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RESEARCH ARTICLE

CENTESIMAL COMPOSITION AND AMINO ACID PROFILING OF WOOD APPLE (*LIMONIA ACIDISSIMA*) SEED: AN UNDERUTILIZED PROTEIN SOURCE

L. Banupriya[*] and T. Poongodi Vijayakumar

Department of Food Science and Nutrition, Periyar University, Salem.

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****Corresponding Author***

L. Banupriya.

Abstract

Under-utilized crops can play a crucial role in food and livelihood security for the poor. These plants risk falling into disuse, yet they often play a crucial role in food security, income generation and the culture of the rural poor. Unfortunately the lack of attention has meant that their potential value is under-exploited, and they are in danger of continued genetic erosion, ultimately leading to disappearance. One such under-utilized fruit is Wood apple (*Limonia acidissima*) seeds, in which protein concentrate and protein isolate were prepared from seed flour. The chemical composition and functionality was analyzed by standard techniques. The highest amount of protein content was found in protein isolate (85.93%) as compared to wood apple seed flour (27.56%) and protein concentrate (76.50%). The WASF, WASPC and WASPI had good functional properties. The essential amino acids (histisine, threonine, valine, leucine, isoleucine and lysine) of the protein were at a higher level in flour, protein concentrate and protein isolates of wood apple seed than the Food and Agriculture Organization/World Health Organization reference pattern. The wood apple seed flour, protein concentrate and isolate were nutritionally comparable to other oilseed flour and can potentially be used in supplementary food formulations.

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Introduction:-

Fruits are undoubtedly very important for nutrition security with high potential of value addition and foreign exchange earnings. Fruits are now considered as an important item of commerce as they have gained enormous market potential. India accounts for 12.5% of the total world population of fruit crops and ranks second with the production of 75 million tons in 2013 (Amee Ravani and Joshi, 2014). Tropical fruits, which are at present underutilized, have an important role to play in satisfying the demand for nutritious, delicately flavoured and attractive natural foods of high therapeutic value. Today, consumers are becoming increasingly conscious of the health and nutritional aspects of their food basket. The tendency is to avoid chemicals and synthetic foods and preference for nutrition through natural resources. The underutilized fruits like aonla, bael, jamun, karonda, passion fruit, phalsa, pomegranate, pumpkin, tamarind, wood apple etc. are the main sources of livelihood for the poor and play an important role in overcoming the problem of malnutrition (Gajanana et al., 2010). Wood apple (*Limonia acidissima*) belongs to the family Rutaceae Swingle. It is also called as wood apple, elephant apple, monkey fruit, curd fruit and *Katha Bel* in India. The original home of wood apple is South India and Sri Lanka (Lande et al., 2010). The wood apple is native and common in dry plains of India and Ceylon. Wood apple is used in the preparation of chutneys and for making jelly and jam (Morton, 1987). Wood apple has got high medicinal value. Every part of the fruit i.e. pulp, seed and oil has got its medicinal property. The seed composition and fatty acid profile were reported as 28% protein and 34% oil (Ramakrishna et al., 1979).

Novel or un-convectional protein sources are continually developed. Due to increasing market demands on protein ingredients, novel proteins have been purified from various sources (El Nasri and El Tinay, 2007; Lamsal et al., 2007; Lokra et al., 2007). However, for a novel protein to be useful for food processing application, it should possess desirable functional and nutritional qualities (Tai-Hua Mu et al., 2009). Seeds are unconventional sources of nutrition which could also provide functional properties after being incorporated in food products. The insertion of seeds in the human diet will be determined on their physical characteristics on their composition. Hence in the present study, the wood apple seed flour, protein concentrate and protein isolate were studied for their centesimal (fat, protein, ash, carbohydrate and moisture), nutritional (fibre and amino acid analysis) and functional (WAC, OAC, emulsification capacity and foaming etc.) properties.

Materials and methods:-

Sample Collection:-

The ripe wood apple (*Limonia acidissima*) fruits were purchased from the daily market of Salem. The sample was botanically identified by the botanist at the taxonomy section, Department of Environmental Sciences, Periyar University, Salem, Tamilnadu. The outer hard shell of wood apple fruit was broken and the pulp was separated from the shell by scooping. The seeds were isolated from pulp and washed under running tap water. The wood apple seeds were dried in sun drying and grinded into flour (WASF) was packed in tight polythene bags for further analysis.

