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Amino acids profile and protein functional properties of *Chrozophora oblongifolia* seeds from Kordofan Region, Sudan

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Abstract. *Abdalgader SIA, Ahmed AI. 2019. Amino acids profile and protein functional properties of* Chrozophora oblongifolia *seeds from Kordofan Region, Sudan. Trop Drylands 3: 17-21.* The present research was conducted to study the amino acids profile and protein functional properties of *Chrozophora oblongifolia* seeds. The fresh seeds samples were procured from North and West Kordofan regions, Sudan. The protein functional properties, i.e., water absorption capacity, oil absorption capacities, emulsifying capacity, foaming stability, bulk density and crude protein were determined, then the amino acids profile was investigated using an amino acids analyzer (L-8900 Hitachi-hitech, Tokyo, Japan) under the experimental conditions recommended for protein hydrolysates. The results found that there were seven essential amino acids namely lysine, histidine, threonine, methionine, valine, isoleucine, and phenylalanine and nine non essential amino acids namely arginine, aspartic acid, serine, glutamic acid, glycine, proline, alanine, cystine and tyrosine were qualified and quantified in present investigation according to the following values: Glycine with a range of 0.99-1.02 g/100g proteins, while arginine with a range of 8.43-8.87 g/100g protein was the highest. Leucine and isoleucine which were limiting amino acid in most foodstuffs, were presented in ranges of 7.27-7.59g/100g protein for leucine and 5.24-56 4.19 g/100g protein for isoleucine. Statistical analysis of the seeds protein concentrates showed that a significant differences ($p \leq 0.05$) in crude protein and water absorption capacities, oil absorption capacities, foaming stability and bulk density were found between the two different collection regions, while there were no significant ($p \leq 0.05$) differences found in emulsifying capacity and foaming capacity in the two different locations of which samples were procured. The protein concentrates indicated higher protein content for North Kordofan seeds (83.33%) than that of 80.6% for West Kordofan seeds. This study concluded that *C. oblongifolia* seeds can be considered as a cheap source of edible protein which had a rich of essential amino acids

Keywords: Amino acids profile, functional properties, *Chrozophora oblongifolia* seeds, Kordofan region, Sudan

INTRODUCTION

Plants have been used for thousands of years to flavor and conserve food, to treat health disorders and to prevent diseases including epidemics, and now to use species of *Chrozophora* genus (Euphobiaceae family) that are distributed in West Africa and Asia. These species are monoecious, shrubby herb and annual plants and their leaves, stems and fruits besides the whole plant have been used in food and traditional medicine for the treatments of infectious diseases; many of these species showed high content of protein and oil with high percentages of fatty acids (Ahmed et al. 2014). *Chrozophora oblongifolia* one of *Chrozophora* genus plants. The biological activity of the *Chrozophora* plants received increased attention to discover new leading compounds for treatment of diverse ailments (Galal and Adam 1988). The methanol extract of the parts of *C. oblongifolia* showed highest antioxidant and hepatoprotective activities and it had a valuable biological source of drugs enhances fertility (Kamel et al. 2018). In1995, a study was conducted at Safiola company-Sudan to screening the seeds oil characteristics. Results showed that it has 0.29% moisture, 2.7% free fatty acids, 1.9 mq/kg peroxide value, 102.9g/100g iodine value close to cotton seed and rape seed oils. Also, the trace metals contents were within the normal limit and its refining and bleaching behaving was very similar to other normal vegetable oils (Galal and Adam 1988).

Thus, the industrial potentiality of *Chrozophora* oil should be explored. Therefore, exploitation of *Chrozophora* seed as alternative source of proteins and oil need to be investigated, so the aim of present work was to analyze amino acids profile and investigate protein functional properties of seeds of *C. oblongifolia* plant.

MATERIALS AND METHODS

Plant materials

The fresh seeds of *C. oblongifolia* was collected from two different locations from West and North Kordofan States, Sudan. The plant materials were air-dried in the laboratory and then ground into powder form using a mortar, shieved, and then stored in air tight bottles pending the analyses.

