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Original Article

Extraction, characterization, nutritional and functional properties of Roselle (*Hibiscus sabdariffa Linn*) seed proteins

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Abstract

Physicochemical, nutritional and functional properties of protein fractions and protein isolate (RSPI) from Roselle seed were investigated. The protein content was 91.50, 93.77, 81.55, 71.30 and 40.83% for RSPI, globulin, albumin, glutelin and prolamin, respectively. The functional properties were variable among samples. Glutelin possessed the highest water holding capacity and albumin the lowest. The oil holding capacity ranged from 3.47 to 7.23 mL/g and the emulsifying capacity from 95 to 18 mL/g. Glutelin had the higher foam capacity, while RSPI showed the more stable foam. The molecular weight of all samples ranged from 55,000 Da to below 14,300 Da. All the estimated nutritional parameters based on amino acids composition suggested that Roselle protein fractions and its isolates have good nutritional quality and could be a good source of protein fortification for a variety of food products for protein deficient consumers as well as a potential food ingredient.

Keywords: Roselle seed, protein fractions, protein isolates, physicochemical properties, nutritional and functional properties

1. Introduction

Hibiscus sabdariffa L. also known as Roselle, sorrel mesta belongs to the family of Malvaceae. The plant is widely distributed in the tropical regions, especially in the Middle Eastern countries (Abu-Tarboush *et al.*, 1997), and it is generally considered as a medicinal plant. The calyces or petals of the flower are extensively used to prepare herbal drinks, cold and warm beverages, as well as making jams and jellies (Rao, 1996; Tsai *et al.*, 2002). The brilliant red color and unique flavor coupled with other organoleptic attributes make them valuable food products (El-Adawy and Khalil, 1994). Plant proteins are extensively recognized as an important source of affordable protein. In many African countries food from animal sources are mainly consumed by households of higher socio-economic status and majority of the population does hardly access these food due to poverty (Al Wandawi *et al.*, 1984). Roselle is rich in protein and abundant in many countries (Africa and Asia). Besides that, in recognition of the worldwide need for cheaper protein sources for low-income groups in developing countries, there have been efforts to develop low-cost protein of plant origin. Previous studies showed that Roselle seeds could be used as a potential source of proteins and oil (El-Adawy and Khalil, 1994; Tounkara *et al.*, 2011). Protein fractions, protein isolates or concentrates obtained from Roselle seeds might be an alternative source of low cost protein substitute in dietary supplement or in ingredients for food industry. This may reduce the heavy dependence on conventional sources such as animal, fish and soybeans.

Although, some nutritional and functional properties of Roselle seed products have been studied (Abu-Tarboush et al., 1997), there is still scanty information currently available especially on the extraction, characterization, nutritional

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and functional properties of Roselle protein fractions and their isolates. Therefore, this study was aimed at characterizing and evaluating Roselle seed proteins (Roselle seed protein isolate, albumin, prolamin, glutelin, and globulin) by investigating some of their nutritional and functional properties. This would provide scientific data regarding their potential application in food processing.

2. Material and Methods

2.1 Materials

Seeds of *Hibiscus Sabdariffa* were obtained from Koutiala, southern region of Republic of Mali and the seeds were transported to Wuxi, China. 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.

2.2 Preparation of defatted Roselle seed flour

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. Thereafter the powder was defatted with n-hexane, following a small-scale hexane extraction method described by Tzeng *et al.* (1990). The oil-free flours was desolventized and stored in desiccator at room temperature for subsequent uses.

2.3 Proximate chemical composition

The proximate analysis of Roselle seed flour (RSF) and defatted Roselle seed flour (DRSF) were determined according to AOAC (2000). The moisture content was determined by drying in oven at 105°C until a constant weight was obtained. Ash was determined by weighing the incinerated residue obtained at 550°C for 8-12 hrs. Total crude protein content was determined using the Kjeldahl method. The total lipid in samples was determined by Soxhlet method. Available carbohydrates were calculated as 100% - [%(moisture + ash + fat + protein)].