Preparation of Wood Apple Seed Protein Concentrate (WASPC):-

The wood apple seeds were defatted using El-Tinay et al., (1988) method. Sample was mixed with n-hexane (1:10 w/v) and stirred for 2 hrs at room temperature, and then the mixture was left overnight. In the next day, the mixture was filtered and the residue was collected and dried for 12 hrs at room temperature. The process was repeated two more times to extract the complete oil from the sample. After sieving (60 mesh), the final product WASPC was kept in the refrigerator for further analysis.

Preparation of Wood Apple Seed Protein Isolate (WASPI):-

Protein isolates are nowadays believed to have played a major role in the development of new class of formulated foods (Olaofe et al., 1998). Protein isolates have been developed from a variety of legumes among which are soy bean, peanut, canola, cashew nut, almonds, sesame, pinto and navy beans (Seyam et al., 1983). The protein isolate was extracted using the protocol of Cilia et al., (2009) with some modifications made by Jyoti and Sudesh, (2013). The total protein content in wood apple seed protein isolate was determined by Kjeldahl method (Sadasivam and Manickam, 2005).

Determination of the centesimal composition of WASF, WASPC and WASPI:-

Centesimal composition such as moisture, total ash, fat and protein contents of the wood apple seed, protein concentrate and protein isolate were assayed by standard methods of Association of the Official Analytical Chemists (AOAC, 1995), and the carbohydrate content was estimated by calculating the difference. The fibre for the samples was determined by the method of AOAC, (1990).

Determination of functional properties :-

Proteins in the form of isolates or concentrates are important ingredients in many food products, where they perform specific functions (Kinsella and Melachouris, 1976). The functional properties of WASF, WASPC and WASPI were studied as follows.

Bulk density (BD)

The bulk density was determined according to the method of Onuma-Okezie and Bello (1988). Ten grammes of the sample were placed in a graduated cylinder and gently packed by tapping the cylinder on the bench top (10 times), the volume of the sample was recorded. Bulk density was expressed as g/ml of the material.

Water absorption capacity (WAC)

WAC was determined by the method of Sosulki (1962). WAC is expressed as the amount of water absorbed by 100 g of samples on moisture free basis.

Fat absorption capacity (FAC)

FAC was determined according to the method of Sosulki et al., (1976) using 1g of samples and refined groundnut oil. The values are expressed as the amount of oil (milliliters) absorbed by 100 g sample on moisture free basis.

Foam measurements

FC and FS were determined according to the method of Lawhon et al., (1972). The samples (3 g) was dispersed in 100mL distilled water, adjusted to pH 7.0, transferred the contents to a waring blender and whipped for 5min at 10,000 rpm. The contents along with the foam were poured into a 250mL measuring cylinder; the foam volume was recorded after 30 mintues. FC is expressed as percentage increase in volume. After 30min the volume of foam was measured and expressed as FS.

Emulsification properties

Emulsification capacity (EC) was determined by the method of Beuchat et al., (1975). To the samples (1 g), 25 ml of distilled water were added and mixed in a blender at low speed. After complete dispersion, refined groundnut oil was added at a rate of 0.4 ml/s; blending was continued until phase separation was seen. EC was expressed as millilitres of oil emulsified per gram of material.

Dispersibility

The dispersibilities of wood apple seed flour, protein concentrate and isolate was measured by the method of Karuna et al., (1991). A 10 g sample was placed in a stopperd measuring cylinder and distilled water was added to reach a volume of 100 ml. The mixture was stirred vigorously and allowed to settle for 3 h; the volume of settled particles was subtracted from 100, and the difference was reported as percentage dispersibility.

Wettability

The wettability was estimated for samples according to the method of Regenstein and Regenstein, (1984). Two grams of the samples were weighed and transferred to beaker containing 80 ml distilled water and magnetic without stirring the water. The behaviour of the samples was observed on the water surface immediately after adding the sample. After 30 min observation, the material was stirred on the magnetic stirrer sufficiently fast to form a vortex which reached the bottom of the beaker. The stirring continued for one min and after which the grade describing wettability was recorded as excellent, good, and fair or poor according to the time and behaviour of the dispersion.

