Food Hydrocolloids 28 (2012) 320–324

Contents lists available at SciVerse ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Chemical, amino acid and fatty acid composition of *Sterculia urens* L. seed

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ARTICLE INFO

Article history:
Received 2 March 2011
Accepted 3 January 2012

Keywords:
Chemical composition
Amino acid
Total lipid
Fatty acid
Sterculia urens seed

ABSTRACT

The chemical, amino acid and fatty acid compositions of *Sterculia urens* seeds are reported. The cotyledons were found to be rich in protein (30.88%) and lipids (39.2%). The major amino acids in defatted *Sterculia urens* cotyledon flour (DSCF) were determined as glutamic acid, arginine and aspartic acid. Cysteine, methionine, tyrosine and histidine were observed in minor quantities. The ratio of essential to non-essential amino acids was observed to be 0.45. Among the essential amino acids, isoleucine was found to be higher than the reported FAO/WHO requirements. The GC-FID and GC–MS analysis revealed that the major fatty acids of the total lipid were stearic acid (31.72%), linoleic acid (28.83%) and palmitic acid (26.79%). Eicosadienoic acid (4.98%) and eicosatrienoic acid (2.96%) were also found in the total lipid.

1. Introduction

Amino acid and fatty acid compositions of any food material are essential parameters to evaluate the quality of raw materials apart from their carbohydrate and mineral constituents. Nutritional value or quality of proteins is governed by their amino acid composition, ratio of essential amino acids to non-essential amino acids, susceptibility to hydrolysis during digestion, source, and the effect of processing (Radha, Ramesh, & Prakash, 2007; Schuster-Gajzago et al., 2006). Similarly, the lipid quality depends on nature of fatty acids present, their quantity, and interaction with other food components particularly proteins (James & Leonard, 2002). Studies on chemical, amino acid and fatty acid composition of common and uncommon sources such as soy, groundnut, jatropha have been reported (Atasie, Akinhanmi, & Ojiodu, 2009; Ebun-Oluwa & Aladesanmi, 2007; Harinder, George, & Becker, 2008; Shaileja, Sucheta, Balwinder, & Satish, 2008; Supradip, Arun, Mahajan, Kundu, & Gupta, 2008).

A 'complete protein' may be defined as a balanced combination of essential amino acids required for healthy living and consuming wide variety of plant foods help deliver all the required essential amino acids (Vijaya, 2001). Determination of amino acid profile is essential to ascertain the nutritional value with respect to the ratio of essential amino acids to non-essential amino acids. Phenylalanine content was reported in defatted groundnut flour and defatted sesame flour to be 960 mg/100 g and 770 mg/100 g respectively (Prabhavat et al., 1999). High glycine (400 mg/100 g) and leucine (1.2 g/100 g) contents were reported in millet by Kalinova and Moudry (2006). Amino acid composition of defatted pumpkin seeds was analyzed by Glew et al. (2006) who found that the seed flour possessed lower lysine and threonine contents as compared to the recommended standards for children (FAO/WHO, 1985). Blending of complementary protein sources to various foods to enhance their nutritive quality was reported to be a practical approach (Friedman, 1996). A weaning food formulation was developed by mixing cowpea, maize, peanut and soya bean to meet the dietary requirement of protein and minerals (Mensa, Phillips, & Eitenmiller, 2003).

Abundant literature is available on the fatty acid composition of plant seeds. Fatty acid composition of sesame seed was reported to contain 41–45% linoleic acid as the major unsaturated fatty acid (Tokusogulu, Unal, & Alakir, 2004). Quamachil (*Pithecellobium dulce* L.) seed was reported to possess 27.3% total lipid which contained major amounts of 18:1 (40.9%), 18:2 (26.5%) and 16:0 (19.4%) and palmitic acid was found to be higher in phospholipids than neutral and glycolipids (Prabhakara Rao, Narsing Rao, Jyothirmayi, Karuna, & Prasad, 2009). Lipid classes and fatty acid composition of Adavi chinta (*Entada pursaetha*) seed oil was reported by Prabhakara Rao, Narsing Rao, Jyothirmayi, Karuna, and Prasad (2010). They found that the palmitic acid (8.8%), oleic acid (37.2%), linoleic acid (43.9%) and eicosanoic acid (1%) were observed in the total lipid.

Sterculia urens is a medium sized tree belonging to the family of *Sterculiaceae*, which grows wildly and has several industrial applications. It is reported that the processed seeds (roasted/cooked) are eaten. The de-hulled seeds consist of 35% protein and 26% oil. The seed oil is reported to be edible and also used in soap manufacture (The Wealth of India, 1952; Yesodharan & Sujana, 2007). Chemical composition of *Sterculia urens* seed from different regions with varying protein content (11.5–30.8%) and oil (24–29%) has been

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doi:10.1016/j.foodhyd.2012.01.003

reported by Vishakha and Bhargava (1999). Chemical, mineral composition and protein solubility of defatted *Sterculia urens* seed flour was studied by Narsing Rao and Rao (2010) who found that the seed was a rich source of protein (20%) and crude fat (29%). Satyanarayana, Subhashini Devi, and Arundhati (2011) reported that *Sterculia urens* seed is a rich source of protein, lipid and carbohydrates.

Interestingly, even though the seed was rich in protein and oil, very little information is available on these aspects. Hence, the present investigation reports the amino acid and fatty acid composition of *Sterculia urens* seed.

2. Materials and methods

2.1. Material

Sterculia urens seeds were procured from M/s. Kovela Foundation, (Visakhapatnam, Andhra Pradesh, India). All chemicals and solvents used in the study were of analytical grade from Sd Fine-Chem Ltd (Mumbai, India). Standard fatty acid methyl esters (C 4–C 24) were procured from Sigma-Aldrich (St. Louis, USA).

