



AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: <http://www.ajptr.com/>

Physico-Chemical, Mineral, Amino Acid Composition, Antioxidant Activity and Sorption Isotherm of *Sterculia foetida* L. Seed Protein Flour

Narsing Rao Galla ^{*1}

1. Council of Scientific and Industrial Research - Central Food Technological Research Institute, Resource Centre, Hyderabad, Habshiguda, Uppal Road, Hyderabad-500 007, Andhra Pradesh, India

ABSTRACT

Sterculia foetida L. seed protein flour (SSPF) was produced by dehulling, defatting, drying, grinding, passing through mesh sieve 240 μ and evaluated its physico- chemical characteristics. The antioxidant activity increases with increasing in concentration of the SSPF. The total polyphenol content was determined by the Folin-Ciocalteu reagent. Amino acid composition by using Biochrom automatic amino acid analyzer and they were detected after post column derivatization with Ninhydrin reagent. The antioxidant activity was determined by DPPH and ferric reducing power methods and storage stability by sorption isotherm method. SSPF was possessed higher contents of protein (40.10%), calcium (58 mg) and phosphorus (1138 mg/100 g). Glutamic acid, arginine, aspartic acid, leucine, lysine, valine and phenylalanine are the major amino acids. The ratio of essential to nonessential amino acids was 0.45. All essential amino acids were higher than the reported amounts for 70 kg person (100 ± 5 g SSPF) as per FAO/WHO/UNU requirements. The seed protein flour was rich in polyphenols, which showed considerable antioxidant activity. SDS-PAGE showed nine protein bands, in which five kDa proteins are matching with molecular marker. The antioxidant activity increases with increasing in concentration of the SSPF. The sorption isotherm studies indicated that the SSPF was non-hygroscopic in nature.

Keywords: Chemical composition, Mineral content, Amino acid composition, Antioxidant activity, *Sterculia foetida* seed protein flour, Sorption isotherm

*Corresponding Author Email: narsing72@sify.com

Received 18 November 2012, Accepted 29 November 2012

Please cite this article in press as: Galla NR., Physico-Chemical, Mineral, Amino Acid Composition, Antioxidant Activity and Sorption Isotherm of *Sterculia foetida* L. Seed Protein Flour. American Journal of PharmTech Research 2013.

INTRODUCTION

Sterculia foetida (Jangli badam/Guttapu badam) is belongs to the malvaceae family is widely distributed throughout tropics, and native to Asia and Sri Lanka. The trees are grown in gardens up to the height of 30 meters, and are known for their handsome foliage and attractive fruits. The tree yields edible seeds rich in fat (30 - 36%), and the seeds contain protein content to an extent of 11.4%¹. Phospholipids of *Sterculia foetida* seed, in which phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol are the major polar components². The de-oiled seed cake was rich in protein 28.89% reported by Vishakha and Bhargava³. The protein solubility studies were carried out by Narsing Rao and Rao⁴ they reported that the seed protein flour possesses higher (90% at pH 12) and lower (25% at pH 6) solubility.

Chemical, mineral and amino acid composition of conventional and unconventional sources such as pulse, oilseeds, milk, fruits and vegetables were reported in the literature. A complete protein and quality of protein depends on their amino acid composition particularly, the ratio of essential amino acids to non-essential amino acids and processing condition for production of proteins and their catabolism after consumption, which also provide the nutritional status of specific proteins. A variety of plant foods help in delivering a balanced combination of essential amino acids required for healthy living. Developed a product to meet a dietary requirement of protein and minerals by blending a suitable combination of cowpea, maize, peanut and soya bean and also improve the nutrition quality of product by blending various foods reported in the literature⁵⁻¹⁵. Interestingly, the *Sterculia foetida* seed lipid characterization, seed protein solubility studies are reported earlier, even though the seed was rich in protein, very little information is available on these aspects. Hence, the further investigation was under taken to determine the physico-chemical, mineral, polyphenol, antioxidant activity, amino acid composition, SDS-PAGE and sorption isotherm of *Sterculia foetida* seed protein flour.

MATERIALS AND METHODS

Materials

Sterculia foetida dry seeds (10 kg) were personally collected from the trees at Indian Institute of Chemical Technology (IICT) and Center for Cellular and Molecular Biology(CCMB) campus Hyderabad, India. Analytical grade chemicals and solvents used in the study were purchased from Sd Fine-Chem Ltd (Mumbai, India). 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and potassium ferricyanide were purchased from Sigma (St. Louis USA). Butylated hydroxytoluene (BHT), Ciocalteu phenol reagent and gallic acid were purchased from Loba Chemie, Mumbai, India.

Standard protein markers of myosin (205 kDa), phosphorylase B (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (29 kDa) and cytochrome C (12 kDa) for SDS-PAGE were purchased from Sigma Chemicals Co., St. Louis, USA.

Preparation of *Sterculia foetida* seed protein flour (SSPF)

Sterculia foetida seed cotyledons were manually separated from three layers of hull. The cotyledons were ground in a laboratory mixer (Sumeet, Nasik, India) and passed through sieve 30 mesh (500 μ). The cotyledon meal was soaked in hexane to remove fat at 26 ± 2 °C, room temperature (RT). The residue was tray dried (Chemida, Mumbai, India) at 45 ± 2 °C, ground to pass through a 60 (240 μ) mesh sieve and the resultant SSPF was packed in metallized polyester polyethylene laminated (MPE) pouches and stored at RT for further experiments. A part of whole seed was also ground in mixer and taken as *Sterculia foetida* seed meal (SSM).