2.4 Preparation of Roselle seed protein isolate

Roselle seed protein isolates (RSPI) were obtained from defatted flour as reported by El-Tinay *et al.* (1988) with some modifications. The defatted flour was dispersed in distilled water at flour to water ratio of 1:10 (W/V); the pH was adjusted to 10 with 1 M NaOH and stirred for 3 hrs at room temperature. The extract was separated by centrifugation at 4,300 x g for 20 min. The residues were re-extracted twice as described above. The extracts were combined and protein was precipitated by adjusting the pH to 3.5 with 1 M HCl before centrifugation at 4,300 x g for 20 min. The protein isolate (precipitate) was washed twice with distilled water. It was then resuspended in distilled water and the pH was adjusted to 7.0 with 1 M NaOH prior to freeze-drying. The dried protein (protein isolates) was stored in desiccator at room temperature for subsequent analyses. The protein content was determined by the Kjeldahl method (AOAC, 2000).

2.5 Protein fractionation

Proteins were extracted from defatted Roselle seed flour based on their solubility at room temperature (25°C) in water, 5% NaCl, 70% ethanol and 0.1 M NaOH using the procedure of Osborne (1909) with minor modifications. The defatted flour was extracted with 400 mL distilled water with stirring for 4 hrs and centrifuged at 3,000 x g for 30 min to obtain the albumin fraction (supernatant). The residue obtained after this step was extracted with 400 mL of 5% NaCl to obtain the globulin fraction. Thereafter the residue was extracted with 400 mL of 0.1 M NaOH (1 hr) to obtain the glutelin fraction, while the residue after glutelin extraction was extracted with 400 mL of 70% ethanol to obtain the prolamin fraction. All the extractions were carried out twice. The albumin, globulin, glutelin and prolamin fractions were then subjected to isoelectric precipitation at pH 3.5 and washed with distilled water. All fractions were freeze dried using a Christ–Alpha 1-4 Freeze dryer (Biotech International, Germany). The determination of protein in various fractions was done using a micro-Kjeldahl method (AOAC, 2000).

2.6 Amino acids analysis

The dried samples were digested with HCl (6M) at 110°C for 24 hrs under nitrogen atmosphere. Reversed phase high performance liquid chromatography (RP-HPLC) analysis was carried out in agilant 1100 (Agilent Technologies, Palo Alto, CA, U.S.A.) assembly system after precolumn derivatization with o-phthaldialdehyde (OPA) (Jarrett *et al.*, 1986). Each sample (1 µL) was injected on a Zorbax 80 A C18 column (i.d. 4.6x180 mm, Agilent Technologies, Palo Alto, CA, U.S.A.) at 40°C with detection at 338 nm. Mobile phase A was 7.35 mM/L sodium acetate/triethylamine/tetrahydrofuran (500:0.12:2.5, v/v/v), adjusted to pH 7.2 with acetic acid, while mobile phase B (pH 7.2) was 7.35 mM/L sodium acetate/methanol/acetonitrile (1:2:2, v/v/v). The amino acid composition was expressed as g of amino acid per 100 g of protein.

2.7 Protein nutritional parameters

The nutritional parameters of Roselle protein isolates and its fractions were calculated using their amino acid composition including:

(1) Proportion of essential amino acids (E) to the total amino acids (T) of the proteins.

(2) Amino acid score (AAS) = (mg of amino acid per g

of test protein/mg of amino acid per g of standard protein) ×100. The FAO/WHO/UNU reference pattern of essential amino acid requirements (g/100g of protein) was used as the standard.

(3) Predicted Protein Efficiency Ratio (PER) values. The predicted PER values of Roselle protein isolates and its fractions were estimated by three regression equations developed by Chavan *et al.* (2001).

I. PER = - 0.684 +0.456(Leu) - 0.047(Pro)
II. PER = -0.468 + 0.454(Leu) - 0.105(Tyr)
III. PER = -1.816 + 0.435(Met) + 0.780(Leu) + 0.211(His) - 0.944(Tyr).

2.8 Water holding capacity

In order to determine the water holding capacity (WHC) of Roselle protein isolate and Roselle protein fractions, the method outlined by Diniz and Martin (1997) with some modifications was used. Triplicate samples (0.5 g) were dissolved with 10 mL of distilled water in centrifuge tubes and vortexes for 30 sec. The dispersions were allowed to stand at room temperature for 30 min, centrifuged at 3,000 x g for 25 min. The supernatant was filtered with Whatman Number 1 filter paper and the volume retrieved was accurately measured. The difference between initial volumes of distilled water added to the protein sample and the volume obtained after filtration was determined. The results were reported as mL of water absorbed per gram of protein sample.

