



Functional Properties of Defatted Roselle (*Hibiscus sabdariffa* L.) Seeds Protein and Its Hydrolysates

**Fatoumata Tounkara^{1*}, Issiaka Togola¹, Lassana Sissoko¹,
Mah Moutaga Fane², Nouhoum Diarra¹ and Nah Traoré²**

¹Laboratory of Plant Biochemistry and Biotechnology, Faculty of Sciences and Techniques (FST), University of Sciences, Techniques and Technologies of Bamako (USTTB), BP: 3206, Bamako, Mali.

²Laboratory of Organic Chemistry and Natural Substances, Faculty of Sciences and Techniques (FST), University of Sciences, Techniques and Technologies of Bamako (USTTB), BP: 3206, Bamako, Mali.

Authors' contributions

This work was carried out in collaboration among all authors. Author FT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors IT and LS managed the analyses of the study. Authors MMF, ND and NT managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2019/v41i530421

Editor(s):

(1) Dr. Moreira Martine Ramon Felipe, Associate Professor, Departamento de Engenharia Química, Universidade de Santiago de Compostela, Spain.

Reviewers:

(1) Valdir Florencio da Veiga Junior, Military Institute of Engineering, Brazil.
(2) Omojokun, Ayomide Olubunmi, Federal University of Technology, Nigeria.
(3) Adekola O. Atanda, Ahmadu Bello University, Nigeria.
Complete Peer review History: <http://www.sdiarticle4.com/review-history/52603>

Original Research Article

Received 14 September 2019

Accepted 21 November 2019

Published 05 December 2019

ABSTRACT

Aim: The purpose of this study was to evaluate the functional properties of Roselle seed protein isolates and its hydrolysates.

Place and Duration of Study: The Roselle seeds were collected in Koutiala (Mali), in November 2018. All analysis were conducted in the Faculty of Sciences and Technics, particularly in the Laboratory of Plant and Food Biochemistry and Biotechnology from January to June 2019.

Methodology: The effect of enzymatic hydrolysis on the functional properties of Roselle seed protein (RSP) was investigated. Defatted Roselle seed flour was used to extract the protein isolates. The protein was digested for 2 hours and 3 hours using pepsin followed by pancreatin.

Results: The 2 hours and 3 hours Roselle seed protein hydrolysates (RSPH2, RSPH3) compare to

*Corresponding author: E-mail: toucoulup@gmail.com;

RSPI, exhibited a good foaming capacity of 300, 315 and 165% respectively. The water holding capacity (WHC) of the RSPI, RSPH2 and the RSPH3 were 2, 2.5 and 2.2 ml/g respectively. The oil holding capacity ranged from 5.75 to 5.32 ml/g, the emulsifying capacity of the RSPH2 was higher than that of the RSPH3 and the RSPI, 105, 97 and 82 ml/g respectively.

Conclusion: The ability of pepsin and pancreatin hydrolysates to be functional is primarily due to their soluble peptide content. The samples have good functional properties. These results proposed that pepsin and pancreatin hydrolysates could be useful as whole or partial replacement of high-price materials such as egg albumen and casein.

Keywords: Roselle; seed-protein; hydrolysis; 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 [1] 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 [2,3]. The brilliant red color and unique flavor coupled with other organoleptic attributes make them valuable food products [4].

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 [5].

Protein isolates are used as additives in food products for improving functional properties, such as the foaming/emulsifying capacity, gel formation, viscosity, texture and water-binding capacity. Protein isolates obtained from defatted maize germ [6], as well as wheat and soybean proteins [7], have been added to a variety of products, usually as replacements for egg albumin. It is possible to enhance their use in different food and non-food applications with diversified and improved functional properties by hydrolysis.

Enzymatic hydrolysis of proteins has a great of potential for modifying functional properties of food proteins. In recent years, production of enzymatic protein hydrolysates has undergone through considerable development. Protein hydrolysates can be classified according to their degree of hydrolysis. Protein hydrolysates with variable degrees of hydrolysis are used as flavorings in soups, sauces, and meat products

[6]. Protein hydrolysates most likely have better functional properties than the original proteins and are commonly used as food ingredients [6]. Protein hydrolysates are widely used as protein supplements or in special medical diets, such as hypoallergenic foods [6].