Determination of amino acid profile:-

The three samples (WASF, WASPC and WASPI) were hydrolysed by incubation in 6 N HCl at 110 ^{o}C for 24 h. The amino acid analysis was performed using an automated precolumn derivation with *O*- phthaldiadehyde (OPA) using high performance liquid chromatography (HPLC) analysis was carried out in an Agilent 1100 (Agilent Technologies, Palo Alto, CA, USA) assembly system after precolumn derivatization with *o*-phthaldialdehyde (OPA). The sample was injected onto a Zorbax 80 A C18 column (i.d., 4.6 ×180 mm, Agilent Technologies) at 40°C with detection at 338 nm. The mobile phase A was 7.35 mM/L sodiumacetate/ 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/m ethanol/acetonitrile (1:2:2, v/v/v). The amino acid composition was expressed as g of amino acid per 100 g of protein (Tidjani et al., 2010).

Statistical analysis:-

The average data (in triplicate) were tabulated, analyzed statistically and interpreted. The statistical analysis like mean, standard deviation, one way analysis of variance (ANOVA) were analyzed by the Statistical Package for the Social Sciences (SPSS) for windows, Version 17.0.

Result and discussion:-

Nutritional composition:-

The centesimal composition of seed flour, protein concentrate and protein isolate of *L. acidissima* seed is represented in Table1. It could be observed that there were significant ($p< 0.05$) differences among the studied wood apple seed flour, protein concentrate and protein isolate in their contents of crude protein, crude lipids, crude fiber, ash, moisture and carbohydrate. The highest amount of protein content was found in protein isolate (85.93%) as compared to wood apple seed flour (27.56%) and protein concentrate (76.50%). This could be due to the removal of fat content. Similar results were found in bambara protein isolate with protein content of 85.97% (Abdel Rahman et al., 2011). The fibre content was higher in WASF (8.2 g/100g) than WASPC (4.6g/100g) and WASPI (0.1g/100g). The ash content was greater in WASPC (4.5%) and lower in WASPI (0.4%) and WASF (3.8%). Narsing Rao et al., (2011) found similar results with *Feronia Limonia* L. seed. The protein content was found to be 33.79 and 77 g/100 g in wood apple seed meal and wood apple protein concentrate respectively. It was also shown that defatted *L. Acidissima* seed contained 49.51 % protein (Sachin et al., 2015). Similar results were also found in prickly pear seed flour and protein concentrate (Nassar, 2008) and apple, papaya and watermelon seed flours contained significantly ($p < 0.05$) higher levels of protein (30.11-33.79%) than other seed flours, Concerning lipids, orange, apricot and watermelon seed flours contained significantly ($p < 0.05$) higher levels (45.05-54.20%), which reflect the importance of such seeds for oil production (Samia et al., 2012). These results indicate that wood apple seeds can be included in food formulations as a source of protein.

Functional properties:-

Functional properties have been categorized as the non-nutritive characters that food constituents play in a food system. Functionality of flour is important in the preparation, processing, storage, quality, and sensory attributes of foods. Knowledge of functional property is critical for the development of new products and the improvement of existing ones (Sachin et al., 2015). The results of water and oil absorption capacity, bulk density, foaming, emulsifying properties, dispersibility and Wettability of the wood apple seed flour, protein concentrate and protein isolate are shown in Table2.

Water absorption capacity (WAC)

WAC was determined in *L. acidissima* seed seed flour, protein concentrate and protein isolate. It was found to be 2.28, 0.9 and 1.15 ml/g for WASF, WASPC and WASPI respectively. Higher water absorption values of proteins indicate the swelling ability and property of dissociation for exposing additional binding sites. Water absorption is influenced by several factors such as the number of hydration positions, physical environment, pH, solvent, presence of lipids and carbohydrates (Kinsella, 1982).

Oil absorption capacity (OAC)

The OAC was observed to be higher in protein isolate (1.46 ml/g) than the protein concentrate (1.06 ml/g) and seed flour (0.9 ml/g). This could result from higher protein content of the protein concentrate leading to a greater extent of hydrophobic interaction between protein and fat. Similar results were found in winged bean seed protein concentrate (Afe Dwiani et al., 2014). OAC of wood apple seed protein isolate was same as sesame protein isolate (1.5 ml/g) (Khalida et al., 2003) and was lower than that of chickpea protein isolate (1.7 ml/g) (Lopez et al., 1991), and carinata protein isolate (2.17 ml/g) (Pedroche et al., 2004). Results obtained in this study indicate that wood apple seed had good oil absorption capacity.