2.2. Methods

2.2.1. Preparation of defatted Sterculia urens cotyledon flour

Sterculia urens seed cotyledons were separated manually and the ground material was defatted according to a reported method by Narsing Rao and Rao (2010) with minor modifications at 26 ± 2 °C room temperature (RT). Cotyledons were coarsely ground in a laboratory mixer/grinder (Sumeet, Nasik, India) and one part of the ground cotyledons were soaked in hexane at RT with occasional stirring for a period of 3 h, solvent was separated and extractions were repeated four times with fresh solvent. The residue was tray dried (Chemida, Mumbai, India) at 45 ± 2 °C, ground to pass through a 60 (240 μ) mesh sieve to obtain defatted *Sterculia urens* cotyledon flour (DSCF). The flour was packed in polyethylene pouches and stored at RT for determining amino acid composition. The other part of the ground cotyledons was used for the investigations of proximate composition and extraction of total lipid.

2.2.2. Proximate analysis

Moisture, total ash, crude fat (soxhlet extraction using hexane), crude protein and crude fibre of *Sterculia urens* cotyledons were determined by standard methods (AOAC, 1995; Pellett & Young, 1980).

The percent carbohydrate content was calculated by difference using the following equation

$$= 100 - (\text{sum of moisture, total ash, crude fat, crude protein and crude fibre contents})$$

The energy value was calculated by using following expression and reported as kcal/100 g sample.

$$= 9 \times \%\ \text{fat} + 4(\%\text{protein} + \%\text{carbohydrates})$$

2.2.3. Estimation of amino acid composition

DSCF equivalent to 5 mg protein was placed in 20 ml vacuum hydrolysis tube, to which 6 N HCl (10 ml) was added. The sample was allowed to hydrolyse in a laboratory oven (Dalal, Mumbai, India) at 110 ± 2 °C for 24 h. The hydrolysed sample was cooled and the volume was made up to 25 ml. The contents were filtered and an aliquot of 5 ml was evaporated under vacuum to dryness. The residue was mixed with 2.5 ml of loading buffer (pH 2.2) and the amino acid profile was determined using an automatic amino acid analyzer (Biochrom 30, Cambridge, England).

An aliquot of 20 μl was injected into automatic amino acid analyzer. All the amino acids were detected after post column derivatisation with Ninhydrin reagent. Elutes were spectrophotometrically monitored at 570 nm and the concentrations of the unknown samples were determined by comparing with standard peak areas (Agilent amino acid standard kit, California, USA).

Cysteine and methionine contents of DSCF were determined after converting them into cysteic acid and methionine sulphone respectively, by oxidizing the sample with performic acid (9:1 mixture of 80% formic acid and 30% hydrogen peroxide) for 18 h at RT following the method of Moore (1963). The excess of oxidizing reagent was removed by evaporation and the mass was hydrolysed with 6 N HCl. Later it was derivatised and eluted as described above. All analyses were conducted in duplicate and mean values were presented.

2.2.4. Extraction of total lipid and determination of fatty acid composition by Gas chromatography (GC), Gas chromatography–Mass spectrometry (GC–MS)

2.2.4.1. Extraction of total lipid. *Sterculia urens* cotyledons (100 g) were coarsely ground and extracted with a mixture of chloroform–methanol (2:1, v/v) at RT (Folch, Lees, & Stanley, 1957). A solid to solvent ratio of 1:3 was maintained and the extraction was repeated 4 times and the pooled extracts were distilled in a rotary vacuum evaporator at ≤ 50 °C to obtain the total lipid.

2.2.4.2. Estimation of free fatty acid (FFA) content. The total lipid was analysed for free fatty acid content (FFA) following a standard method (Ranganna, 1986). The total lipid (1 g) was dispersed in previously neutralized ethanol. The contents were heated on a hot plate for complete dispersion of lipid in solution and were titrated with 0.1 N KOH using phenolphthalein as indicator. The percent FFA content was calculated using following formula and expressed as percent oleic acid.

$$\frac{282.5^{a} \times \text{Titer volume (ml)} \times \text{Normality of KOH} \times 100}{1000 \times \text{weight of the lipid taken for estimation}}$$

[a]Equivalent of oleic acid

2.2.4.3. Estimation of peroxide value. The peroxide value of total lipid was estimated by a reported method (Ranganna, 1986). The lipid sample (2 g) was added to 25 ml solvent mixture (acetic acid:chloroform, 60:40, v/v). The contents were treated with 1 ml potassium iodide solution (4 g KI in 3 ml water) and kept at room temperature in dark for 10 min. The contents were mixed with 50 ml distilled water and titrated with 0.1 N sodium thiosulphate. The end point was noted using starch as indicator. The peroxide value was calculated and expressed as meq O_2/kg lipid.

2.2.4.4. Gas chromatography and Gas chromatography–mass spectrometry (GC, GC–MS). Fatty acid methyl esters (FAMEs) of the total lipid were prepared by refluxing with 2% sulphuric acid in methanol (Christie, 2003). The FAMEs were extracted into ethyl acetate and thoroughly washed with water to make them free of acid and dried over anhydrous sodium sulphate and the solvent was distilled off in a rotary flash evaporator. GC and GC–MS analyses were performed following a reported method (Prabhakara Rao, Jyothirmayi, Karuna, & Prasad, 2010). The FAMEs were dissolved in chloroform and their GC-FID analysis were performed with an Agilent 6850 series gas chromatograph equipped with an FID detector using a DB-225 capillary column (Agilent Technologies, Palo Alto, USA). The column temperature