Studies have shown that functional properties of food proteins can be improved through enzymatic hydrolysis. Mannheim and Cheryan [6] suggested that solubility of enzymatic hydrolysate of proteins from corn gluten meal was better than from the unmodified proteins. Though foam formation was shown to be improved by enzymatic hydrolysis of whey and casein proteins, foam stability of the hydrolysates was poorer than those of unmodified proteins [8]. Qi et al. [7] suggested that the emulsifying activity index of soy protein modified with pancreatin was better than that of soy protein isolate, and thus the potential use of soy protein hydrolyzed in formulation of emulsified food products. Reports showed that the low-molecular-weight fraction of soybean glycinin protein hydrolysate had more poor emulsion capacity when compared to the unhydrolyzed glycinin.

There is currently few information available especially, on the functional properties of the Roselle seed protein. Therefore, this study is undertaken to evaluate the functional properties of Roselle seed protein and its hydrolysates obtained at different hydrolysis time for the purpose to be used in food formulation systems.

2. MATERIALS AND METHODS

2.1 Materials

Seeds of *H. sabdariffa* were obtained from Koutiala, Southern region of Republic of Mali. All enzymes used were of food grade. Pepsin and pancreatin were purchased from Sigma Chemical Co. (St. Louis, USA). All the other

chemicals used in the experiments were from commercial source and of analytical grade.

2.2 Methods

2.2.1 Preparation of defatted Roselle seed flour

Roselle seeds were winnowed and destoned. 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 twice with n-hexane at room temperature at a ratio of 1:10 (w/v) and stirred for 7 h for each time. The oil-free flours was desolventized and stored in desiccator at room temperature for subsequent uses.

2.2.2 Proximate composition

The proximate analysis of Roselle seed flour (RSF) and defatted Roselle seed flour (DRSF) were determined according to [9]. 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 hours. Total crude protein content was determined using the Kjeldahl method. The total lipid in samples was determined by Soxhlet method. Available carbohydrate content was calculated as:

$$100\% - [\% (\text{moisture} + \text{ash} + \text{fat} + \text{protein})]$$

2.2.3 Preparation of Roselle seed protein isolate

Roselle seed protein isolates (RSPI) were obtained from defatted flour as reported by [10, 11], with some modifications. The defatted flour was dispersed in distilled water; the pH was adjusted to 10 with 1 M NaOH and stirred for 3 hours at room temperature. The suspension was then centrifuged at 4000 rpm for 20 min. The residues were re-extracted for the second time as described above. The supernatants were combined and protein was precipitated before centrifugation at 4000 rpm for 20 min. The protein isolate (precipitate) was washed twice with distilled water then resuspended in distilled water and the pH was adjusted 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 using Kjeldahl method, AOAC [9].

2.2.4 Preparation of protein hydrolysates

To produce hydrolysates from RSPI, enzymatic hydrolysis was performed using two enzymes (pepsin followed by pancreatin) under their optimal conditions. The RSPI samples were divided in to two groups and hydrolyzed in 500ml reactor under temperature and pH control devices. Sample 1 was hydrolyzed by pepsin (1% at PH 2) for 1 hour followed by pancreatin (2% at PH 7) for 1 hour. Sample 2 was hydrolyzed (under the same condition) by pepsin for 1 hour followed by pancreatin 2 hours. Conditions were constantly monitored and maintained throughout the process. Upon completion of the hydrolysis, the enzymes were deactivated by heating in a boiling water bath for 10 min. The reaction mixtures were then centrifuged at 7000xg and the supernatants were collected. The protein hydrolysates obtained were freeze-dried and stored at -20°C for subsequent analysis.

2.2.5 Amino acids analysis

The dried samples (100 mg) were subjected to acid hydrolysis using 5 ml of 6 M HCl under nitrogen atmosphere for 24 hours at 110°C. The hydrolysate was washed into a 50 ml volumetric flask and made up to the mark with distilled water. The amino acids were subjected to RP-HPLC analysis (Agilent 1100, USA) after precolumn derivatization with o-phthalaldehyde (OPA). Each sample (1 µl) was injected into a Zorbax 80 A C18 column (i.d. 4.6X180 mm, Agilent Technologies, Palo Alto, CA, USA) at 40°C with detection at 338 nm. The Amino acid composition was expressed as g per 100 g protein.

2.2.6 Protein nutritional parameters

The nutritional parameters of the Roselle seed protein isolate (RSPI) and its hydrolysates 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 FAO/WHO/UNU standard reference pattern) × 100.
- (3) Predicted protein efficiency ratio (PER). The predicted PER values of the Roselle protein isolates and its hydrolysates were estimated by three regression equations developed by [12].