Foaming properties

The foaming capacity (FC) of a protein refers to the amount of interfacial area that can be created by the protein and foam stability (FS) refers to the ability of protein to stabilize against gravitational and mechanical stresses (Fennema, 1996). Foam formation and foam stability are a function of the type of protein, pH, processing methods, viscosity and surface tension (Fekria et al., 2012). The WASPI, because of its higher protein content, possessed good amount of foaming capacity (174 mL) with greater stability (169 mL after 30 min.) when compared with WASPC and WASF which showed only 138 mL and 85 mL foam volume and the stability was also poor (6 mL and 13 mL after 30min.). Similar results were found in wood apple seed protein concentrate with 58 mL of foaming capacity and 50 mL of foaming stability after 75 mins (Narsing Rao et al., 2011).

Emulsion capacity (EC)

The emulsion capacity of WASF was higher (53.6 ml/g) than the protein concentrate (30.3 ml/g) and protein isolate (36.6 ml/g). Defatted C. Lanatus and L. acidissima flour showed an emulsion activity 47.58 and 40.60 % respectively (Sachin et al., 2015). Kiosseoglou et al., (1999) reported that the emulsifying properties of seed proteins are related to the processing procedure and to the protein composition. Several other authors have reported that different factors may affect emulsifying properties, such as net charge, pH, interfacial tension and protein conformation (Hung and Zayas, 1991; Lawal et al., 2004). The capacity of proteins to enhance the formation of emulsion is important for many applications in cakes, coffee whiteners, and frozen desserts (Elkhalifa and Bernhardt, 2010).

Bulk density

The bulk density of WASF, WASPC and WASPI was low (0.37, 0.55 and 0.75 g/ml). It was reported that the low values of bulk densities make the flour suitable for high nutrient density formulation of foods (Shad et al., 2011). Similar results were found in sesame protein isolate and soy protein isolate with lower bulk density (0.16 and 0.21 g/ml) respectively (Kanu et al., 2007). Some reports have attributed to the fact that since protein isolate is rich in protein, it will have a low bulk density as there will be little amount of carbohydrate that increases the bulk density of food products (Krause et al., 2002).

Dispersibility

The dispersibility of a mixture in water indicates its reconstitutability (Kullarni et al., 1991). The dispersibility of wood apple seed is shown in Table 2. Highest dispersity was obtained at wood apple seed protein isolate (74.2%) while for protein concentrate at 65.9%. The lowest dispersibility was 41.7% obtained for wood apple seed flour. Similar results were found in Fenugreek proteins concentrate (Nazar et al., 2007). High dispersibility enhances other functional properties like emulsifying and foaming properties during processing for the manufacture of cookies (Kinsella, 1979).

Wettability

Wettability is an important property when protein powders are dispersed to produce aqueous beverages and batters (Zayas, 1997). Wettability of proteins is affected by surface polarity, topography, texture, area and by the size and microstructure of the protein particles (Hagerdal and Lofqvist, 1978). Wettability grade of wood apple seed protein isolate was excellent since it wet slightly when it comes in contact with water and after 30 min the wetted sample and the powder sunk to the bottom. The WASF and WASPC had good wettability property as 80% of sample got wet in 30mins. Stirring dispersed the sample. The findings obtained in this report agree with the observations of Hassan (1994) and Osman (2004) for watermelon protein isolate and chickpea flour, respectively.

Amino acid analysis:-

Nutritional qualities of proteins are based on their amino acid composition. The quality of proteins is very much determined by the amino acid composition, as amino acids are fundamental building blocks of protein. Table 3 shows the amino acid content of WASF, WASPC and WASPI respectively. As a reference, the FAO/WHO (2007) recommended mode of the essential amino acid for (2–5 years old) child and adult were also given. The essential amino acids (histisine, threonine, valine, leucine, isoleucine and lysine) of the protein were at a higher level in flour, protein concentrate and protein isolates of wood apple seed than the Food and Agriculture Organization/World Health Organization reference pattern (FAO, 2007). The WASPI has higher amount of alanine and arginine was higher in WASF (85g/100g) and WASPI (62.9 g/100g). High arginine content in the seeds is indicative of possession of therapeutic value (Jyothi lakshmi and Purnima Kaul, 2011). Similar results were found in *Citrullus collosynthis* and *Citrullus vulgaris*, with arginine values (66.10±0.57 mg/g) and (63.55±0.78) respectively (Ogundele et al., 2012). Arginine is an essential amino acid for children's growth (NRC, 1989; Olaofe et al., 2008). Thus wood apple seed showed good sources of essential and non essential amino acids and can be used to supplement food substances for proper growth of children and good health of adults.

