and Aletor, 2005), 11 cultivars of lupin seed (Eggum *et al.,* 1993) and japonica and indica cooked milled rices (Boisen *et al.,* 2001), but it exhibited higher values of BV. Moreover, heat treated cowpea revealed higher values of AD and TD compared to the raw bean (Giami, 2005). The results of Giami's (2005) study are contradictory to our findings. This might be due to the differences of primary, secondary and tertiary protein structures of the Roselle seeds. Enzymatic hydrolysis towards digestibility of protein are directly related to it structure (Rasco, 1998). In addition, chemical changes in Roselle seeds during the boiling process could reduce the protein susceptibility to digestive enzymes.

CONCLUSION

This study has shown that *Hibiscus sabdariffa* L. seeds grown in Malaysia have a good protein quality just as good as that of casein. Moreover, protein quality of Roselle seeds was found to increase after boiling at 100^{o}C. Thus, Roselle seeds have the potential of becoming an economical protein source for human consumption. However, further studies are required to investigate the presence of oligosaccharides and anti-nutrients that may affect the capability of the Roselle seeds.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the National Council for Scientific Research and Development (NCSRD) for their financial support under the Intensification of Research in Priority Areas (IRPA) (No: 06-02-04-0804-EA001).

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