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Research Article

Physico-chemical Amino acid composition, fatty acid profile, functional and antioxidant properties of *Spinacia oleracea* L. leaf

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ABSTRACT

Spinach (*Spinacia oleracea* L.) leaf powder (SLP) was prepared and its physicochemical (proximate composition, colour, mineral content, EMC-RH, protein solubility, amino acid content and fatty acid profile), functional (water absorption, fat absorption, emulsification and foam capacities) and antioxidant (DPPH, ABTS and Iron reducing power) properties were studied. SLP possessed a protein content of 31.72%, calcium (1335 mg/100g), iron (29 mg/100g) and phosphorous (278 mg/100g). Moisture sorption isotherms of SLP indicated its non-hygroscopic nature. Solubility of SLP protein in distilled water was 39.66%, which got enhanced to 72.83% in 0.1M NaCl at pH 12. The spinach leaf lipid was found to be rich in ω-3 fatty acids (67.4%). Leucine (11.87%), phenylalanine (5.9%) and valine (9.24%) were the major essential amino acids observed in SLP. Functional properties of SLP exhibited good water absorption, fat absorption and foam capacities. Antioxidant activity of SLP determined by the 2,2-azino-bis (3-ethyl benzothiazoline 6- sulphonic acid (ABTS) assay was found higher, when compared to the activities determined by diphenyl picrylhydrazyl (DPPH) and ferric reducing power assays. Protein solubility, amino acid content, fatty acid profile, functional properties and antioxidant characteristics encouraging for food and pharmaceutical applications.

Keywords: *Spinacia oleracea;* physicochemical characteristics; protein solubility; amino acid content; fatty acid profile; functional properties; antioxidant properties

1. Introduction

Leaf and algal proteins form the alternative sources of proteins for humans who consume pulses, milk and meat products for their protein requirements. In nature, leaves are abundantly available, economical and are the highly renewable sources for many valuable nutrients. Alfalfa leaf proteins were studied for their physico-chemical and functional properties (Lamsal, Koegel & Gunasekaran, 2007). Leafy vegetable species namely *Vernonia amygdalina* (Bitter leaf), *Solanum africana*, *Amaranthus hybridus* (Green tete) and *Telfaria occidentalis* (Fluted pumpkins) were evaluated for their physico-chemical and mineral composition (Aletor & Ipinmoroti, 2002). It was reported that they possessed 33.3 g/100 g crude protein (31.7–34.6 g/100 g) and 8.4 g/100 g (7.4–9.8 g/100 g) crude fibre on dry matter basis.

Leaf protein powders were prepared and evaluated for their, chemical, mineral, amino acid compositions and functional properties. Leaves of *S. Africana* and *V. amygdalina, potato, carrot and tomato* (Carlsson, 1989); alfalfa (Lamsal *et al.*, 2007); *Asphodeline* (*Liliaceae*) and *Sophora japonica* L. (Gokhan *et al.*, 2012) were reported. Moringa leaf meal as a protein source replacing grass diet was evaluated in lactating dairy cows and was compared with a concentrate containing 20% soybean meal (Bryan *et al.*, 2011). Mineral nutrient content of commonly consumed leafy vegetables namely *Hibiscus sabdariffa*, *Amaranthus spinosus*, *H. cannabinus*, *Solanumm*

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acrocapon and *Vigna unguiculata* (Amagloh & Nyarko, 2012) were also reported.

Chamnamul (*Pimpinella brachycarpa*) and fatsia (*Aralia elata*) common leafy vegetables consumed in East Asia (Kim *et al.,* 2013), *Paederia foetida* and *Erechtites hieracifolia* wild leafy vegetables of Indonesia (Srianta *et al.,* 2012) and coloured leaves of amaranth, spinach and beet (Ali *et al.,* 2009) were found to exhibit high antioxidant properties. The total lipid extracted from amaranthus leaves using chloroform: methanol (2:1, v/v) yielded 10.6% lipid with 53.6% non-polar fraction, 33.8% glycolipids and 12.6% phospholipids (Lakshminarayana *et al.,* 1984).

Literature on characterization of spinach leaf powder for protein and lipid composition is limited and hence, the present investigation was undertaken to prepare spinach leaf powder and investigate its physicochemical composition, minerals, sorption studies, amino acid composition fatty acid composition, , functional characteristics and antioxidant activity.