2.9 Oil holding capacity

Oil holding capacity (OHC) was determined using the method of Chakraborty (1986). One gram of protein (W_0) was weighed into pre-weighed 15 mL centrifuge tubes and thoroughly mixed with 10 mL (V_1) of soybean refined pure oil using vortex mixer. Samples were allowed to stand for 30 min. The protein-oil mixture was centrifuged at 3,000 x g for 20 min. Immediately after centrifugation, the supernatant was carefully poured into a 10 mL graduated cylinder, and the volume was recorded (V_2). Fat absorption capacity (milliliter of oil per gram of protein) was calculated as $OHC=(V_1-V_2)/W_0$. Analysis was performed in triplicate.

2.10 Emulsifying capacity

Emulsifying capacity was measured using the procedure described by Rakesh and Metz (1973), with modification. One gram of each freeze–dried sample was transferred into a 250 mL beaker and dissolved in 50 mL of 0.5 N NaCl and then 50 mL of soybeans pure oil was added. The homogenizer equipped with a motorized stirrer driven by a rheostat Ultra-T18 homogenizer (Shanghai, China) was immersed in the mixture and operated for 120 sec at 10,000 rpm to make an emulsion. The mixture was transferred to centrifuge tubes, maintained in water- bath at 90°C for 10 min and then centrifuged at 3,000 x g for 20 min. Emulsifying capacity was calculated as in equation: $EC=(V_A-V_R)/W_S$, where V_A is the volume of oil added to form an emulsion V_R, is the volume of oil released after centrifugation, and W_S is the weight of the sample. Analysis was performed in triplicate.

2.11 Foaming capacity and foaming stability

Foaming capacity (FC) was determined in triplicate using the method described by Makri *et al.* (2005). Concentrations of 1% proteins were prepared in deionized water and adjusted to pH 7.4 with 1.0 N NaOH and 1.0 N HCl. A volume of 100 mL (V_1) of protein concentrate suspension was blended for 3 min using a high speed blender, poured into a 250 mL graduated cylinder, and the volume of foam (V_F) was immediately recorded. Foaming capacity was calculated using the following equation: FC (%)=(V_F/V_1)x100. Foam stability was determined by measuring the fall in volume of the foam after 60 min.

2.12 SDS PAGE analysis

The SDS-PAGE was done on 12% separating and 4% stacking gels according to Laemmli (1970) using low molecular weight (14300-97200 Da) markers obtained from Sigma Aldrich (St. Louis, MO, U.S.A.). The lyophilized crude extract powder (0.005 g) was dissolved in 1 mL of 20 mM Tris-HCl buffer at pH 7.1. The solution was then centrifuged at 12,000 x g for 2 min to obtain the analytical sample. The purified inhibitor was applied at a concentration of 0.15 mg/mL. Coomassie brilliant blue R-250 was used for staining.

2.13 Statistical analysis

All experiments were conducted at least in triplicate with SPSS software (version 191 16.0, the predictive Analytics Company, Chicago, U.S.A.). The data were subjected to a one way analysis of variance (ANOVA), followed by Duncan's multiple range test.

3. Result and Discussion

3.1 Proximate chemical composition

Preliminary studies were conducted to assess the major nutrient composition of the studied samples. The Roselle seed flour and defatted Roselle seed flour were analyzed for moisture, crude protein, crude fat and ash using AOAC (2000). Available carbohydrates were calculated as 100%- [%(moisture+ash+fat+protein)]. The results are shown in Table 1. The undefatted RSF contained 27.32% and 39.24% of protein and carbohydrates respectively, but were both elevated significantly (P<0.05) to 36.39% and 46.03% after defating. The fat content was 20.83% for RSF and 1.36% for DRSF. Defating is a purification step which when carried out, increases the concentrations of nutrients such as proteins, carbohydrates and other major components of food compo-

Table 1. Proximate chemical composition of Roselle seed and defatted Roselle seed flours (g/100 g).

Samples	Parameters[1]				
	Protein(N x 6.25)	Moisture	Fat	Ash	Carbohydrate
RSF	27.32±0.39^{b}	8.14±0.10^{b}	20.83±0.55^{a}	4.47±0.11^{b}	39.24
DRSF	36.37±0.10^{a}	10.64±0.17^{a}	1.36±0.09^{b}	5.58±0.13^{a}	46.03

[1]All values are Means and standard deviations of three replicates. Mean values in rows with different letters were significantly different (P<0.05). RSF: Roselle seed flour, DRSF: defatted Roselle seed flour.

nents. Moreover, defating probably limits or minimizes fat molecules interaction with proteins and carbohydrate groups within the material, which when present, could interfere with the extraction of such components. The results of our study were within the range reported for other samples studied (Abu-Tarboush *et al.*, 1997). Other researchers found that the carbohydrates were mainly composed of dietary fibers (Rao, 1996).