Determination of physico-chemical characteristics

The bulk density of SSPF was measured by noting the volume occupied by 20 g sample in a 100 mL graduate cylinder and expressed as g/cc. Moisture, total ash and crude protein of SSPF were carried out using standard methods¹⁶. Protein content was estimated by using standard micro-Kjeldahl method and the protein conversion factor used is 6.25¹⁷. The nitrogen and protein, % were calculated using following equations.

$$\text{Nitrogen, \%} = \frac{14 \times \text{TV} \times \text{N of HCl} \times \text{Volume made up of digest}}{1000 \times \text{ATD} \times \text{WS}} \times 100$$

TV: Titre volume

N: Normality

WS: Weight of the sample taken for digestion.

ATD: Aliquot taken for distillation

$$\% \text{ Protein} = \% \text{ Nitrogen} \times 6.25 \text{ (Factor)}$$

Determination of mineral content

The mineral content in the SSPF was determined from the total ash of the sample and reported as mg/100 g. Total ash was prepared by igniting 5 g sample in silica dish and further combusting in a muffle furnace at 550 °C for 6 h. The ash was digested with 25 mL of 6 M hydrochloric acid on water bath for 30 min and made up to 50 mL in a standard flask with acid¹⁶. The above ash solution was taken for estimation of cadmium, chromium, copper, iron, lead magnesium, potassium and zinc by atomic absorption spectroscopy (Shimadzu AA 6701 F, Atomic Absorption Flame Emission Spectrophotometer, Shimadzu Ltd, Kyoto, Japan) equipped with

hollow cathode lamp. A 1000 µg/mL of standard metal solution (Shimadzu, Japan) was diluted successively to 2, 1, 0.5, 0.4, 0.2, 0.1 µg/mL and aspirated before analysis of test samples ¹⁸.

Determination of calcium content

The calcium content was estimated by titrimetric method ¹⁶. Ash solution (20 mL) contain calcium was precipitated as calcium oxalate by dispersing in equal amount of water followed by addition of ammonium oxalate, ammonia solution (1:4) and acetic acid (1:4). The contents were heated to boil and allowed to precipitate calcium oxalate for 12 h. The precipitate was dissolved in hot dilute H₂SO₄ and titrated with standard potassium permanganate and expressed as mg calcium/100g sample.

Determination of phosphorous content

Phosphorous content was analyzed by using Fiske-Subbarow reagent ¹⁹. Ash solution of 5 mL was treated with 5 mL of molybdic acid to form a phosphomolybdate complex and the complex is reduced with 2 mL aminonaphtholsulphonic acid (Fiske - Subbarow reagent) to the complex molybdenum blue. The contents were thoroughly mixed, and the volume was made up to 50 mL. The blank also was prepared similarly using water in place of the ash solution. Reaction was allowed to continue for 10 min at RT. Later the optical density of colour was read at 650 nm in UV-Visible spectrophotometer (Shimadzu, UV-160A model). The setting for the blank at 100% transmission was noted using a standard graph was drawn using potassium dihydrogen phosphate (0.1-0.6 mg/mL). The blue colour developed was read at 650nm in UV-Visible spectrophotometer (Shimadzu, UV-160A).

Determination of total polyphenol content

The total polyphenol content was determined by extracting 1 g of SSPF with 100 mL, 80% ethanol in water (v/v) and treating with Folin–Ciocalteu reagent (phenol reagent). A standard calibration curve was drawn by using pure gallic acid 19-76 µg/ mL and the optical density was recorded at 675 nm. The total polyphenol content was expressed as mg of gallic acid equivalents /100 g sample ²⁰. The total polyphenol content was calculated and expressed as mg of gallic acid / 100 g SSPF as follows:

$$\text{Polyphenols (mg / 100 g)} = \frac{\mu\text{g of polyphenols} \times \text{Total volume in the aliquot} \times \text{of solution (25 mL)}}{1000 \times \text{Weight of the sample}} \times 100$$

Determination of amino acids

The amino acid profile was determined by analysing the hydrolysed samples on Biochrom Automatic amino acid analyzer (Biochrom 30, England). The vacuum tubes were purged with

nitrogen to displace air. SSPF equivalent to 5 mg protein was placed in 20 mL vacuum hydrolysis tube, to which 6 N HCl (10 mL) was added. The tubes were evacuated and sealed under vacuum. The sample was allowed to hydrolyse in a Lab model oven (Dalal, Mumbai, India) at $110 \pm 2^\circ\text{C}$ for 24 h. The hydrolysed reaction mass was cooled, volume was made up to 25 mL and filtered. An aliquot of 5 mL was evaporated under vacuum to dryness and the residue was mixed with 2.5 mL of loading buffer (pH 2.2).

Aliquot (20 μL) was injected into automatic amino acid analyzer (Biochrom 30, England). All the amino acids were detected after post column derivatization 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, USA). Cysteine and methionine content in SSPF was determined according to the reported method 21 by converting 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 and followed by derivatization and elution as described above.