Amino acids analysis

The amino acid content (except for tryptophan) in seeds of *C. oblongifolia* plant was determined using an amino acid analyzer (L-8900 Hitachi-hitech, Tokyo, Japan) under the experimental conditions recommended for protein hydrolyzates. Samples containing 5.0 mg of protein

were acid hydrolyzed with 1.0 ml of 6 N HCl in vacuum-sealed hydrolysis vials at 110°C for 22 h. Ninhydrine was added to the HCl as an internal standard. The tubes were cooled after hydrolysis, opened and placed in a desiccator containing NaOH pellets under vacuum conditions until dry (5-6 days). The residue was then dissolved in a suitable volume of NaS buffer, pH 2.2 filtered through a Millipore membrane (0.22-lm pore size, Millipore, Billerica, MA, USA) and analyzed for amino acids by ion-exchange chromatography in a Beckman (model 7300, Pickering Laboratories, Inc. Mountain View, CA, USA) instrument, equipped with an automatic integrator. Amino acid nitrogen was determined by multiplying the concentration of individual amino acids by corresponding factors calculated from the percentage N of each amino acid (Mosse 1990). The ammonia content was included in the calculation of protein nitrogen retrieval, as it comes from the degradation of some amino acids during acid hydrolysis (Yeoh and Truong 1996; AOCS 1993). The ammonia nitrogen content was calculated by multiplying the ammonia content by 0.824 (N = 82.4% NH_3).

Amino acids calculation

The amount of amino acid obtained was calculated (g/100g) by the formula:

$$X = \frac{\text{Area of Asp in the sample X Area of internal std (AABA) X amount of std X dilution factor}}{\text{Area of Asp std X Area of sample internal std X sample weight}}$$

Where:

X = represents the amount of amino acid (g/100g)

Preparation of *Chrozophora oblongifolia* seeds protein concentrate

Protein was extracted from *C. oblongifolia* protein concentrate using alkali solution with isoelectric precipitation and freeze drying. The dried defatted seeds was weighed and suspended in distilled water in 1: 10 (w/v) ratio using magnetic stirrer, the mixture was stirred for 1 hour, while adjusting the pH at 9.0 using sodium hydroxide NaOH solutions (4M). Then, the mixture was centrifuged at 3500 rpm for 15 minutes at ambient temperature. The supernatant was transferred into a beaker and stirred for another 30 minutes and the pH was adjusted into 4.5. The supernatant was left undisturbed for cold precipitation overnight in 4°C freezer. After that, the supernatant was carefully siphoned off and the protein slurry was washed 3 times with distilled water by adding distilled water and centrifuging at 3500rpm for 10 minutes at 4°C. The pellets was then mixed together and some distilled water added in there. The pH was adjusted at 7.0. The slurry was kept overnight inside -80°C freezer before it was freeze dried (Chandi and Sogi, 2007). The sample inside the freeze dryer took 2 to 3 days before it was completely dried. The protein concentrates obtained from seeds were weighed using analytical balance.

Crude protein content

The crude protein content was determined in protein concentrate of *C. oblongifolia* plant seeds by macro-Kjeldahl method according to the official methods analysis (AOAC 2005).

Functional properties of protein concentrate

*Water absorption capacity***:** The water absorption capacity was determined according to method described by Jyothirmayi et al. *(*2006) as follows: Only 0.1 g of *C. oblongifolia* protein concentrate was taken from the sample mixed with 1 ml of distilled water. The slurry was centrifuged at 3000 rpm for 15 minutes. The supernatant was removed, then the pellets were drained for 30 minutes and the gain weight per unit weight was reported as water absorption capacity (g/g).

*Oil absorption capacity***:** One gram of *C. oblongifolia* protein concentrate was taken mixed with 10 ml of refined sunflower oil, vortex thoroughly, and centrifuged at 3000 rpm for 15 minutes. The oil absorbed by the samples was noted and expressed as oil absorption capacity (g/g) (Beuchat 1977).

*Foaming capacity and foam stability***:** Foaming capacity of *C. oblongifolia* protein concentrate was determined by measuring the volume of foam immediately after the introduction of air (90 cm^3/min) for 15 seconds into 5ml of 0.2% protein solution in 0.05M phosphate buffer (pH 7.4) (Kato 1989). Foam stability was calculated from the following equation:

$$FS = V0\ (\Delta t/\Delta V)$$

Where F: foaming, S: stability, ΔV the change in the volume of foam (V), occurring during the time interval, Δt (30 min), and V0 is the volume of foam at 0 time.

*Emulsifying capacity***:** For the determination of emulsifying capacity, 50 g of protein suspension were transferred into a blender vat and the sunflower oil was added, under continuous mixing until the emulsion was destroyed (Beuchat, 1977). Measurements were performed at 22 ± 1°C and the emulsifying capacity was expressed as ml of oil used for the emulsification of 1 g of *C. oblongifolia* protein derivatives.