3.2 Fractionation of defatted Roselle flour

Fractionation of proteins is often employed to quantify protein types within food materials. The results of the extracted protein fractions from defatted Roselle seed are summarized in Table 2. Globulin, albumin, glutelin and prolamin were observed in DRSF and quantified accordingly. The data showed that globulin was the major protein in the Roselle seed comprised of 31.18% of total protein, followed by albumin (16.47%), glutelin (10.20%) and prolamin (5.57%). The present results are in agreement with those of El-Adawy and Khalil (1994), who found that Roselle seeds contained globulin as the major protein fraction. The Roselle seed protein isolate (RSPI) and globulin fraction showed significant (P>0.05) amount of protein content with 91.50% and 93.77%, respectively (Table 2). However, the data of the present revealed that the protein content of RSPI was higher than that reported by Abu-Tarboush *et al*. (1997) (88.15%). These differences could be attributed to Roselle cultivars, extracting procedures and meal preparation methods. Furthermore, our result was less than soybean protein isolate (94%) reported by Al-Kahtani and Abou-Arab (1993).

3.3 Amino acids composition

The nutritional value of proteins is based on their amino acid composition and is crucial in physicochemical functions such as water holding capacity, oil holding ability and foaming properties among others. In order to appreciate the physicochemical properties of the samples, amino acid compositional analysis was carried out. Apparently, the four Roselle seed protein fractions and its protein isolate were observed to have similar amino acid composition. The results of the amino acids tests were shown in Table 3. Glutamic acid was the major amino acid in nearly all the four fractions. In general, arginine, aspartic acid and glutamic acid were predominant in all the samples. Roselle is considered to be related to okra and results from this study on amino acid composition of Roselle seed proteins were in agreement with the finding of Al-Wandawi (1983) for okra seeds. According to Murray and Roxburgh (1984) high levels of albumin will elevate sulfur-containing amino-acids. In the present study, the albumin content is lower when compared to globulin. This might explain the low values of cysteine and methionine found in Roselle seed (Hainida *et al.*, 2008; Tounkara *et al.*, 2011).

3.4 Nutritional parameters

Protein is one of essential nutrients in the human diet. Both the amount and quality of protein provided by a food are important. The protein quality, also known as the nutritional or nutritive value, depends on the level at which essential amino acids needed for overall body health maintenance

Table 2. Distribution of protein fractions.

Parameters[1]	samples				
	Globulin	Albumin	Glutelin	Prolamin	Protein isolates
PC (%)	93.77±1.60^{a}	81.55±1.34^{b}	71.30±1.08^{c}	40.83±1.89^{d}	91.50±1.50^{a}
TP (%)	31.18±1.16^{a}	16.47±0.85^{b}	10.20±0.67^{c}	5.57±0.51^{d}	-

[1]All values are Means and standard deviations of three replicates. Mean values in rows with different letters were significantly different (P<0.05). Protein content (PC) % = g of proteins in 100 g of extracted solids. % of total protein (TP) = (total protein (g) of each fraction extracted from 100 g of meal/total proteins (g) of (100 g of defatted Roselle seed flour) x100.

Table 3. Comparative amino acid profiles of globulin, albumin, glutelin, prolamin fractions and their protein isolates (g/100 g of protein).