DETERMINATION OF ANTIOXIDANT ACTIVITY

Determination of DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The DPPH radical scavenging activity of the SSPF was measured according to the method reported by Theodore et al.²² with some modifications. Varying quantities of SSPF from 3-15 mg were taken in different test tubes and were dissolved in 1 mL of water. Methanolic solution, (4 mL) of DPPH (0.004% solution) was added to the test tubes and the contents were incubated at room temperature for 30 min. and the absorbance was read at 517 nm against a blank. The solution without SSPF was measured as control. The DPPH radical scavenging activity was compared with the activity of commercial antioxidant butylated hydroxytoluene (BHT) as a positive reference. The inhibition of the DPPH free radical was calculated using the following equation:

$$\text{I\%} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Determination of ferric reducing power

The ferric reducing power of the SSPF was measured by dispersing 3-15 mg in a mixture of 1 mL water, 2.5 mL of phosphate buffer in different test tubes. Potassium ferricyanide (2.5 mL) 1% solution was added and the contents of test tubes were incubated for 20 min at 50°C . After incubation 2.5 mL of 10% tri-chloroacetic acid was added to each test tube and centrifuged at 8000 rpm for 10 min. 2.5 mL of upper layer was mixed with 2.5 mL of distilled water and 0.5

mL of 0.1% ferric chloride and the absorbance was read at 700 nm. The reducing power activity was compared with the commercial antioxidant BHT as a positive reference ²².

Determination of peptides by polyacrylamide gel electrophoresis

SSM and SSPF were examined for relative molecular weights using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) ²³. Protein samples (0.5 g) were dispersed in 25 mL SDS (2 g/100 g), vortexed and centrifuged at 5000 rpm for 30 min. An aliquot (400 µl) of the supernatant was treated with 100 µL sample loading buffer containing 60 mmol Tris-HCl buffer (pH 6.8), 25 mL glycerol, 2 g SDS, 2.5 mL - mercaptoethanol and 0.1 mL bromophenol blue. The samples were heated in a water bath maintained at 95 °C for 5 min. SDS-PAGE was performed using stacking gel (4 g/100 g) and separating gel (10 g/100 g) polyacrylamide gels on Mini Slab Gel Electrophoresis Unit (Balaji Scientific Services, Chennai, India) with loading of 25 µL in each well. Electrophoresis was carried out at 10 mA and 25 °C. Protein bands were stained with 0.1 g Coomassie brilliant blue R-250, 20 mL acetic acid, 30 mL water and 50 mL water, and de-staining was carried out in a solution containing glacial acetic acid, methanol and water (2:3:5, v/v/v). Relative molecular masses were compared using a wide range MW marker (Sigma Chemicals Co., St. Louis, USA) consisting of myosin (205 kDa), phosphorylase B (97 kDa), Bovine serum albumin (66 kDa), ovalbumin (45 kDa) and carbonic anhydrase (29 kDa). Cytochrome C (12 kDa) (Sigma Chemicals Co.) was also mixed in the marker solution.

Determination of sorption isotherm

SSPF (5 g), each in glass petri plates were exposed to the different relative humidity (RH) conditions of 10, 30, 50, 70, 90 and 100% were maintained by using appropriate concentrations of sulphuric acid (39, 29.6, 23, 16.6, 7.8 and 0 M) to determine the equilibrium moisture content (EMC) and relative humidity ¹⁶. The sample was observed visually for change in colour, lump formation and mold growth during the study. The samples which were exposed to different RHs were weighed at regular intervals on electronic monopan balance (Shimadzu, Model Libror AGE 220, Asia pacific Singapore) till they reached a constant weight (no gain or loss). A graph was plotted between EMC and RH to obtain sorption isotherm of SSPF. The EMC content was calculated using following formula:

$$\text{Equilibrium moisture content, \%} = \frac{D - C}{D} \times 100$$

$$\text{Dry matter of the sample C} = \frac{A \times 100 - B}{100}$$

Weight of sample taken for experiment (A)

Initial moisture content (IMC) of sample (B)

Weight of sample after equilibration (D)

Statistical analysis

The results were expressed as mean \pm SD (standard deviation), which was carried out at RT in three replications. The values were computed using MS Excel, 2007 and presented. In duplicate analyses was performed for the amino acid composition and average values were presented. Antioxidant activity was determine in three replications and expressed as mean \pm SD. Sorption isotherm studies were done in triplicate and mean \pm SD with error bars presented.

RESULTS & DISCUSSION

Physico-chemical composition

The various unit operations involved in the production of *Sterculia foetida* seed protein flour (SSPF) is presented as flow chart in Figure 1.

