Preparation and physicochemical properties of protein concentrate and isolate produced from *Acacia tortilis* (Forssk.) Hayne ssp. *raddiana*

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Abstract The composition and physicochemical properties of defatted acacia flour (DFAF), acacia protein concentrate (APC) and acacia protein isolate (API) were evaluated. The results indicated that API had lower, ash and fat content, than DFAF and APC. Also, significant difference in protein content was noticed among DFAF, APC and API (37.5, 63.7 and 91.8%, respectively). Acacia protein concentrate and isolates were good sources of essential amino acids except cystine and methionine. The physicochemical and functional properties of acacia protein improved with the processing of acacia into protein concentrate and protein isolate. The results of scanning electron micrographs showed that DFAF had a compact structure; protein concentrate were, flaky, and porous type, and protein isolate had intact flakes morphology.

Keywords *Acacia tortilis* seeds · Protein concentrate · Protein isolate · Chemical composition · Functional properties

Introduction

The lack of available protein in developing and undeveloped countries continues to be of great concern. In fact, the limitation and the high cost of animal protein in developing countries have promoted the need to develop new, inexpensive sources of protein for human consumption. Recently, attention has been focused on alternative protein sources, especially from plants. In this regard, various studies have assessed the properties of new proteins from untapped seeds of a wide variety of plants. For instance, almond seeds (Yusuf 2003), kenaf seeds (Mariod et al. 2010), grape seeds (Zhou et al. 2011) and watermelon seeds (Wani et al. 2011) have been reported to be new, relatively inexpensive sources of proteins with good physicochemical properties.

The genus *Acacia* (family *Fabaceae*) embraces different species; many of which are used as medicines (Agrawal and Gupta 2013). The seeds of some other acacia species are used as food source for humans (Siddhwaju et al. 1996). In Egypt, acacia [*Acacia tortilis* (Forssk.) Hayne ssp. *raddiana*] is grown in the Sinai area and acacia seeds are used for animal feed (Embaby and Rayan 2016). Our previous study (Embaby and Rayan 2016) offers the scientific basis for use the *Acacia tortilis* seeds in human diets, based on their nutritional composition. Also, according to our results, *Acacia tortilis* seeds had a high level of protein (27.21%) and they can be included in foods as a source of protein. Hence, this study was designed to evaluate protein concentrates and isolates derived from acacia seeds as a potential source of protein that could be used in food processing. To the best of our knowledge no other such study exists that systematically investigates the properties of acacia seed protein. Thus, the aim of this research was to characterize the physicochemical,

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functional and nutritional properties of defatted seed flour, protein concentrates and isolates from acacia seeds. This study provides information for the potential applications of this protein source as an ingredient in foods.

Materials and methods

Dry and mature seeds of *Acacia tortilis* (Forssk.) Hayne ssp. *raddiana* were collected from the campus of Suez Canal University, Ismailia, Egypt. Whole seeds were ground using an electric mill. The seed flour was defatted with hexane (flour/hexane ratio was 1:10 w/v). The defatted flour was air dried and ground, then stored at -20°C for further use.

Preparation of protein concentrate

The protein concentrate of acacia seeds was prepared according to the method of Wolf (1970). Defatted seed flour was mixed with 95% aqueous alcohol (1:20, w/v) and stirred for 1 h at ambient temperature. The suspension was filtered and the residue was air dried and dispersed in de-ionized water (1:20, w/v). The pH of the dispersion was adjusted to 4.5 and stirred using a magnetic stirrer for 2 h. The slurry was then centrifuged and the precipitate was washed with de-ionized water, neutralized to pH 7.0, and then freeze-dried.

Preparation of protein isolate

The protein isolate from acacia seeds was prepared according to the method of Wolf (1970). The defatted seed flour was stirred for 2 h at room temperature with de-ionized water (1:20, w/v) adjusted to pH 11.0 (with 1 N NaOH). The slurry was centrifuged, and the precipitate was re-dissolved with de-ionized water adjusted to 11.0 with centrifugation again. The supernatants were mixed, and adjusted to pH 4.5 (with 1 N HCl), and then stirred for 2 h at room temperature and subsequently centrifuged. The precipitate was washed, re-dissolved in water, neutralized to pH 7, and then freeze-dried.

Proximate analysis

Moisture, ash, fat and protein contents were determined by using the standard Association of Official Analytical Chemists (AOAC) methods (2005) and carbohydrates were calculated by difference.