Amino acids[1]	Protein isolates	Globulin	Albumin	Glutelin	Prolamin	FAO /WHO Child (Adult)
Essential amino acids						
Lysine	4.91±0.04	4.53±0.04	6.20±0.05	4.07±0.03	5.19±0.01	4.8(4.5)
Histidine	2.41±0.02	2.49±0.01	2.30±0.03	2.38±0.02	2.38±0.04	1.6(1.5)
Leucine	7.88±0.02	8.06±0.01	7.28±0.01	8.11±0.01	9.06±0.01	6(5.9)
Isoleucine	4.19±0.01	4.36±0.03	4.13±0.01	4.33±0.01	5.12±0.02	3(3)
Phenylalanine	5.25± 0.02	5.52±0.01	4.81±0.01	5.04±0.05	5.66±0.05	
Phe +Tyr	8.18±0.01	7.66±0.01	8.17±0.03	8.28±0.09	9.32±0.01	4.1(3.8)
Methionine	2.40±0.02	2.04±0.01	2.15±0.05	2.34±0.02	0.70±0.03	
Met+Cys	3.54±0.01	2.88±0.04	3.25±0.02	3.57±0.01	1.90±0.01	2.3(1.6)
Valine	5.56±0.01	5.06±0.02	6.36±0.01	5.69±0.02	7.35±0.04	2.9(3.9)
Threonine	3.53±0.04	3.85±0.01	3.80±0.06	4.62±0.01	5.69±0.03	2.5(2.3)
Tryptophan	0.07±0.01	0.09±0.01	0.07±0.01	0.08±0.01	0.07±0.01	0.66(0.6)
Non-essential amino acids						
Glycine	3.61±0.04	4.24±0.02	4.32±0.02	4.87±0.01	5.13±0.03	
Cysteine (Cys-S)	1.14±0.03	0.84±0.04	1.10±0.05	1.23±0.03	1.20±0.06	
Aspartic acid	10.02±0.01	9.79±0.01	9.68±0.02	10.25±0.1	9.19±0.01	
Glutamic acid	21.10±0.04	20.61±0.02	21.00±0.01	20.09±0.09	14.48±0.06	
Serine	4.75±0.02	4.66±0.05	4.41±0.02	5.07±0.02	5.00±0.02	
Arginine	11.35±0.03	12.31±0.01	9.53±0.01	9.64±0.05	8.30±0.02	
Alanine	4.76±0.01	4.28±0.02	4.50±0.02	5.14±0.01	6.82±0.02	
Tyrosine	2.93±0.03	2.17±0.01	3.36±0.02	3.24±0.01	3.66±0.05	
Proline	4.14±0.04	5.10±0.03	5.00±0.01	3.82±0.04	5.02±0.02	

[1]All values are Means and standard deviations of three replicates.

and growth (Zhu *et al.*, 2006). Since a direct assessment of protein nutritional value in human subjects is impractical for regulatory purposes, methods based on in vitro and in vivo bioassays for assessment of protein quality have been developed. In our study, amino acid composition has been used as a basis for estimating the nutritional quality of Roselle seed protein isolates and its fractions. Results of the ratio of essential to total amino acids (E/T), amino acid score (AAS) and protein efficiency ratio (PER) of Roselle seed protein isolate (RSPI), globulin, albumin, glutelin and prolamin fractions are shown in Table 4. In all samples the ratio of essential to total amino acids (E/T) was higher than the pattern recommended by FAO/WHO/UNU (at least 36%), and prolamin fraction had the highest ratio with 41.22% (Table 4). In general, the protein efficiency ratio below 1.5 implies a protein of low or poor quality, while PER between 1.5 and 2.0 indicates an intermediate protein quality and then PER above 2.0 means protein of high quality (Friedman, 1996). The predicted PER values of all the samples are in range of high quality (Table 4). The PER values of RSPI and its fractions were quite satisfactory compared with a standard casein PER of 2.5 (Friedman, 1996) and were higher than the findings reported by Abu-Tarboush *et al.* (1997) PER of 2.0 and 2.06 for Roselle protein concentrate (RPC) and RSPI respectively. Bryant *et al.* (1988) reported PER of 2.17 and 2.14 for okra and soybean protein isolates, respectively. However, the total essential amino acid scores for all samples reached the FAO/WHO requirement (2007) for the essential amino acids for children except tryptophan (Table 4).

3.5 Water and oil holding capacity

The functional properties of proteins in a food system depend in part on the water protein-interaction. WHC refers to the ability of the protein to imbibe water and retain against gravitational force. Intrinsic factors affecting the WHC of food protein include amino acid composition, protein conformation and surface hydrophobicity (Barbut, 1999). Interactions of water and oil with proteins are very important in the food systems because of their effects on the flavor and texture of foods. The results on the WHC and OHC are shown in Table 5. The glutelin fraction showed the highest WHC 3.92 mL/g, followed by globulin 2.21 mL/g, RSPI 2 mL/g, prolamin 0.86 mL/g and albumin 0.74 mL/g with no significant difference (P>0.05) between albumin and prolamin fractions (Table 5). The value of OHC in decreased order was 7.23, 6.93, 5.75, 4.86 and 3.23 mL/g for glutelin, albumin, RSPI, globulin, and prolamin, respectively, with no significant

Table 4. Nutritional parameters of Roselle (*Hibuscus Sabdariffa L.*) protein isolate and its fractions.