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).

2.2.7 Water holding capacity (WHC)

The Water Holding Capacity (WHC) of the Roselle protein isolates and its hydrolysates, was determined as described by Diniz and Martin [13] with some modifications. Triplicate samples (0.5 g) were placed in centrifuge tubes, dissolved with distilled water and vortexed for 30 sec. The mixture was allowed to stand at room temperature for 30 min and later centrifuged at 3000 rpm for 25 min. The supernatant was filtered using 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 retrieved was determined. The results were reported as ml of water absorbed per gram of protein sample.

2.2.8 Oil holding capacity (OHC)

Oil holding capacity (OHC) was determined as described by the method of [14]. One gram of each sample (W_0) was weighed into pre-weighed 15ml centrifuge tubes and thoroughly mixed with 10ml (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 3000 rpm for 20 min. The supernatant was immediately poured into a 10 ml graduated cylinder, and the obtained volume reading was recorded (V_2). Oil holding capacity (ml of oil per g of protein) was calculated as:

$$OHC = (V_1 - V_2) / W_0$$

2.2.9 Emulsifying capacity (EC)

Emulsifying capacity of the samples was measured as described by Turgeon et al. [15] with some modifications. 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 into centrifuge tubes, kept under a water-bath at 90°C for 10 min and then centrifuged at 3000 rpm for 20 min. Emulsifying capacity was calculated using the 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

W_S is the weight of the sample.

2.2.10 Foaming capacity (FC)

Foaming capacity (FC) was determined using the method described by Makri et al. [16] with some modifications. Sample concentrates of 1% were prepared in de-ionized 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 the 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:

$$FC (\%) = (V_F / V_1) \times 100$$

2.3 Statistical Analysis

Results were expressed as the mean values \pm standard deviation (S.D.) of three separate determinations. The data were averages of triplicate observations and were subjected to a one way analysis of variance (ANOVA), followed by Duncan's multiple range test. The data was subjected to correlation analysis, using SPSS software (version 16.0).

3. RESULTS AND DISCUSSION

3.1 Proximate Composition

The results are shown in Table 1. The undefatted RSF contained 27.00% and 39.15% of protein and carbohydrates respectively, but were both elevated to 37.25% and 46.67% after defatting. The fat content was 21.83% for RSF and 2.04% for DRSF. Defatting is a purification step which when carried out, increases the concentrations of nutrients such as proteins, carbohydrates and other major components of food components.

Moreover, defatting 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 [1]. Other researchers found that the carbohydrates were mainly composed of dietary fibers [2].

3.2 Amino Acids Analysis

The biological activity of protein is more related to its amino acid make up. In order to appreciate the physicochemical properties of our samples, amino acid composition analysis was carried out. The obtained amino acid test results were shown

in Table 2 along with FAO/WHO/UNU [17] recommended essential amino acid composition values. The results suggest that the Roselle protein isolates and its hydrolysates contained a good proportion of all essential amino acids. Glutamic acid was the major amino acid in the protein and both hydrolysates. In general, arginine, aspartic acid and glutamic acid were predominant in all the samples. The amino acid compositions of Roselle seed protein and its hydrolysates in this study were in agreement with the findings of [4-18] for Roselle seed protein. On the other hand, the results of all our samples comply with the findings of Abu-Tarboush et al. (1997) this was the same for Roselle seed protein concentrates and Roselle seed protein isolates [1].

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

Composition (g/100 g)	RSF	DRSF
Moisture	8.45±0.18	9.55±0.15
Ash	5.47±0.11	5.59±0.13
Protein (N x 6.25)	27.00±0.39	36.15±0.69
Fat	21.83±0.55	2.04±0.16
Carbohydrate	37.25±0.60	46.67±0.97

All values are means and standard deviations of three replicates

Table 2. Comparative amino acid profiles of Roselle seed protein isolate and its hydrolysates (g/100 g of protein)