Table 1: Proximate composition of wood apple seed flour, protein concentrate and protein isolate (g/100g)

Parameters (%)	**WASF**	**WASPC**	**WASPI**
Moisture	4.06 ± 0.05^{c}	1.7 ± 0.05^{b}	0.08 ± 0.00^{a}
Ash	3.83 ± 0.05^{b}	4.56 ± 0.05^{c}	0.40 ± 0.00^{a}
Protein	27.56 ± 0.05^{a}	76.50 ± 0.34^{b}	85.93 ± 0.11^{c}
Fat	33.43 ± 0.05^{c}	1.49 ± 0.00^{b}	0.01 ± 0.00^{a}
Carbohydrates	23.10 ± 0.00^{c}	11.10 ± 0.00^{b}	0.50 ± 0.00^{a}
Fibre	08.20 ± 0.08^{c}	4.69 ± 0.05^{b}	0.10 ± 0.00^{a}

Values are the average of three determinants. The alphabets following the values indicates LSD between groups at $p<0.05$.

Table 2: Functional properties of wood apple seed flour, protein concentrate and protein isolate

Functional properties	WASF	WASPC	WASPI
Water absorption capacity (ml/g)	2.28 ± 0.01^{c}	0.9 ± 0.01^{a}	1.15 ± 0.00^{b}
Fat absorption capacity (ml/g)	0.92 ± 0.00^{a}	1.06 ± 0.00^{b}	1.46 ± 0.00^{a}
Foaming capacity (mL)	85.00 ± 1.00^{a}	138.00 ± 0.34^{b}	174.00 ± 0.05^{c}
Foaming stability (mL)	72.00 ± 0.00^{a}	132.00 ± 0.05^{b}	169.33 ± 0.01^{c}
Emulsifying capacity (%)	53.66 ± 0.50^{c}	30.33 ± 0.00^{a}	36.67 ± 0.01^{b}
Bulk density (g/ml)	0.37 ± 0.00^{a}	0.55 ± 00^{b}	0.75 ± 0.00^{c}
Dispersibility (%)	41.70 ± 0.01^{a}	65.90 ± 0.02^{b}	74.23 ± 0.00^{c}

Values are the average of three determinants. The alphabets following the values indicates LSD between groups at $p<0.05$.

Table 3: Amino acid patterns of flour, protein concentrate and protein isolate of wood apple seed (g/100 g protein)

Amino acid	WASF	WASPC	WASPI	FAO/WHO/UNU*	
				Child	Adult
Essential amino acid					
Histidine	63	53	28.4	1.9	1.6
Threonine	17	13	14	3.4	0.9
Methionine	10	8	16.4		
Leucine	23	25	11.8	6.6	1.9
Isoleucine	14	12	15.4	2.8	1.3
Phenylalanine	12	10	13.5		
Lysine	14	9	9.5	5.8	1.6
Valine	12	11	15.6	3.5	1.7
Non essential amino acid					
Aspartic acid	43	37	18.6		
Glutamic acid	50	44	23.6		
Serine	22	19	16.3		
Glycine	12	15	15.7		
Alanine	15	30	96.5		
Argnine	85	32	62.9		
Tyrosine	10	12	17.2		

*FAO/WHO/UNU energy an d protein requirements (2007)

Conclusion:-

Wood apple (*Limonia acidissima*) seeds are high in protein and fat, on isolating protein, it can find application as a protein source in various food formulations. Protein and fat together accounts for 3/4th the weight of the seeds and is grouped under oilseed. The WASF, WASPC and WASPI contained good amount of nutritional and functional properties. The essential amino acid pattern of wood apple seed suggests the possible use as a supplementary source to most legumes and that of the essential amino acids are above the WHO/FAO/UNU (2007) requirements for humans. So it can be completely utilize to obtain unconventional protein source.

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