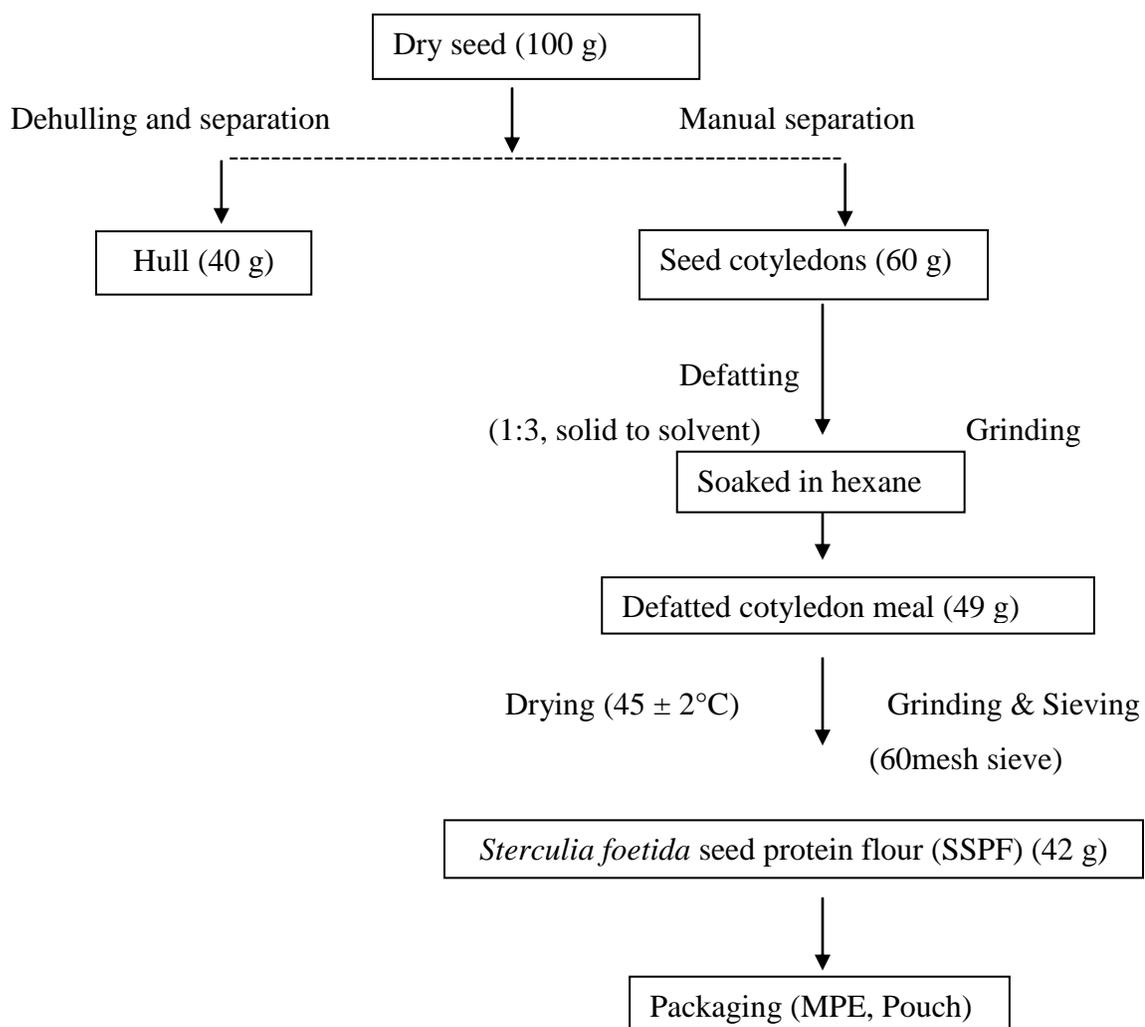


Figure 1: Flow chart for the preparation of *Sterculia foetida* seed protein flour (values in parenthesis are actual yields)

Sterculia foetida seeds yielded 60% cotyledons after processing obtained 42 g of SSPF (Table 1). Various stages of material during seed processing like whole seed, de-skinned, de-hulled seed, cotyledons, SSPF and fruiting branch of plant are presented (Figure 2a: fruiting branch and 2b: seed material during processing). The bulk density showed the seed protein flour is 0.32. Bulk density measurements will help the processors in the industry for packaging and dispensing units to determine the size and shape of the container/columns/reactors or for selection of films for package. SSPF was rich in protein (40.10%) and mineral matter (7.88%).

Table 1: Physico-chemical, mineral and total polyphenol content^a of SSPF

Parameters	SSPF	Reported values for other seed proteins
% Yield	42.00 ± 0.45	
Bulk density g/cc	0.32 ± 0.01	
Moisture %	8.80 ± 0.35	
Total ash, %	7.88 ± 0.33	
Protein, % (N x 6.25)	40.10 ± 0.74	
Minerals (mg /100 g sample)		
Calcium	54.69 ± 0.48	56.0 (<i>Cicer arietinum</i>) ³⁶
Copper	0.27 ± 0.21	
Iron	4.15 ± 0.53	4.4 (<i>Vigna radiata</i>) ³⁶
Magnesium	1.11 ± 0.24	
Phosphorus	1138 ± 0.56	670.0 (<i>Arachis hypogaea</i>) ³⁶
Potassium	8.14 ± 0.80	
Zinc	2.56 ± 0.39	
Total polyphenol (mg /100 g sample)	228.00 ± 0.92	400.0 (<i>Phaseolus vulgaris</i>) ³⁷

^aValues are means of triplicate analyses with SD standard deviation; SSPF: *Sterculia foetida* seed protein flour. %, Yield: Yield of seed protein flours on whole seed basis



Figure 2: (a) Photograph depicting the fruiting branch of *Sterculia foetida* and (b) various samples during seed processing (i) whole seed; (ii) de-skinned seed (iii) de-hulled seed; (iv) seed cotyledons (v) *Sterculia foetida* seed protein flour

The protein (40.10%) and mineral matter (7.88%) of SSPF are comparable with the reported values for seed protein flours of *Sterculia foetida* (protein content 40%), *Sterculia urens* (40%) and *Glycine max* (55%)^{3, 5, 7, 24}.