2. Materials and methods

2.1. Materials

2.1.1. Spinach leaf

Fresh spinach bunches (20 kg) were collected from vegetable market in Hyderabad, India.

2.1.2. Chemicals

Fresh spinach bunches (20 kg) were collected from vegetable markets in Hyderabad, India. Chemicals and solvents used in the study were of analytical and laboratory grade and were procured from Sd. Fine-Chem. Ltd., Mumbai, India. DPPH (2, 2-diphenyl-1-picrylhydrazyl) and ABTS (2, 2-azinobis {3-ethyl-benzothiazoline-6-sulfhonic acid}), butylatedhydroxyltolulene (BHT) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxilic acid (Trolox) were procured from Sigma Aldrich, Bangalore, 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 spinach leaf powder (SLP)

Fresh spinach leaves separated from stems were washed with water, soaked in sodium hypochlorite 40ppm for 20 min, again washed with water and dried in a cabinet tray dryer (Chemida, Mumbai, India) at 45 ± 2 ℃ for 10 h. The dried material was ground to powder using a high-speed mixer (M/s. Sumeet, Nasik, India), passed through 210µ mesh to obtain uniform dehydrated spinach powder (DSP). The dehydrated spinach powder was subjected to lipid removal using isopropyl alcohol (IPA) at room temperature (RT) of 28 ± 2 ℃. The residue was dried in a vacuum dryer at 50 ± 2 ℃ for five hours and ground to obtain spinach leaf powder (SLP). The powder was packed in metallised polyester polyethylene (MPE) laminate pouch and stored at 4 ℃.

2.2.2. Physico-chemical composition

Physicochemical properties were evaluated using standard analytical methods reported by Ranganna (1986) and colour was measured using a Hunter Lab Ultra-Scan XE colourimeter. The bulk density was measured by noting the volume occupied by 25 g powdered sample in a 100 ml graduated cylinder. Protein content was estimated using micro-Kjeldahl method employing the conversion factor of 6.25 (Pellett & Young, 1980).

2.2.3. Determination of mineral content

Minerals such as calcium, iron and phosphorus were determined by standard reported methods (Ranganna, 1986; AOAC, 1995) and expressed as mg/100g SLP.

2.2.3.1. Preparation of SLP ash solution

Weight of 5g SLP was taken in silica crucible heated on Bunsen flame to remove volatile matter. The crucible with sample was placed in muffle furnace at 450 ℃ for 6 h. The white ash was dissolved in 6 N HCl and heated on a boiling water bath for 30 minutes and the solution was made up to 100 ml volume (ash solution).

2.2.3.2. Determination of calcium content

Calcium is precipitated as calcium oxalate which was dispersed in hot diluted H_2SO_4 and titrated with standard potassium permanganate according to the standard method reported by Ranganna (1986). Ash solution 20 ml was taken in a 250 ml conical flask and added 20 ml distilled water, 10 ml saturated ammonium oxalate and 2 drops methyl red indicator. The mixture was made alkaline with ammonia and acidified with acetic acid and the contents were heated to boil. The mix was allowed at RT for overnight to precipitate out calcium. The solid was filtered, washed with water till the absence of oxalates in filtrate by checking using silver nitrate. The white solid was dispersed in hot dilute sulfuric acid (1:4) and titrated with 0.01 N $KMnO_4$ to the permanent pink colour. The calcium contant was calculated using following formula:

$$\text{Calcium mg/100 g} = \frac{\text{Titer volume ml (0.01 N } KMnO_4) \times 0.2 \times \text{Total volume of ash solution}}{\text{Ash solution taken for estimation} \times \text{Weight of SLP taken for ashing}}$$