Parameters	Protein isolate	Globulin	Albumin	Glutelin	Prolamin
E/T %	36.20	36.00	37.10	36.66	41.22
Estimated PER					
I	2.71	2.75	2.40	2.83	3.21
II	2.80	2.96	2.48	2.87	3.25
III	3.11	3.84	2.11	2.87	2.60
Amino acid scores					
Leucine	133.56	136.61	123.39	137.45	153.56
Histidine	160.67	166.00	153.33	158.67	158.57
Threonine	209.13	219.13	246.09	200.87	247.39
Valine	142.56	129.74	163.07	145.89	188.46
Met + Cys	221.25	180.00	203.12	223.12	118.75
Isoleucine	139.66	145.33	137.67	144.33	170.67
Phe+Tyr	215.26	201.58	215.00	217.891	245.26
Lysine	109.11	100.67	137.77	90.44	115.33
Tryptophan	11.66	15.00	11.66	13.33	11.67

E/T: Proportion of essential amino acids (E) to total amino acids (T), PER: protein efficiency ratio

Table 5. Functional properties of Roselle seed protein isolates and its fractions.

Functional properties[1]	Protein isolate	Globulin	Albumin	Glutelin	Prolamin
Water holding capacity (mL/g)	2.00±0.023^{c}	2.21±0.12^{b}	0.74±0.11^{d}	3.92±0.27^{a}	0.86±0.06^{d}
Oil binding capacity (mL/g)	5.75±0.30^{b}	4.86±0.14^{c}	6.93±0.29^{a}	7.23±0.44^{a}	3.47±0.23^{d}
Emulsifying capacity (mL/g)	75.33±5.0^{b}	50.67±3.10^{c}	56.67±3.10^{c}	95.3±3.1^{a}	18.0±2.0^{d}
Foaming capacity (%)	155±5.0^{d}	145±5.0^{c}	176±3.60^{b}	186±3.60^{a}	140±5.0^{e}

[1]All values are Means and standard deviations of three replicates. Mean values in rows with different letters were significantly different (P < 0.05).

difference (*P*>0.05) between albumin and glutelin fractions (Table 5). The oil absorption capacity of albumin fraction was higher than those of RSPI and globulin fraction. Similar results have been reported showing that oil absorption capacities of African locust bean albumin are higher than that of globulin (Lawal *et al.*, 2005). It is suggested that the high oil absorption capacity, may give an advantage to Roselle seed proteins in the formulation of food systems like sausages, cake, batters, mayonnaise and salad dressings.

3.6 Emulsifying capacity

Food emulsions are thermodynamically unstable mixtures of immiscible liquids (water and oil). The formation and stability of emulsion is very important in food systems such as salad dressings. Proteins constitute an important group of emulsifiers because they reduce interfacial tension, form rigid interfacial films and possess charged groups. Emulsifying capacity of Roselle seed protein fractions and protein isolate is shown in Table 5. The highest emulsifying activity was observed in glutelin fraction (95 mL/g), followed by RSPI (75.33 mL/g). For albumin, globulin and prolamin fractions the emulsifying activity was 56.66 mL/g, 50.66 ml/g and 18 mL/g, respectively, with no significant difference (*P*>0.05) between albumin and globulin fractions. The emulsion activity of albumin fractions was comparable to previous finding (Xu and Diosady, 1994) where, it was reported that Roselle seed protein isolate was similar to that of Chinese rapeseed protein isolate, but was higher than that of soybean protein isolate and lower than that of chickpea protein isolate (Paredes-Lopez *et al.*, 1991).