The preliminary data obtained on the composition is encouraging and substantiates the need to carry out further research on mineral, amino acid, SDS-PAGE and storage stability through sorption isotherm studies.

Mineral and total polyphenol contents

SSPF mineral content is presented in Table 1. The ash content was (7.88%) very high, which indicated that these flours are good source of mineral matter. It was further confirmed that seed protein flour was found to be considerably rich in minerals such as Ca (54 mg/100 g), Fe (4.15 mg/100 g), K (8.14 mg/100 g) and P (1138 mg/100 g). Toxic minerals like Cd, Cr and Pb were not found in SSPF. Phosphorous content of watermelon (1279), pumpkin (1090) and paprika (989 mg/100 g) seed meal was reported by Tarek and Khaled²⁵. Gopalan et al.²⁶ also reported the similar Ca, Fe and P contents for cereal grains. In general, wheat flour used in the baking industry is deficient in some minerals like Ca and Fe and is a poor source of proteins and minerals²⁷. Hence, fortification of wheat flour with SSPF could improve their nutritional quality, and as a protein supplementation in cereal based foods.

The total polyphenol (228 mg /100 g) content was observed in SSPF (Table 1). A higher polyphenols reported in *Aegle marmelos* (745 mg/100 g), *Feronia limonia* (358 mg/100 g) and *Sterculia urens* (316 mg/ 100 g) seed protein flours 7, 28, 29. A very high polyphenol content of 4200 mg/100 g was reported in sunflower seed meal. Sesame seed and groundnut contained 89 and 95 mg of polyphenol per 100 g. The total polyphenol content of mustard seed protein flour was reported to be in the range of 370 - 460 mg/100 g. The polyphenols are responsible for antioxidant activity was reported by Amarowicz et al.³⁰ from the solvent extract of legume seeds.

Amino acid composition

The total amino acids accounted for 85.17 g/ 100 g protein is presented (Table 2.) Higher amounts of glutamic acid (17.69 g/100 g), arginine, aspartic acid, arginine lysine and valine were found in SSPF. In the present study significant quantities of leucine (5.30 g/100 g) and isoleucine (3.75 g/100 g) were observed in SSPF. The ratio of essential to non essential amino acids in SSPF was found to be 0.45. The total amino acids accounted for 85.17 g/ 100 g protein and the rest of the nitrogen could be from tryptophan which was not determined in this method and non-protein nitrogen such as alkaloids, ammonia, purines, pyrimidines, vitamins and amino sugars.

The higher essential amino acid content (26.633 g/ 100 g) indicates the superior protein quality of SSPF.

Table 2: Amino acid composition of SSPF (g /100 g protein) and related references

Amino Acid	SSPF	SF ²⁶	SPI ³²	PM ²⁶	g/day for 70 kg adult) ³³
Alanine	4.34	NA	3.83	NA	
Arginine	9.13	7.2	7.57	4.8	
Aspartic acid ^b	8.56	NA	11.81	NA	
Cysteine	1.26	1.6	0.06	2.24	
Glutamic acid ^c	17.69	NA	21.29	NA	
Glycine	3.88	NA	3.86	NA	
Histidine	2.00	2.4	2.9	2.08	
Isoleucine	3.75	5.12	4.48	6.4	1.40
Leucine	5.30	7.68	7.00	11.04	2.73
Lysine	5.40	6.4	5.39	3.52	2.10
Methionine	1.54	1.28	0.93	3.36	1.05 (includes cysteine)
Phenylalanine	4.03	4.8	5.30	4.96	1.75 (includes tyrosine)
Proline	4.82	NA	5.29	NA	
Serine	4.62	NA	5.48	NA	
Threonine	2.86	3.84	4.10	3.84	1.05
Tyrosine	2.24	3.36	3.71	3.52	
Valine	3.75	5.12	4.41	7.68	1.82

^aValues are means of duplicate analyses, SSPF: *Sterculia foetida* seed protein flour, ^bAspartate + Asparagine ^cGlutamate + Glutamine, SF: Soy flour, SP: Soy protein isolate, M: Pearl millets, NA: Not available. Tryptophan and hydroxyproline were not determined

Higher amounts of glutamic acid (17.69 g/100 g), arginine, aspartic acid, arginine lysine and valine were found in SSPF. Similar, in content of glutamic acid (17.9 g) was reported in bael seed protein powder²⁸. The ratio of essential to non-essential amino acids in SSPF was found to be 0.45, when comparable to the reported value (0.42) for defatted sesame seed meal and 0.46 for groundnut meal³¹. The mustard seed proteins were well-balanced in amino acid composition was also reported in the literature¹⁰. Mustard was observed to be rich in glutamic and aspartic acids similar to other vegetable proteins. They reported that sulphur containing amino acids and lysine were high in sesame, when compared to vegetable proteins.