2.2.3.3. Determination of iron content

Iron was determined by converting into ferric form using potassium persulphate and treating with potassium thiocyanate to form red ferric thiocyanate which is measured at 480 nm using spectrophotometer. An aliquot of ash solution 5 ml was taken in a 20 ml test tube added 0.5 ml H_2SO_4 potassium persulphate (1ml) and treating with potassium thiocyanate (2ml) and the volume was made up to 15 mL using distilled water. The optical density (OD) of developed colour was measured at 480 nm. A standard was also prepared using ferrous ammonium sulphate (0.1 mg/ ml) and blank was prepared without ash solution and standard iron. The iron content was calculated using following expression:

$$\text{Iron mg/100 g} = \frac{\text{OD of sample} \times 0.1 \times \text{Total volume of ash solution}}{\text{OD of Standard} \times 5 \times \text{Weight of SLP taken for ashing}}$$

2.2.3.4. Determination of phosphorous content

Phosphorous content in SLP was determined by reacting ash solution with molybdate reagent and amino naphthol sulphonic acid and colour developed was read at 650 nm. The ash solution of the sample in hydrochloric acid (5 ml) was allowed to react with 5 ml of molybdate reagent and 2 ml of amino naphthol sulphonic acid solution in 50 ml standard flask. The contents were shaken thoroughly, and the volume was made up to 50 ml. Reagent blank was prepared without the ash solution. The absorbance of colour developed was read at 650 nm the reagent blank.

A standard graph was plotted using diluted working standard of potassium phosphate solution (1ml = 0.02 mg phosporous). Colour was developed using 5 to 40 ml of aliquots from the working standard with the addition of 5 mL of molybdate reagent and 2 ml of amino naphthol sulphonic acid reagents. They were mixed thoroughly and volume was made up to 50 ml. The developed colour was measured and a graph was plotted with concentration versus absorbance for computing sample concentration. The phosphorous content was calculated using following formula:

$$\text{Phosphorus mg/100 g} = \frac{\text{mg of P in the aliquot of ash solution} \times \text{Total volume of ash solution}}{\text{OD of Standard} \times \text{Weight of SLP taken for ashing}} \times 100$$

2.2.4. Equilibrium moisture content-relative humidity (EMC-RH) studies

EMC-RH studies of SLP were carried out according to a reported method by Ranganna, (1986). SLP (5 g) was exposed to different relative humidity (RH) ranging from 10 to 100 maintained by using appropriate concentrations of sulphuric acid in air-tight glass desiccators at room temperature (RT) 28 ± 2°C to determine their sorption behavior. The samples were weighed at regular intervals till they attained constant weight or change in colour or lump formation or mold growth during the study. Moisture sorption isotherms were drawn by plotting equilibrium moisture content (EMC) against the corresponding equilibrium relative humidity (ERH).

2.2.5. Determination of protein solubility

2.2.5.1. Effect of water, pH and ionic NaCl on SLP protein solubility

The protein solubility was evaluated by a method reported by Narsing Rao and Govardhana Rao (2010). One gram SLP was extracted in water, 0.1, 0.5 and 1.0 M NaCl solutions and pH were adjusted to 2–12 pH using 0.5 M hydrochloric acid (HCl) or 0.5 M sodium hydroxide (NaOH). After extraction, the suspensions were centrifuged at 5500 × g at RT for 15 min, and the supernatant was collected. Protein content in supernatant was determined using Biuret method (Sadasivam & Manickam, 1997).

2.2.5.2. Buffer capacity

The buffer capacity was estimated following the method described by Narsing Rao and Govardhana Rao (2010). One gram of SLP was dispersed in 40 ml distilled water and known volumes of 0.5 M NaOH or 0.5 M HCl were added and corresponding changes in pH in both alkali and acid ranges were noted. The quantity of alkali and acid added was plotted against pH. Buffer capacity in each range was expressed as mmol of NaOH or HCl per gram of SLP required for changing the pH value by one unit.

2.2.5.3. Protein precipitation

Protein precipitation studies were carried out according to the method reported by Taher *et al.* (1981). SLP 10 g was dispersed in distilled 400 ml distilled water and the pH was adjusted to 10 using 0.5 M NaOH and stirred using magnetic stirrer for the optimum extraction time (30 min). The suspension was centrifuged at 5500 × g at room temperature (RT) for 15 min to obtain clear solution. The clear supernatant 20 ml was taken in graduated centrifuge tubes and adjusted to the desired pH values of 2.0–6.5 using 0.5 M HCl. The suspensions were centrifuged at 5500 × g at RT for 15 min and the protein content was determined and protein precipitation was calculated using the formula:

$$\frac{V_1P_1\ V_2P_2}{V_1P_1} \times 100$$

where V_1 and V_2 are the volumes of the supernatant (ml) before and after precipitation, and P_1 and P_2 are mg protein in 1 ml of V_1 and V_2, respectively