*Bulk density***:** The bulk density was determined according to the methods outlined by Okaka and Potter (1977). Ten grams of protein isolate were put into 100ml measuring cylinder, then tapped several times on the laboratory bench till the isolate stopped settling, the values were expressed as g/cm^3.

RESULTS AND DISCUSSION

Amino acids profile

Most of the amino acids were found to be presence in *C. oblongifolia* seeds samples investigated except tryptophan (Table 1). A total of 17 amino acids consisting of eight essential namely lysine, histidine, threonine, methionine, valine, isoleucine, leucine, and phenylalanine and non-essential amino acids namely arginine, aspartic acid, serine, glutamic acid, glycine, proline, alanine, cystine and tyrosine were presented in Table 1, which

showed the highest value of essential amino acid were found in North Kordofan region seeds that of leucine, isoleucine and valine, while the highest value of essential amino acids of *C. oblongifolia* seeds in West Kordofan were found in that of leucine, isoleucine, valine and thrionine. On the other hands the lowest values of essential amino acids from both samples were histidine and methionine. Non-essential amino acids arginine , alanine and aspartic acid from *C. oblongifolia* seeds in North and West Kordofan were found in the highest value compared to the lowest values of serine, glycine, glutamic acid, tyrosine and cystine from both samples regions. The amino acid with the least concentration was glycine with a range of 0.99-1.02 g/100 g proteins, while arginine with a range of 8.43-8.87 g/100g protein was the highest (Table 1). The ranges of essential amino acids obtained from the different locations of *C. oblongifolia* seeds samples in present study were in agreements with *Chrozophora brocchiana* seeds amino acids reported by Ahmed (2014) and also was within the same range for reference pattern protein by FAO (1981) standards which was indicated for leucine and isoleucine. These were limiting amino acids in most feed stuffs that were presently ranging from 7.03g/100g protein for leucine and 4.19 g/100g protein for isoleucine in those mentioned in the FAO standards. The value of essential amino acids concentration obtained in this study were in line with Ahmed (2014) who showed that *C. oblongifolia* has had higher protein contents of essential amino acids such as, leucine, isoleucine and valine. The results of this study showed that the protein of *C. oblongifolia* seeds had higher quantity of amino acids such as arginine , alanine leucine, aspartic acid, isoleucine, and valine.

Table 1. Amino acids profile (g/100g crude protein) of *C. oplongifolia* seeds

Amino acid	Samples source	
	North Kordofan	West Kordofan
Essential		
Lysine	2.31	3.23
Histidine	1.80	1.16
Thereonine	3.16	3.87
Valine	4.13	5.33
Methionine	1.02	1.91
Isoluecine	5.24	5.56
Leucine	7.27	7.59
Phenylalanine	3.12	3.57
Non-essential		
Aspartic Acid	6.11	6.74
Alanine	8.10	7.23
Serine	1.46	1.03
Tyrosine	1.09	1.04
Glutamic Acid	1.51	1.75
Glycine	1.02	0.99
Ammonia	6.21	6.32
Arginine	8.43	8.87
Cystine	1.65	1.55
Proline	3.02	2.97

Protein content of the concentrates

Protein contents in those protein concentrates had a high amounts in both North and West Kordofan regions which were 83.33 and 80.66%, respectively (Table 2); the variations in protein contents of different protein isolates could possibly be due to extent of soluble proteins present in raw materials. The variations in protein contents are attributed to genetic makeup of the sources of proteins along with some environmental factors. The proteins known as polymers of amino acids and their relatives proportion represents its quality that is dependent on genetic makeup of sources of proteins such as (legumes). This result is similar to some legumes such as pea protein concentrate of 83.61% and pigeon pea of 82.92% which were reported by the above mentioned authors (Masood and Rizwana 2010).

Functional properties

Water and oil absorption capacity

Seeds protein concentrates obtained from North Kordofan seed exhibited water absorption capacity of 2.9 g/g, and West Kordofan seeds protein was absorbed 2.26 g/g (Table 2). However, these values were higher than 1.45 for peanut powder reported by Monteiro and Prakash (1994). On the otherhand, this value is in agreements with the 2.62 g/g in mung been protein concentrates revealed by Du et al. (2018). Therefore, the protein concentrates obtained from defatted *C. oblongifolia* seeds had low water absorption, but are still considered high in the range of water absorption capacity of glutinous food, while the oil absorption capacity of protein concentrate was 3.73 g/g for North Kordofan seed oil and 3.13 g/g for West Kordofan seeds oil. The low value of oil absorption may be attributed to the presence of large proportion of hydrophilic groups and polar amino acids on the surface of the protein molecules (Sathe et al. 1982) as we knew that protein has both hydrophilic and hydrophobic properties, thereby can interact with water and oil in food. The varied values of WAC might be due to the protein structure and amount of polar amino acids, whereas the OAC difference might be due to the difference in nonpolar side chains binding the oil (Yi-Shen 2018).