A product with balanced essential amino acid profile by mixing a suitable combination of soy, sesame and peanut flours in 1.1:1.7:0.7 ratios were reported in the literature¹¹. In our study, SSPF was observed to contain essential amino acids leucine (5.30 g) and lysine (5.40 g) followed by phenylalanine (4.03 g) and valine (3.75 g), when compared to soy protein isolate (SPI) and casein wherein leucine (7.0 and 8.4 g), lysine (5.39 and 7.1 g) and phenyl alanine (5.3 and 4.5 g) were reported by Tang, et al.³². The proportions of the essential amino acids available in the protein were very much higher when comparable to those of the required amino acids as per

FAO/WHO/UNU³³. Hence the seed protein can be termed as a balanced protein due to considerable quantities of required essential amino acids per 100 g protein.

The higher essential amino acid content (26.633 g/ 100 g) indicates the superior protein quality of SSPF. The cysteine content (1.2g) found in SSPF was comparable to soy flour (1.6 g) reported²⁶. In this study it was found that 100 g SSPF protein provides good quantities of leucine (5300 mg) and isoleucine (3750 mg). The recommended values of essential amino acids (mg/ kg body weight per day) for adult humans are isoleucine (20), leucine (39), lysine (30), methionine and cysteine (15), phenylalanine and tyrosine (25), threonine (15), tryptophan (4) and valine (26)³³. A 70 kg/ day adult required 100 ± 5 g of SSPF to meet the daily required essential amino acids. Amino acid composition revealed that there is a scope to prepare protein concentrates, isolates and hydrolysates from SSPF.

Antioxidant activity

The results of DPPH radical scavenging activity in terms of inhibition percent and ferric reducing power optical density are presented in Table 3. DPPH radical scavenging activity was found to be 28.42% inhibition for 3 mg and with 15 mg it was 68.49%. BHT showed 89% inhibition at 0.2 mg level and 95% at 0.4 mg. The reducing power was found to an optical density of 0.081 was read for 3 mg and for 15 mg it was 0.299. The activity measured was much lower, when compared with synthetic BHT, which gave an optical density of 0.42/0.05 mg.

The antioxidant activity of SSPF is supported by SDS-PAGE profiles and total polyphenols (294 mg /100 g). The lower molecular weight peptides (10, 12, 20 and 29 kDa) may be responsible for antioxidant activity in SSPF. The free amino acids, type of proteins, nature of peptides present in a sample and method opted for preparations of particular proteins are also influence the antioxidant activity³⁴. The polyphenols are responsible for antioxidant activity was reported by Saxena et al.³⁵.

Table 3: Antioxidant activity of *Sterculia foetida* seed protein flour^a

Weight of the sample (mg)	DPPH (Inhibition, %)	Ferric reducing power (OD)
3	28.42 ± 1.16	0.081 ± 0.012
6	33.78 ± 1.46	0.107 ± 0.006
9	44.86 ± 1.50	0.161 ± 0.014
12	55.13 ± 1.20	0.267 ± 0.009
15	68.49 ± 0.81	0.299 ± 0.010

^aValues are means of triplicate analyses and expressed on samples basis with ± SD, OD: Optical density

SDS-PAGE profile

SDS-PAGE profile of SDS soluble SSM and SSPF was presented in Figure. 3. In total, nine

protein bands were observed, whereas five of them were matching with the molecular marker, which were found to be 12, 29, 45, 66 and 97 kDa in SSPF. Apart from these, other protein bands were also noticed below 12, 29, 45, 66 and 97 kDa and absent of 97 and 205 kDa was noticed in SSM. The SDS-PAGE profile indicated that the lower and higher molecular protein bands were present in the *Sterculia foetida* seed. SDS-PAGE profile showed that lower and higher molecular peptides are present in of SDS soluble SSM and SSPF Similar protein band trend was observed in aegle and hemp seed protein powders^{28, 31} and concluded that defatting process might have some effect on appearance of protein bands.

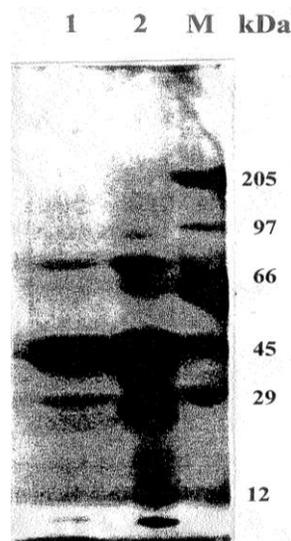


Figure 3: SDS-PAGE Pattern of *Sterculia foetida* seed protein (1) SDS soluble SSPF (2) SDS soluble SSM M: Broad band standard molecular weight protein markers (myosin 205 kDa, phosphorylase B 97 kDa, bovine serum albumin 66 kDa, ovalbumin 45 kDa, carbonic anhydrase 29 kDa and cytochrome C 12 kDa)