Amino acid analysis

Samples of defatted seed flour, protein concentrate and protein isolate were hydrolyzed with 5 mL of 6 M HCl at

110°C for 24 h. Samples were re-dissolved in sodium citrate buffer, filtered and then injected into a High-Performance Amino Acid Analyzer (Biochrom 20, Auto sampler version, Amer-sham Pharmacia Biotech., Sweden). The proportions of recovered amino acids were presented as grams per 100 g of protein and were compared with the FAO/WHO (1990) reference pattern.

Physicochemical and functional properties

Water absorption capacity was determined by the method of Rodriguez-Ambriz et al. (2005). Oil absorption capacity was determined by the method of Lin and Zayas (1987). Emulsifying activity and stability were determined according to the method of Pedroche et al. (2004). Foaming capacity and stability were determined by the method of Sze-Tao and Sathe (2000). Protein solubility was determined by the method of Rodriguez-Ambriz et al. (2005).

Morphological analysis

Scanning electron microscopy (SEM) of defatted seed flour, protein concentrates and protein isolates was carried out using a JSM-5800 LV microscope (JXA-840A ELECTRON PROBE MICROANALYZER, JEOL, TOKYO, JAPAN).

Statistical analysis

All determinations were carried out in triplicate and the results are presented as mean $\pm$ SD. The data were subjected to ANOVA accompanied with Duncan test using SPSS software (version 16.0 for Windows, SPSS Inc., Chicago) and the significance was set at $p < 0.05$.

Results and discussion

Proximate composition

Proximate analysis of defatted acacia flour (DFAF), acacia protein concentrate (APC) and acacia protein isolate (API) is presented in Table 1. DFAF, APC and API showed moisture 7.41, 4.81 and 3.99%, respectively, ash content of 6.34, 4.69 and 2.84%, respectively and fat content of 1.84, 0.47 and 0.42%, respectively, and API had the lowest levels for each of these constituent. Thus, acacia proteins were less susceptible to attack by microorganisms because of the lower moisture. Also, the low ash content were within the general range for some common edible seeds, making acacia proteins suitable for human consumption and animal feed. Acacia seeds have been reported as food source for human due to the high amount of protein

Table 1 Chemical composition of defatted *Acacia tortilis* flour (DFAF), *Acacia* protein concentrate (APC) and *Acacia* protein isolate (WPI)

Component (%)	DFAF	APC	WPI
Moisture	7.41 ± 0.05 ^a	4.81 ± 0.17 ^b	3.99 ± 0.32 ^c
Ash	6.34 ± 0.05 ^a	4.69 ± 0.06 ^b	2.84 ± 0.06 ^c
Fat	1.84 ± 0.00 ^a	0.47 ± 0.02 ^b	0.42 ± 0.42 ^c
Protein	37.5 ± 0.75 ^c	63.7 ± 0.74 ^b	91.8 ± 0.03 ^a
Carbohydrates	54.3 ± 0.74 ^a	31.1 ± 0.77 ^b	4.91 ± 0.03 ^c

Results represent the average of three determinations ± SD, values in the same row with different letters are significantly different ($p < 0.05$)

(Vijayakumari et al. 1994; Siddhwaju et al. 1996; Embaby and Rayan 2016). The low levels of fat found in acacia proteins was due to their association with the proteins as lipoprotein and these polar lipids have an important role in the flavour of the proteins (Sanchez-Vioque et al. 1999). Significant differences in protein content were noticed among DFAF, APC and API (37.5, 63.7 and 91.8%, respectively), with API exhibiting the highest protein content. These protein values were high and may enhance the use of acacia seed as a good protein source in food formulation and in meeting the growing protein need of the world as a substitute for animal protein. The protein

content of API was similar to that reported for other sources including field pea (90.8–94.7%), kidney bean (92.2%), mung bean (93.9%) and chickpea (89.9–94.4%) (Shevkani et al. 2015; Tang 2008; Kudre et al. 2013; Kaur and Singh 2007); this fulfilled the minimum requirement of protein (90%) for commercial protein isolates (Shevkani et al. 2015).

Carbohydrate content of DFAF, APC and API were significantly different ($p < 0.05$), with values of 54.3, 31.1 and 4.91%, respectively. In general, the differences in chemical composition for DFAF, APC and API may be attributed to the difference methods of protein extraction used. Thus, the extraction methods increased the levels of proteins, but decreased the levels of moisture, ash, fat and carbohydrates. The present results were in good agreement with those reported by Jain et al. (2015) for groundnut proteins and Mao and Hua (2012) for walnut protein concentrate and isolate.