Amino Acids	RSP1	RSPH2	RSPH3	FAO / WHO Child (Adult)
Essential Amino Acids				
Lysine	4.78±0.04	4.58±0.02	4.54±0.01	4.8 (4.5)
Histidine	2.43±0.02	2.24±0.02	2.18±0.02	1.6 (1.5)
Leucine	7.78±0.02	7.85±0.04	6.66±0.03	6 (5.9)
Isoleucine	4.27±0.01	3.52±0.01	3.83±0.02	3 (3)
Phenylalanine	5.25± 0.02	5.41±0.01	4.31±0.02	
Phe +Tyr	8.18±0.01	8.19±0.01	6.33±0.01	4.1 (3.8)
Methionine	2.40±0.02	1.79±0.03	2.36±0.02	
Met+Cys	3.54±0.01	3.34±0.01	3.98±0.02	2.3 (1.6)
Valine	5.63±0.01	5.16±0.02	5.21±0.01	2.9 (3.9)
Threonine	3.59±0.04	3.25±0.03	3.66±0.02	2.5 (2.3)
Tryptophan	0.07±0.01	0.08±0.01	0.068±0.01	0.66 (0.6)
Non-Essential Amino Acids				
Glycine	3.51±0.04	4.13±0.03	4.69±0.02	
Cysteine (Cys-S)	1.14±0.03	1.25±0.01	1.62±0.02	
Aspartic acid	10.12±0.01	10.27±0.02	11.59±0.02	
Glutamic acid	21.08±0.04	23.48±0.03	24.58±0.04	
Serine	4.78±0.02	4.43±0.01	4.68±0.02	
Arginine	11.32±0.03	10.27±0.02	11.69±0.04	
Alanine	4.72±0.01	4.58±0.02	4.84±0.03	
Tyrosine	2.93±0.03	2.78±0.02	2.02±0.01	
Proline	4.18±0.04	4.70±0.03	5.36±0.04	

All values are means and standard deviations of three replicates

3.3 Protein 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 are needed for overall body health maintenance and growth [19]. In this study, amino acid composition was used as a basis for estimating the nutritional quality of the Roselle seed protein and its hydrolysates. Results of the ratio of essential to total amino acids (E/T), amino acid score (AAS) and protein efficiency ratio (PER) of the hydrolysates are shown in Table 3. In all samples the ratio of essential to total amino acids was higher than 36% (the recommended value by FAO/WHO/UNU), and RSPI, when compared to its hydrolysates had the highest ratio of 42.91% (Table 3). In general PER 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 indicates protein of high quality [19]. The predicted PER values of all the samples were in range of the high quality (Table 3). The AAS results showed that all our samples had well balanced amino acid compositions and their ratio of amino acids were close to that of protein quality for adult recommended by the Food and Agricultural Organization / World Health Organization reference [17].

3.4 Water Holding Capacity

Water and oil interactions with proteins are essential in food systems as they have a great impact on food organoleptical attributes. Intrinsic factors affecting water holding capacities of food proteins include amino acid composition, protein conformation, and surface polarity/hydrophobicity [20]. The ability of protein to absorb water and retain it against a gravitational force within a protein matrix is WHC. The WHC of the RSPI RSPH2 and RSPH3 were 2, 2.5 and 2.2 ml /g (Table 4) respectively. The result of WHC of the Roselle protein isolates and its hydrolysates observed in this study is in agreement with the previous reports [1-21,22]. Water holding capacity is an important factor for protein additives used in food systems. The water holding capacity of the samples can be used to define how those proteins can be added to formulated foods and how they can replace animal proteins traditionally used [21,22].

3.5 Oil Holding Capacity

The ability of protein and /or the hydrolysates to absorb oil is an important functionality that influences organoleptical properties in various food products. The OHC for the RSPI, RSPH2 and RSPH3 were 5.75, 5.47g and 5.32 ml/g, respectively (Table 4). It is suggested that the high oil absorption capacity, may give an advantage to Roselle seed protein and its hydrolysates in the formulation of food systems like sausages, cake, mayonnaise and salad dressings. Wasswa et al. [23] also produced protein hydrolysates with high OHC.

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. Interactions between proteins and lipids are common in many food systems, and thus the ability of proteins to form stable emulsions is important. Emulsions are formed due to the presence of hydrophobic and hydrophilic groups of proteins. The emulsion formation is mainly dependent on the diffusion of peptides at oil-water interfaces. Hydrolysates with high solubility and smaller molecular size should facilitate that diffusion and enhance the protein-lipid interaction [24]. As shown in Table 4, the Roselle protein and its hydrolysates were good emulsifiers with EC of 82 mL/g, 105 mL/g and 97 mL/g for the RSPI, RSPH2 and RSPH3 respectively. Roselle seed protein digested using pepsin followed by pancreatin were degraded into polypeptides and oligopeptides, thus increasing the protein solubility and resulting in increased EC and stable emulsion. Hydrolysis was effective in improving the EC of hydrolysates. Related studies have demonstrated that enzymatic hydrolysis of tilapia [24] and grass carp skin [23] produced hydrolysates with higher emulsifying capacity.