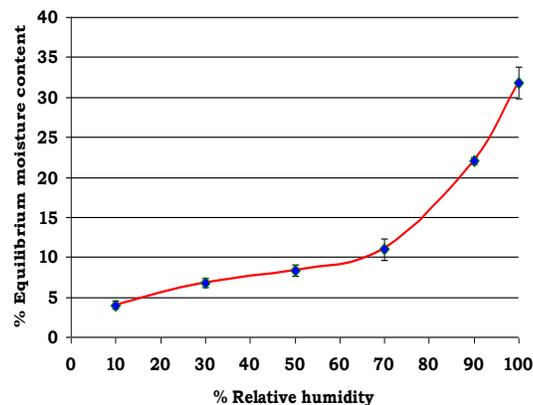


Figure 4: Experimental sorption isotherm of SSPF, mean of triplicate analyses are presented and error bars show standard deviation

The sorption isotherm of SSPF was presented in Figure. 4. SSPF had an initial moisture content (IMC) of 8.80%, which equilibrated at 62% RH at RT. The critical moisture content (CMC) was noted from the isotherms in the zone where there was sudden rise in the moisture content for a small change in RH. The CMC was 11.14%, which equilibrated at 71% RH. The SSPF gained moisture and discoloration was observed above 70% RH.

Sorption isotherm

The sorption isotherm of SSPF was presented in Figure 4. SSPF had an initial moisture content (IMC) of 8.80%, which equilibrated at 62% RH at RT. The critical moisture content (CMC) was noted from the isotherms in the zone where there was sudden rise in the moisture content for a small change in RH. The CMC was 11.14%, which equilibrated at 71% RH. The SSPF gained moisture and discoloration was observed above 70% RH.

The water activity of the SSPF was calculated to be 0.71 using sorption isotherm data. The water activity data is very much useful in the preparation of food formulations. If the water activity value is less than 0.4, the product will pick up moisture rapidly and lead to mold growth. Similar results were also observed in *Sterculia urens*, *Aegle marmelose* and *Feronia limonia* seed protein flours reported to possess CMC 9.2, 8.76 and 9.9%, which equilibrated at 72, 68 and 69% RH 7, 28, 29 The EMC - RH study showed that the SSPF was non-hygroscopic in nature and can be packed in polyethylene pouches for storage at RT.

CONCLUSIONS

Sterculia foetida seed protein flour preparation is easy and economical, which is rich source of protein, minerals like calcium, potassium, iron and phosphorous and considerable quantities of polyphenols. SSPF is rich in essential amino acids, which is comparable to conventional seeds and FAO/WHO/UNU standards. enhance, SSPF could be a good source of protein powder for supplement/blending with seed meals in food formulations to enhance the nutritional value and also to get an optimum amino acid balance. The SSPF is valuable protein and antioxidant source for human food and animal feed. The seed is rich in total lipid as literature report, which also further can be studied on utilization in bio-diesel production or soap manufacture. The knowledge derived on chemical composition and antioxidant activity, would be helpful in the preparation of protein isolates/ concentrates and hydrolysates.

ACKNOWLEDGEMENTS

The author thanks Central Food Technological Research Institute, Mysore, India for providing facilities to carry out work. I also thank National Institute of Nutrition, Hyderabad, India for

providing amino acid composition.

REFERENCES

1. The Wealth of India. Raw Materials Council of Scientific and Industrial Research. New Delhi, India, 1976: 43-48.
2. Prasad RBN, Nagender RY, Venkob RS. Phospholipids of palash (*Butea monosperma*), papaya (*Carica papaya*), jangli badam (*Sterculia foetida*), coriander (*Coriandrum sativum*) and carrot (*Daucus carota*) seeds. J Ami Oil Chem Soc 1987; 64: 1424-1427.
3. Vishakha K, Bhargava A. Studies on the nutritional composition of *Sterculia* species. J Food Sci Technol 1999; 38 (6): 542-544.
4. Narsing Rao G, Rao DG. Physico-chemical and functional properties of defatted *Sterculia foetida* seed flour. J Food Sci Technol 2009; 46(3): 225-227.
5. Obulesu M, Bhagya S. Biochemical, functional and nutritional characteristics of soy protein concentrate prepared by thermal processing. J Food Sci Technol 2006; 43: 161-166.
6. Tokusogulu O, Unal MK, Alakir A. Proximate chemical composition, amino acid and fatty acid profiles of sesame seed flours. J Food Sci Technol 2004; 41: 409-412.
7. Narsing Rao G, Rao DG. Chemical and functional characterization of *Sterculia urens* (*Sterculia urens* L.) seed meal. Food Hydrocolloid 2010; 24: 479-485.
8. Narsing Rao G, Rao DG, Jyothirmayi T. Protein solubility and functional properties of Quamachil (*Pithecellobium dulce* L.) seed flour. J Food Sci Technol 2008; 45(6): 480-483.
9. Harinder PSM, George F, Becker K. Protein concentrate from *Jatropha curcas* screw-pressed seed cake and toxic and antinutritional factors in protein concentrate. J Sci Food Agric 2008; 88: 1542-1548.
10. Schuster-Gajzago I, Kiszter AK, Toth-Markus M, Barath A, Markus-Bednarik Z, Czukor B. The effect of radio frequency heat treatment on nutritional and colloid-chemical properties of different white mustard (*Sinapis alba* L) varieties. Innovat Food Sci Emerg Tech 2006; 7: 74-79.
11. Radha C, Ramesh P, Prakash V. Preparation and characterisation of protein hydrolysate from an oil seed flour mixture. Food Chem 2007; 106: 1166-1174.
12. Mensa W, Phillips RD, Eitenmiller, RR. Formulation and evaluation of cereals/legumes based weaning food supplements. Plant Foods Human Nutr 2003; 58: 1-14.