Amino acid composition

The nutritional value of acacia protein was based on the amino acid composition. Table 2 lists amino acid composition for DFAF, APC and API of acacia seeds. Sulphur amino acids (cystine and methionine) were the most

Table 2 Amino acid composition of defatted *Acacia tortilis* flour (DFAF), *Acacia* protein concentrate (APC) and *Acacia* protein isolate (WPI)

Amino acid ^{a,b}	DFAF	APC	WPI	FAO/WHO reference ^c
Essential				
Cystine	0.82 ± 0.02 ^c	0.55 ± 0.02 ^b	0.63 ± 0.04 ^a	2.5 (1.7)
Methionine	0.00 ± 0.00 ^c	0.73 ± 0.05 ^a	0.39 ± 0.02 ^b	
Valine	4.34 ± 0.10 ^a	3.30 ± 0.04 ^c	3.88 ± 0.04 ^b	3.5 (1.3)
Isoleucine	1.62 ± 0.08 ^b	2.23 ± 0.04 ^a	2.33 ± 0.02 ^a	2.8 (1.3)
Leucine	4.85 ± 0.05 ^c	6.60 ± 0.03 ^b	6.98 ± 0.05 ^a	6.6 (1.9)
Tyrosine	3.53 ± 0.07 ^c	4.37 ± 0.06 ^b	5.05 ± 0.04 ^a	6.3 (1.9)
Phenylalanine	3.21 ± 0.04 ^c	3.66 ± 0.04 ^b	4.27 ± 0.05 ^a	
Histidine	2.94 ± 0.10 ^b	2.92 ± 0.03 ^b	3.52 ± 0.03 ^a	1.9 (1.6)
Lysine	3.72 ± 0.08 ^b	4.74 ± 0.08 ^a	4.65 ± 0.03 ^a	5.8 (1.6)
Therionine	2.38 ± 0.03 ^c	2.55 ± 0.05 ^b	2.69 ± 0.04 ^a	3.4 (0.9)
Nonessential				
Aspartic acid	4.59 ± 0.06 ^c	5.47 ± 0.05 ^b	7.76 ± 0.04 ^a	
Proline	20.2 ± 0.07 ^a	13.8 ± 0.04 ^b	6.20 ± 0.05 ^c	
Serine	3.18 ± 0.06 ^c	3.65 ± 0.04 ^b	3.89 ± 0.04 ^a	
Glutamic acid	7.75 ± 0.11 ^c	9.47 ± 0.04 ^b	9.70 ± 0.04 ^a	
Glycine	3.78 ± 0.10 ^c	4.38 ± 0.06 ^b	4.66 ± 0.05 ^a	
Alanine	3.48 ± 0.16 ^c	4.73 ± 0.08 ^b	5.42 ± 0.03 ^a	
Arginine	8.56 ± 0.12 ^c	9.12 ± 0.06 ^b	10.5 ± 0.04 ^a	

^aAll amino acid (AA) values are expressed as grams per 100 g of protein

^bValues are mean ± SD of three determination, Mean values with different letters in the same row are significantly different ($p < 0.05$)

^cNumbers in parentheses of FAO/WHO recommended pattern (1990) represent essential amino acid for adults and numbers outside the parentheses represent essential amino acid for pre-school child (2–5 years)

limiting amino acids in DFAF, APC and API. Methionine and cystine have been reported to be the limiting amino acids in pulses (Tiwari and Singh 2012), lentil and horse gram (Ghumman et al. 2016). Significant differences were noted in amino acid content among the different protein forms (DFAF, APC and API). The DFAF had higher levels of valine and histidine than those reported in FAO reference pattern (FAO/WHO 1990). Also, the level of leucine was close to that reported in the reference pattern. However, all other essential amino acids were lower than the reference levels, indicating that DFAF requires supplementation with complementary protein for adequate nutrition. On the other hand, both APC and API had higher levels of all essential amino acids than those of DFAF. These differences may be due to the chemical modification of some amino acids during the extraction process (i.e., alkaline and acid treatments) (Moure et al. 2006). Also, for APC and API the levels of valine, leucine and histidine were higher than those listed in the FAO pattern, but the levels of other essential amino acids were close to those in the reference pattern except for the sulphur amino acids. Conclusively, both APC and API had improved amino acid profiles and can meet the recommended requirements reported by FAO (FAO/WHO 1990) except for sulphur amino acids. Similar results were found by Wani et al. (2011) for amino acid analysis of watermelon seed protein concentrate and isolate.