2.2.6. Amino acid

Amino acid content was analysed using an automatic amino acid analyzer employing high-performance liquid chromatography (Shimadzu Model LC 10A, Japan) (Bidlingmeyer *et al.,* 1984). Hydrolysis of the samples was carried out using 6 N HCl under vacuum maintaining the temperature at 100 °C for 48 h. Phenylthiocarb-amyl derivatives were prepared using phenyl isotyhiocyanate and using C-18 reverse phase column. Amino acids were detected after post column derivatisation with Ninhydrin reagent (Agilent amino acid standard kit, California, USA). Cysteine and methionine contents were determined in hydrolysed sample were determined according to the method reported by Moore (1963).

2.2.7. Determination of fatty acids

Total lipid and fatty acid composition were analysed according to the reported method with minor modification (Folch *et al.*, 1957). The individual fatty acids were confirmed by comparison of retention times as well as fragmentation pattern of authentic

standards. Dehydrated spinach powder (100g) was extracted with a mixture of solvents (chloroform-methanol, 2:1, v/v) at room temperature (RT). The extraction was carried out using a magnetic stirrer maintaining DSP to solvent ratio at 1:3 and the extraction were repeated for four times and pooled extracts were washed with water. The solvent was dried over anhydrous sodium sulphate, and total lipid was recovered by distillation in a rotary vacuum evaporator at □ 50 °C.

The total lipid extracted from DSP was converted into fatty acid methyl esters (FAME) by refluxing along with a mixture of sulphuric acid in methanol (2, v/v) for 6 h. The FAMEs were analysed by Gas Chromatography and Gas chromatography - Mass spectrometry with an Agilent 6850 series gas chromatograph equipped with a FID detector. A DB-225 capillary column (30 m × 0.25 mm i.d) in GC and Agilent (Palo Alto, USA) 6890N gas chromatograph equipped with a HP-5 MS capillary column connected to an Agilent 5973 mass spectrometer operating in the EI mode (70 eV; *m*/*z* 50–550). The individual fatty acids were confirmed by comparison of retention times as well as fragmentation pattern of authentic standards.

2.2.8. Functional properties

2.2.8.1. Water absorption capacity

The water absorption capacity (WAC) and fat absorption capacity (FAC) were determined using a reported method by Narsing Rao and Govardhan Rao (2010). Dispersing 1 g of SLP in about 10 mL of distilled water or sunflower oil. The contents were vortexes for 2 min and centrifuged at 5500 × g for 15 min at RT. The supernatant water or oil was decanted and the residue weight was noted. The weight difference was noted before and after water or oil process. Calculated WAC and OAC and expressed as g/ g SLP.

2.2.8.2. Emulsification capacity (EC)

Emulsification capacity was determined following a reported method in literature (Gagne and Adambounou, 1994). . SLP 1 g was taken, homogenate and slowly adding of vegetable oil while stirring until the separation of oil globules was observed. The oil volume was noted after separation and emulsifying capacity was expressed as mL of oil/g SLP.

2.2.8.3. Foam measurements

Foam capacity (FC) and foam stability (FS) of SLP were measured by following the methods described by Lawhon *et al*. (1972). One gram of SLP dispersed in100 ml distilled water and whipping for 10 min. The increase in volume by foam was measured, calculated and expressed as foam per cent. Foam volume was measured during a time intervals such as 15, 30, 45 and 60 min, calculated the foam volume and expressed foam stability.