13. Mapekula M, Chimonyo M, Mapiye C, Dzama K. Fatty acid, amino acid and mineral composition of milk from Nguni and local crossbred cows in South Africa. *J Food Comp Anal* 2011; 24: 529-536.
14. Chew LY, Nagendra Prasad K, Amin I, Azrina A, Lau CY. Nutritional composition and antioxidant properties of *Canarium odontophyllum* Miq. (dabai) fruits. *J Food Comp Anal* 2011; 24: 670-677.
15. Halinski LP, Paszkiewicz M, Golebiowski M, Stepnowski P. The chemical composition of cuticular waxes from leaves of the gboma eggplant (*Solanum macrocarpon* L.) *J Food Comp Anal* 2012; 24: 670-677.
16. Ranganna S. *Hand Book of Analysis and Quality Control for Fruits and Vegetable Products*. 2nd ed. Tata McGraw-Hill Publishing Company Ltd., New Delhi, India 1986.
17. Pellett LP, Young VR. *Nutritional evaluation of protein foods*. University Publication. UN 1980.
18. AOAC. *Official methods of analysis of Association of Official Analytical Chemists International* 16th ed., Washington, D.C., U.S.A 1995.
19. Raghuramulu N, Madhavan NK, Kalyanasundaram S. *A Manual of Laboratory Techniques*. National Institute of Nutrition, Hyderabad, India, 2003; Pp. 181-182.
20. Sadasivam S, Manickam A. *Biochemical Methods*, 2nd ed. New age international publishers. New Delhi, India, 1997; Pp. 193-194.
21. Moore S. On the determination of cystine as cysteic acid. *J. Biolo. Chem.* 1963; 238: 235-237.
22. Theodore AE, Sivakumar R, Kristinsson HG. Antioxidative activity of protein hydrolysates prepared from alkaline-aided channel catfish protein isolates. *J Agricul Food Chem* 2008; 56(16): 7459-7466.
23. Laemmli UK. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* 1970; 227: 680-685.
24. Narsing Rao G, Rao DG. Physico-chemical and functional properties of defatted *Sterculia foetida* seed flour. *J. Food Sci. Technol.* 2009; 46(3): 225-227.
25. Tarek A, Khaled MT. Characterisation and composition of watermelon, pumpkin and paprika seed oil and flour. *J Agric Food Chem* 2001; 49: 1253-1259.
26. Gopalan C, Rama Sastri BV, Balasubramanian SC, Narasing Rao BS, Deosthale YG, Pant KC. *Nutritive value of Indian Foods*. National Institute of Nutrition, Council of Medical Research. Hyderabad: India Indian 2007.

27. Akpapunam MA, Darbe JW. Chemical composition and functional properties of blends of maize and bambara groundnut flours for cookie production. *Plant Foods Human Nutr* 1994; 46: 147-155.
28. Narsing Rao G, Balaswamy K, Rao DG, Prabhakar Rao PG, Satyanarayana A. Nutritional quality, fatty acid, amino acids and functional characteristics of bael (*Aegle marmelos* L.) seed protein concentrate. *J Food Drug Anal* 2011; 19 (4): 528-535.
29. Narsing Rao G, Prabhakar Rao PG, Rao DG. Preparation of wood apple (*Feronia limonia* L.) seed protein concentrate and evaluation of its nutritional and functional characteristics. *Int Food Res J* 2011; 18: 914-920.
30. Amarowicz R, Troszynsa A, Barylko-Pikielna N, Shahidi F. Polyphenolic extracts from legume seeds: correlations between total antioxidant activity, total phenolics content, tannins content and astringency. *J Food Lipid* 2004; 11: 278-286.
31. Udayasekhara Rao P. Chemical composition and biological evaluation of debitterized and defatted neem (*Azadirachta indica*) seed kernel cake. *J Am Oil Chem Soc* 1987; 64: 1348- 1351.
32. Tang CH, Ten Z, Wang XS, Yang XQ. Physico-chemical and functional properties of hemp (*Cannabis sativa* L.) seed protein isolate. *J Agricultural Food Chem* 2006; 54: 8945-8950.
33. FAO/WHO/UNU. Protein and Amino Acid Requirements in Human Nutrition. WHO, Geneva. 2007; Pp. 150.
34. Amarowicz R. Antioxidant activity of protein hydrolysates. *Eur J Lipid Sci Technol* 2008; 110: 489-490.
35. Saxena R, Venkaiah K, Anitha P, Venu L, Raghunath M. Antioxidant activity of commonly consumed plant food of India: contribution of their phenolic content. *Int J Food Sci Nutr* 2007; 58: 250-260.
36. Singh U, Rao PV, Seetha R, Jambunathan R. Nutrient losses due to scarification of pigeonpea (*Cajanus cajan* L) cotyledons. *J Food Sci* 1989; 54: 974-981.
37. Scalbert A, Manach C, Morand C, Remesy C, Jimenez L. Dietary polyphenols and the prevention of diseases. *Crit Rev Food Sci Nutr* 2005; 45: 287-306.