3.7 Foaming Capacity

The formation of foam is analogous to the formation of emulsion. The capacity of proteins to form stable foams with gas by forming impervious protein films is an important property and it was likely due to the increased net charges on the protein, which weakened the hydrophobic interactions but increased the flexibility of the protein.

Table 3. Nutritional parameters of Roselle (*Hibiscus sabdariffa* L.) seeds protein isolates and its hydrolysates

Parameters	RSPI	RSPH2	RSPH3
E/T %	42,91	42.52	38.23
Estimated PER			
I.	2.65	2.70	2.10
II.	2.75	2.80	2.31
III.	3.03	3.17	2.72
Amino acid scores			
Leucine	129.67	130.83	111.00
Histidine	151.87	140.00	136.25
Threonine	143.60	130.00	146.40
Valine	194.14	177.93	179.65
Met + Cys	153,91	145.22	173.04
Isoleucine	142.33	117.33	127.67
Phe+Tyr	199.51	199.75	154.39
Lysine	99.58	95.42	94.53
Tryptophane	10.60	12.12	10.45

Data are mean of 3 replications. E/T, Proportion of essential amino acids (E) to total amino acids (T), PER, predicted protein efficiency ratio

Table 4. Functional properties of Roselle seed protein isolates and its hydrolysates

Functional properties	RSPI	RSPH2	RSPH3
Water holding capacity (ml/g)	2.00±0.02	2.5±0.02	2.20±0.10
Oil holding capacity (ml/g)	5.75±0.30	5.47±0.30	5.32±0.14
Emulsifying capacity (ml/g)	82.00±5.00	105±4.00	97±3.10
Foaming capacity (%)	165±5.00	300±3.50	315±3.50

All values are means and standard deviations of three replicates

Results in Table 4 suggested that all the hydrolysates were good foaming agents with a FC of 315% and 300% for the RSPH3 and RSPH2 respectively, these results corroborate with the findings of [24] who reported that enzymatically-modified food proteins improved foaming properties. Moreover, the FC of the hydrolysates was improved by hydrolysis. However, it should be noted that the FC may be influenced by the molecular size, protein structure and hydrophobicity of the hydrolysate [25,26] which are highly dependent on the parent protein from which they are obtained and the hydrolysis procedure. The effect of hydrolysis on foam stability seems to depend on the degree of hydrolysis [24,25,26,27]. The short chain molecules form a wicker interaction network, which result in to a less stable foam [26].

4. CONCLUSION

From the results obtained in this study it can be concluded that, Roselle protein isolate and its hydrolysates could have excellent applications for future product development by virtue of their functional properties. The amino acid pattern of

all samples was higher than FAO/WHO requirement. All the finding results showed that Roselle seed protein isolates and its hydrolysates have good functional properties and suggests their possible use as a supplementary protein source.

ACKNOWLEDGEMENTS

This study was financially supported by the University of Sciences, Techniques and Technologies. Authors are grateful to the workers of the Faculty of Sciences and Techniques, particularly the members of Laboratory of Plant and Food Biochemistry and Biotechnology.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Abu-Tarboush HM, Ahmed AA, Kahtani HA. Some nutritional and functional properties of Karkade (*H. sabdariffa*) seed