Emulsion absorption capacity

The emulsion absorption, for both samples revealed almost a similar absorption rate of 3.33 g/g for North Kordofan protein concentrates and 3.00 g/g for West Kordofan protein concentrates. Results are comparable to the earlier findings of Bugis (2009) who reported a value of 1.32 g/g emulsion absorption for lupine protein isolates. These findings are in line with Makri et al. (2005) who stated that protein being the surface active agents can form and stabilize the emulsion by creating electrostatic repulsion on oil droplet surface.

Table 2. Protein functional properties of *Chrozophora oblogifolia* seeds

Parameter	North Kordofan	West Kordofan
Protein content %	(83.33)a ±1.52	(80.66)b ±1.52
Water absorption capacity g/g	(2.90) a ±0.10	(2.26)b ±0.25
Oil absorption capacity g/g	(3.73)a ±0.25	(3.13)b ±0.15
Emulsion absorption g/g	(3.33)a ±0.25	(3.00)a ±0.10
Foaming capacity %	(21.66)a ±0.57	(20.00)a ±1.00
Foaming stability g/ml	(2.33)b ±1.52	(2.43)a ±1.52
Bulk density g/cm3	(0.73)a ±0.04	(0.56)b ±0.05

Note: *All Determinations were carried out in triplicate and mean value ± standard deviations (SD). Means not sharing superscript letters in a column were significant different

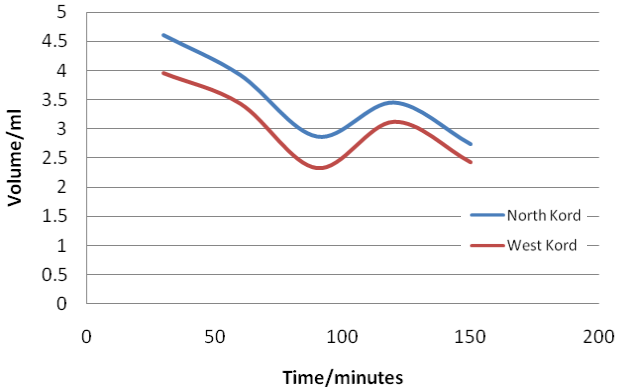

Figure 1. Foaming stability of protein concentrate of *Chrozophora oblongifolia* seeds

Foaming capacity and stability

Foaming capacity of protein concentrates of North Kordofan and West Kordofan seeds were 21.66% and 20%, respectively, where it reached a maximum at pH 9. The foaming capacity of both locations of protein concentrates was affected by pH and it tended to be decreased with pH decrease. The same trends were found in foaming stability. The foaming stability of North Kordofan protein was reported at a time of 30, 60, 90, 120 and 150 minutes were 4.60, 3.92, 3.43, 2.87, 2.33 g/ml, respectively, for the same mentioned times the West Kordofan protein was 3.95, 3.45, 3.12, 2.74 and 2.43g/ml, respectively (Figure 1). The foaming capacity of *C. oblongifolia* protein concentrates in present investigation was found to be lower value when compared with the results of the commercial protein for albumin studied by Moharram et al. (1984), while the foaming capacity declined due to several factors, including the source and composition of protein as well as the temperature and the solubility (Jasim 1983).

Bulk density

The bulk density of proteins concentrates from two different regions of North Kordofan and West Kordofan seeds were found to be 0.73 g/cm^3 for North Kordofan and 0.56 g/cm^3 for West Kordofan (Table 2). These results were in agreement with Masood and Rizwana (2010) who reported a value of 0.71 g/cm^3 for bulk density of legumes protein isolates.

In conclusion, the amino acid with the lowest concentration was glycine, while arginine had the highest value found in the two different production regions. The range of essential amino acids obtained in this study were within the same range for reference pattern protein required by FAO standards specially leucine and isoleucine which were limiting amino acid in most feedstuffs. This study investigated the nutritional value of *C. oblongifolia* seeds as a cheap source of edible protein which had a rich of essential amino acids. Characterization of the properties of protein concentrates obtained from *C. oblongifolia* seeds expected to improve the industrial application of crude protein substances. More studies to elaborate the use of this protein mixed with other food materials and their effects in consumer acceptability are needed in future work as recommended point of view.

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