Physicochemical and functional properties

Water absorption capacity (WAC)

As seen in Table 3, water absorption capacity (WAC) of DFAF, APC, and API was significantly different ($p < 0.05$). API exhibited the highest WAC of 7.660 mL H₂O/g compared with those in the APC (4.876 mL H₂O/g) and the DFAF (3.505 mL H₂O/g). In general, protein concentrate has poor water-holding capacity compared to protein isolates (Ogunwolu et al. 2009), and this was attributed to high ability of protein isolate to swell, dissociate and unfold exposing additional binding sites, however

the other non-protein components and the insufficient disruption of structure of the protein concentrates can impair this process (Jain et al. 2015). The WAC values for APC and API were higher than those reported by Lin and Zayas (1987) for commercial protein concentrate and isolate by Zhu et al. (2010) for commercial soy protein isolate and by Jain et al. (2015) for groundnut protein concentrate and isolate. On the other hand, WAC value obtained for DFAF is close to that obtained by Embaby and Rayan (2016) for acacia seed flour. The higher WAC and OAC of API make it a suitable ingredient for food products such as breads, cakes and muffins where hydration and shortening properties are desirable (Shevkani et al. 2015).

Oil absorption capacity (OAC)

The oil absorption capacity (OAC) of DFAF, APC and API was 1.286, 2.803 and 6.801 mL/g, respectively (Table 3). OAC for DFAF was lower than that of cashew nut powder (Ogunwolu et al. 2009) but comparable to that of acacia seed flour (Embaby and Rayan 2016). Also, the OAC of both APC and API was higher than that reported for soybean flour (Al-Kahtani and Abou-Arab 1993), walnut flour (Mao and Hua 2012) and groundnut flour (Jain et al. 2015). In fact, proteins have the ability to bind fat, thus can enhance the flavour retention, and improve the texture of the processed food (Kinsella et al. 1985). Moreover, high OAC of the protein isolate makes it a good ingredient for the cold meat industry where the protein can bridge fat and water in these products (Ogunwolu et al. 2009).

Emulsifying properties

DFAF had the highest level of emulsifying activity index (EAI) (59.60%), and that of APC (54.01%) was found to be somewhat higher than API (49.56%). On the other hand, the emulsion stability index (ESI) of API was the highest, and that of APC was higher than DFAF with significant differences (Table 3). Similarly, Kinsella et al. (1985) and Chove et al. (2001) found that the emulsifying capacity of protein decreased as protein concentration increased. The

Table 3 Physicochemical and functional properties of DFAF, APC and API

Property	DFAF	APC	API
Water absorption index (g/g)	3.505 ± 0.072 ^c	4.876 ± 0.044 ^b	7.660 ± 0.196 ^a
Oil absorption capacity (mL/g)	1.286 ± 0.081 ^c	2.803 ± 0.022 ^b	6.801 ± 0.114 ^a
Emulsifying activity index (%)	59.60 ± 0.40 ^a	54.01 ± 0.76 ^b	49.56 ± 0.44 ^c
Emulsifying stability (%)	68.21 ± 0.36 ^c	71.13 ± 0.29 ^b	78.95 ± 1.25 ^a
Foaming capacity (%)	8.33 ± 0.00 ^c	22.84 ± 2.09 ^b	39.58 ± 2.08 ^a
Foaming stability (%)	67.2 ± 0.60 ^c	71.1 ± 0.80 ^b	78.15 ± 0.35 ^a

Results are mean of three different determinations ± SD. Mean values of each row followed by different superscript letter are significantly different $p < 0.05$

results also agree with those reported for groundnut (Jain et al. 2015), and cashew nut protein concentrate and isolate (Ogunwolu et al. 2009). The protein adsorption is diffusion controlled at low protein concentration since it will spread over the surface before it can be adsorbed. On the other hand, the activation energy barrier did not allow protein migration to take place at high protein concentration (Sze-Tao and Sathe 2000), therefore the EAI decreased as protein concentration increased.