3.7 Foaming capacity and foaming stability

Proteins are good foaming agents, since they can rapidly diffuse to the air-water interface and they form a strong cohesive and elastic film by partial unfolding. Foaming properties are correlated with amount of hydrophobic amino-acids exposed at surface of protein molecule (Wang *et al.*, 1999). Dispersed protein lowers the surface tension at water-air interface, thus creating foaming capacity (Turgeon *et al.*, 1992). In order to have foam stability, protein molecules

should form continuous intermolecular polymers enveloping the air bubbles, since intermolecular cohesiveness and elasticity are important to produce stable foams (Kamara *et al.*, 2009). A significant higher value was observed in the foaming capacity (FC) for glutelin (186%), followed by albumin (176%), RSPI (155%) compared to globulin (146%) and prolamin (140%). The values were significantly different at $P<0.05$ (Table 5). The foam stability (FS) of RSPI was higher when compared to that of the fractions. The FS values ranged from 155 to 135, 146 to 110, 176 to 147, 186 to 140, and 140 to 105% for RSPI, globulin, albumin, glutelin and prolamin, respectively (Figure 1).

3.8 SDS PAGE Analysis

The SDS-PAGE profiles of RSPI, globulin, albumin and glutelin fractions are presented in Figure 2. The molecular weight of all samples ranged from 55,000 Da to below 14,300 Da. The RSPI (Line 7 and 8) possessed ten intense polypeptide bands and are listed according to the molecular weights as follows: 55,000, 41,000, 35,000, 29,000, 25,000, 23,000, 22,000, 17,300, 16,300 and below 14,300 Da. As observed in Figure 2 there is no much distinct differences in the SDS pattern of the globulin fraction (Line 5 and 6) and the glutelin fraction (Line 1 and 2). The globulin fraction showed ten major polypeptide bands and their corresponding estimated molecular weights were; 55,000, 41,000, 40,000, 29,000, 25,000, 23,000, 22,000, 17,300, 16,300 and below 14,300 Da. In the case of the glutelin fraction, eight polypeptide bands were shown with estimated Mw at 55,000, 41,000, 35,000, 28,000, 23,500, 22,000, 20,000, 19,000 and below 14,300Da. Compared with the RSPI, globulin and glutelin; albumin fraction revealed less polypeptide bands with estimated molecular weights at Mr 55,000, 29,000, 24,500, 21,000 and below 14,300 Da. However, the protein subunits distribution profiles for RSPI, globulin and glutelin showed two predominant doublet-like bands between 16,300 and 23,000 Da, which did not appear in the albumin fraction (Line 3 and 4).

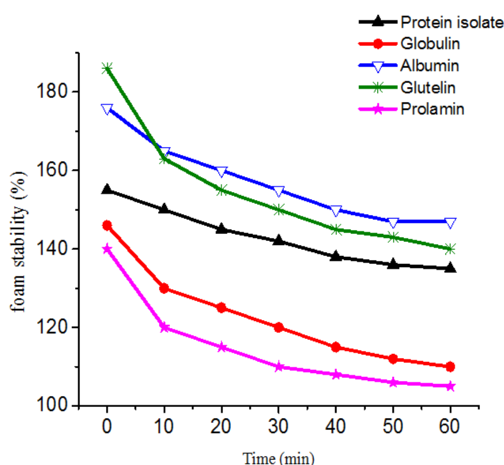

Figure 1. Foaming stability of Roselle seed protein isolates, Globulin, Albumin, Glutelin and Prolamin. Values represent the means ± SD of triplicates.

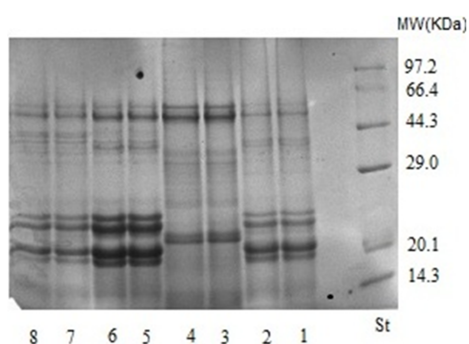

Figure 2. SDS-PAGE profile of protein isolates and protein fractions extracted from Roselle seed. Standard (St); Glutelin (1, 2); Albumin (3, 4); Globulin (5, 6); and Protein isolates (7, 8).

4. Conclusions

From the results of our study it is evident that Roselle protein fractions and its protein isolates have potential as a functional ingredient. The amino acid pattern of all samples was higher than FAO/WHO requirement. All the estimated nutritional parameters based on amino acids composition showed that Roselle seed protein fractions and their isolates have a good nutritional quality and suggests their possible use as a supplementary protein source. The fractions were found to have a high concentration of low molecular weight polypeptides. The Roselle protein isolate and its fractions could have excellent applications for future product development by virtue of their nutritional and functional properties. This would add some economic value to the existing uses of the plant and expand to cultivation.

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