2.2.9. Determination of antioxidant activity

2.2.9.1. DPPH radical scavenging activity

Radical scavenging activity was measured using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (Nanjo *et al*., 1996). SLP extracts in distilled water ranging from 0.3-1.8 mg dispersed in ml of methanol to which 4 ml of methanolic solution of DPPH (0.004) was added and the contents were incubated at RT for 30 minutes. The optical density of the samples was read at 517 nm. The solution without SLP extract was taken as control. The DPPH radical scavenging activity of SLP was compared with a synthetic phenolic antioxidant like butylated hydroxytolulene (BHT) and Trolox. The percentage inhibition was calculated using the following expression.

$$\text{Inhibition, \%} = \frac{\text{Absorbance of control} - \text{Absorbance of SLP extract}}{\text{Absorbance of control}} \times 100$$

2.2.9.2. ABTS Assay

ABTS (2, 2-azinobis {3-ethyl-benzothiazoline-6-sulfhonic acid}) assay was carried out according to the method reported in the literature by Re *et al.* (1999). The ABTS solution (7 mM) and potassium persulphate (2.45 mM) were mixed and then incubated at RT for 16 h. The mixture was diluted with water (1:40, v/v) to obtain the ABTS reagent with an absorbance of 0.700 at 734 nm. ABTS reagent (4 ml) was mixed with 1 ml sample containing of 0.3-1.8 mg SLP and shaken vigorously. The blank was prepared using water instead of samples and its absorbance was recorded at 734 nm after 10 min and the ABTS assay was calculated and compared with BHT and Trolox.

2.2.9.3. Iron reducing power

The iron reducing power was measured according to a reported method (Yildrim *et al.*, 2001). Extract of 0.3 to 1.8 mg/ml were taken in 20 ml test tubes and the volume was made up to 1 ml with water. Phosphate buffer (2.5 ml) and 1 solution of potassium ferricyanide (2.5 ml) were added and the contents of test tubes were incubated for 20 min at 50 °C. Trichloroacetic acid (10, 2.5 ml) was added to each test tube and centrifuged at 5000 x g for 15 min. Supernatant from each test tube (2.5 ml) was dispersed in 2.5 ml of distilled water to which 0.5 ml of 0.1 ferric chloride was added and thoroughly vortexed for 2 min. The optical density of the colour developed was measured at 700 nm. The reducing power of standard synthetic antioxidant, BHT was measured for quantities ranging between 30-180µg for comparison.

2.10. Statistical analysis

Physicochemical composition, sorption isotherm, protein solubility, amino acid composition, functional, and antioxidant properties were carried out in triplicate and mean values with a standard deviation (SD) were computed by using MS Excel, 2007. Fatty acid profile of total lipid was carried out in duplicate and average values were presented. The antioxidant activity of SLP extract was compared with the standard butylatedhydroxyltolulene (BHT) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxilic acid (Trolox).

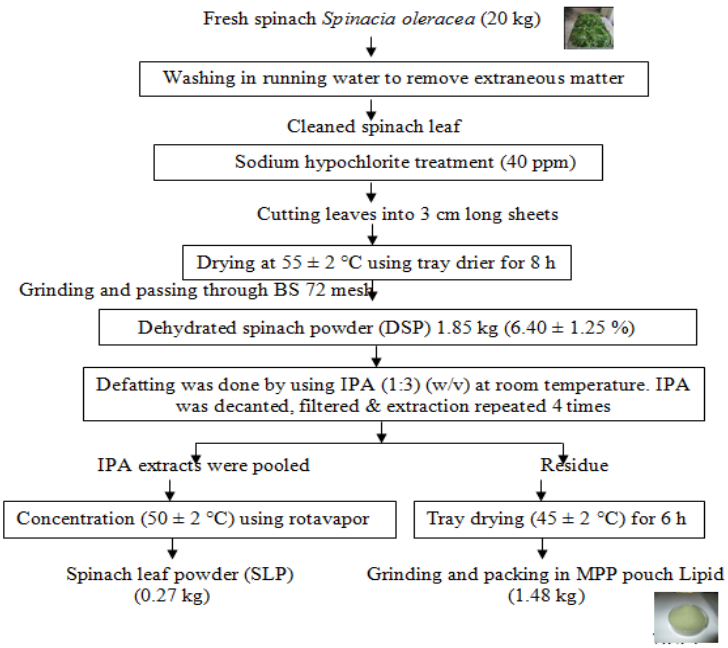


Fig 1. Flowchart of unit operations during the preparation of spinach leaf powder (SLP)

3. Results and Discussion

3.1. Physicochemical and mineral composition

Unit operations involved in the production of spinach leaf powder and total lipid are presented in Fig 1. Fresh spinach leaves yielded 4.57% of spinach powder possessing 28.23% protein and de-fatting yields SLP with a protein content of 31.72%. SLP was found to be rich in mineral matter 19.21% and fiber 10.24%. The Hunter colour units of SLP were L* (56.33), a* (-1.26) and b* (3.51) (Table 1). Higher L* values observed in SLP can be attributed to the presence of bound carotenoids. Higher calcium (1348 mg/100g) and iron (28.65mg/100g) was found in SLP.