Foaming properties

Foaming capacity and stability are functional characteristics of protein that determine their uses in food systems such as whipped toppings, baked foods and ice-cream mixes where aeration and overrun is required (Shevkani et al. 2015). Table 3 shows the foaming capacity (FC) and foaming stability (FS) for DFAF, APC and API. The results indicate that API had a higher level of FC (39.58%) than APC (22.84%) and DFAF (8.33%). Like FC; FS of API (78.15%) was significantly higher than that of APC (71.1%) and DFAF (67.2%). Similar results were reported for cashew nut powder (Ogunwolu et al. 2009) and walnut flour (Mao and Hua 2012). The factors; including transportation, penetration and reorganization of the molecule at the air–water interface regulate the foam formation (Ogunwolu et al. 2009). Thus, proteins must be capable of migrating at the air–water interface, unfolding and rearranging at the interface, to have good foaming (Zhou et al. 2011). Also, by higher protein concentration, the viscosity and the formation of a multilayer protein film increased, therefore, foaming capacity and stability are enhanced (Mao and Hua 2012). Additionally, the higher charge on protein molecules may have weakened the hydrophobic interaction and increased protein solubility and flexibility, give it the possibility to spread more quickly on the air water interface, encapsulating air particles and thus increased foam formation (Lawal et al. 2005).

Protein solubility

The nitrogen solubility of DFAF, APC and API at different pH (from 2 to 12) is shown in Fig. 1. The minimum solubility of DFAF was 14.5% at pH 4, but the solubility was 37.4% at pH 2. Also, the solubility of DFAF increased from pH 6 to pH 12, and the maximum solubility (47.8%) was obtained at pH 12. Similar trends for solubility at different pH levels for APC were found (Fig. 1). The solubility was 39.2% at pH 2 which decreased to the minimum level (5.9%) at pH 4.0. The solubility thereafter increased from pH 6 to pH 12 to reach the maximum solubility of 64.1% at pH 12. Similarly, API exhibited high solubility at both acidic and basic pH and the minimum

Fig. 1 Effects of pH on solubility of defatted acacia flour (DFAF), acacia protein concentrate (APC), and acacia protein isolate (API)

solubility of 3.8% was observed at pH 4, but the maximum solubility of 72.1% was noticed at pH 12. The increases in protein solubility at higher pH values could be attributed to the increase in protein charges which favored the electrostatic repulsion between the molecules (Shevkani and Singh 2015).

Conclusively, the solubility of DFAF, APC, and API in water at different pH showed the same U-shaped pattern, which were similar to many profiles reported for other proteins such as peanut (Monteiro and Prakash 1994), cashew (Ogunwolu et al. 2009), baru nuts (Guimarães et al. 2012), walnut (Mao and Hua 2012) and kidney bean and field pea (Shevkani et al. 2015). Also, acacia protein had a minimum solubility at pH of 4.0, indicating that acacia protein was acidic in nature and the isoelectric point of acacia protein was in the range of pH 4.0–5.0. In fact, the majority of food proteins are acidic in nature therefore they have the minimum solubility at pH 4–5, and the solubility increases as the pH value increases (Damodaran 1997). Moreover, the minimum solubility occurs at the isoelectric point, and this is due to the net charge of peptides and the surface hydrophobicity (Sorgentini and Wagner 2002).

Morphological analysis

Scanning electron micrographs of DFAF, APC and API are illustrated in Fig. 2. The defatted acacia flour showed a compact structure of dense cells of starch granules surrounded by a protein matrix (Fig. 2a). The protein concentrate showed small flake-like particles with a porous type morphology (Fig. 2b). The protein isolate showed an intact flake-like structure (Fig. 2c). These microstructures are similar to those reported for acacia seed flour (Embaby and Rayan 2016), defatted, protein concentrate and protein isolate of groundnut (Jain et al. 2015) and defatted, protein concentrate and protein isolate of walnut (Mao and Hua

Fig. 2 Scanning electron microscope pictures ($\times 200$ magnifications, bar 100 μm) of DFAF (**a**) APC (**b**) and API (**c**)

2012). It was reported that the porous and flake-like structure of both protein concentrate and protein isolate contributes to improved digestibility and solubility of protein (Mao and Hua 2012; Jain et al. 2015).

Conclusion

The composition and structure of acacia protein concentrate (APC) and protein isolate (API) were different when compared with defatted acacia flour (DFAF). Furthermore, acacia protein isolates and concentrates could be good sources of essential amino acids except cystine and

methionine, and could be considered as a rich source of vegetable proteins similar to other seed proteins. The functional properties of acacia protein improved with the processing of acacia into protein concentrate and protein isolate. The solubility of DFAF, APC, and API in water at different pH values indicated that acacia proteins are acidic in nature and pH 4.0 was the isoelectric point for acacia proteins. The results therefore revealed that protein isolates and concentrates of suitable functional properties could be produced from the acacia as a good source of protein ingredient in food systems.

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