- products. Cereal Chem. 1997;74(3):352-355.
2. Rao PU. Nutrient composition and biological evaluation of mesta (*Hibiscus sabdariffa*) seeds. Plant Foods Hum. Nutr. 1996;49(1):27-34.
 3. Tsai P J, McIntosh J, Pearce P, Camden B, Jordan BR. Anthocyanin and antioxidant capacity in Roselle (*Hibiscus sabdariffa* L.) extract. Food Res. Int. 2002;35(4):351-356.
 4. El-Adawy TA and Khalil A H. Characteristic of Roselle seeds as a new source of protein and lipid. J. Agric. Food Chem. 1994;42(9):1896-1900.
 5. Al Wandawi H, Al-Shaikhly K. and Abdul-Rahman M. Roselle seed: A new protein source. J. Agric. Food Chem. 1984;32(3): 510-512.
 6. Mannheim A, Cheryan M. Enzyme modified proteins from corn gluten meal: Preparation and functional properties. J. Am. Oil Chem. Soc. 1992;69(12):1163-1169.
 7. Qi M, Hettiarachchy NS, Kalapathy U. Solubility and emulsifying properties of soy protein isolates modified by pancreatin. J. Food Sci. 1997;62:1110-1115.
 8. Vioque J, Sánchez-Vioque R, Clemente A, Pedroche J, Millan F. Partially hydrolyzed rapeseed protein isolates with improved functional properties. Journal of American Oil Chemists Society. 2000;77(4):447-450.
 9. A.O.A.C. Official method of analysis. The Association of official Analytical Chemists. 16th Ed. Washington; 2002.
 10. El-Tinay AH, Nour AM, Abdel-Karim SH, Mahgoub SO. Aqueous protein and gossypol extraction from glanded cottonseed flour: Factors affecting protein extraction. Food Chem. 1988;29(1):57-63.
 11. Emmy H, Amin I, Normah H, Mohd-Esa N. Nutritional and amino acid contents of differently treated Roselle (*Hibiscus sabdariffa* L.) seeds. Food Chemistry. 2008a;111(4):906-911.
 12. Chavan UD, Mckenzie DB and Shahidi F. Functional properties of protein isolates from beach pea (*Lathyrus maritius* L.). Food Chem. 2001;74(2):177-187.
 13. Diniz FM, Martin AM. Effects of the extent of enzymatic hydrolysis on the functional properties of shark protein hydrolysate. LWT- Food Sci. Technol.1997;30(3):266-272.
 14. Tounkara F, Amza T, Lagnika C, Le GW, Shi YH. Extraction, characterization, nutritional and functional properties of Roselle (*Hibiscus sabdariffa* Linn) seed proteins. Songklanakarin J. Sci. Technol. 2013a;35(2):159-166.
 15. Turgeon SL, Gauthier SF, Paquin P. Emulsifying Property of whey peptide fractions as a function of pH and ionic strength. J. Food Sci. 1992;57(3):601-604.
 16. Makri E, Papalamprou E, Doxastakis G. Study of functional properties of seed storage proteins from indigenous European legume crops (lupin, pea, broad bean) in admixture with polysaccharides. Food Hydrocolloids. 2005;19(3):583-594.
 17. WHO. Protein and amino acid requirements in human nutrition. WHO Technical Report series N°935, Report of a joint WHO/FAO/UNU expert consultation. Geneva, Switzerland; 2007.
 18. Tounkara F, Amadou I, Le GW, Shi YH. Effect of boiling on the physicochemical properties of Roselle seeds (*Hibiscus sabdariffa* L.) Cultivated in Mali. African Journal of Biotechnology. 2011;10(79): 18160-18166.
 19. Friedman M. Nutritional value of proteins from different food sources. J. Agric. Food Chem.1996;44(1):6-29.
 20. Barbut S. Determining water and fat holding. In Methods of testing protein functionality. Blackie Academic and Professional. U.S.A. 1999;186-225.
 21. Amadou I, Amza T, Foh MBK, Kamara T, Le GW. Influence of *Lactobacillus plantarum* LP6 on the functional properties of soybean protein meal. Em. J. of Food and Agri. 2010;22(6):456-65.
 22. Bryant LA, Montecalvo JJR, Morey KS, Loy B. Processing and nutritional properties of okra seed products. Journal of Food Science.1988;53(3):810-816.
 23. Wasswa J, Tang J, Gu XH. Functional properties of grass carp (*Ctenopharyngodon idella*), Nile perch (*Lates niloticus*) and Nile Tilapia (*Oreochromis niloticus*) Skin Hydrolysates. Int. J. of Food Properties. 2008;11(2):339-350.
 24. Foh MBK, Amadou I, Foh BM, Kamara T, Xia W. Functionality and antioxidant properties of tilapia (*Oreochromis niloticus*) as influenced by the degree of hydrolysis. Int. J. Mol. Sci. 2010;11(4):1851-1869.
 25. Wang M, Hettiarachchy NS, Qi M, Burks W, Siebenmorgen TJ. Preparation and functional properties of rice bran protein

- isolate. J. Agric. Food Chem. 1999;47(2): 411-416.
26. Van der Ven C, Gruppen H, De Bont DBA, Voragen AGJ. Correlations between biochemical characteristics and foam forming and stabilizing ability of whey and casein hydrolysates. J. Agric. Food Chem. 2002;50(10):2938-46.
27. Rakesh J, Metz A. Acid precipitated fish Protein isolate exhibits good functional properties. Food Product Development. 1973;7:18-24.

© 2019 Toukara et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

*The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/52603>*