Table 1. Physicochemical and mineral composition of spinach leaf powder (SLP)[a]

Parameter, %	SLP
Yield	4.57 ± 0.55
Bulk density (g/ml)	0.65 ± 0.01
Hunter colour units	
L*	56.33 ± 1.42
a*	-1.26 ± 0.06
b*	13.51 ± 0.27
Moisture	13.03 ± 0.68
Total ash	19.21 ± 0.39
Crude protein	28.23 ± 0.87
Crude fiber	10.24 ± 0.10
Minerals (mg/100g)	
Calcium	1348.91 ± 3.39
Iron	28.65 ± 1.93
Phosphorus	278.7 ± 2.49

[a] Values are average of triplicate analysis with ± SD; DSLP: Defatted spinach powder; Yield: Based on fresh spinach

3.2. Sorption isotherms

Experimental sorption isotherms of SLP indicated that the sample was more stable at RT (Fig 2). Initial moisture content (IMC) and critical moisture content (CMC) of 13.03 and 17.04%, which equilibrated at 63 and 71% RHs respectively. The data generated shows that the sample was non-hygroscopic in nature.

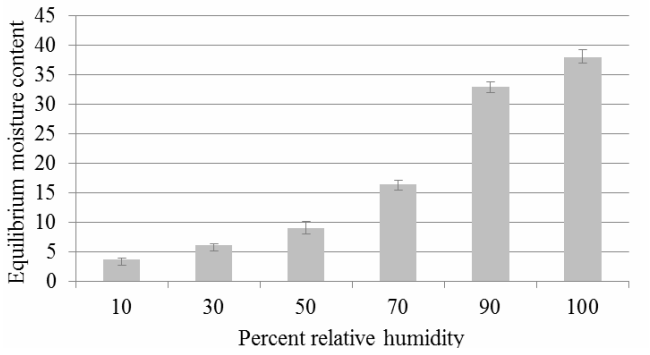
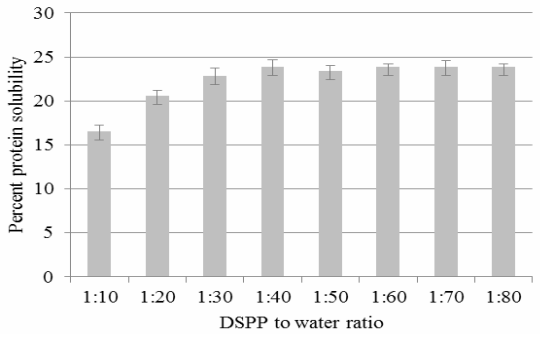


Fig 2. Sorption isotherm of SLP

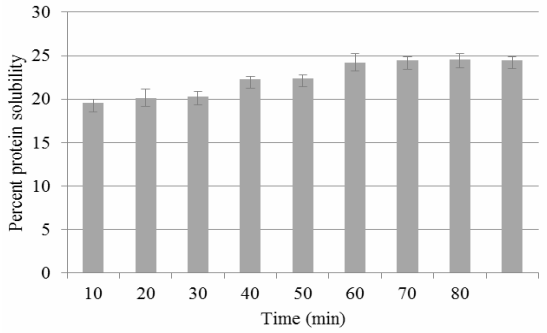
3.3. Protein solubility

Solubility is one of the most important functional properties of proteins, especially for making emulsions and foams. The protein solubility of SLP in the pH range of 2-12 is shown in Fig 3. Protein solubility was minimal over a wide pH range with not more than 12.47% solubility at pH 4. The lower solubility at pH 4 might be attributed to the difference in the isoelectric points of the peptides. The highest solubility of protein was found to be 39.66% at pH 12. Ionic NaCl showed a positive impact on the solubility of protein up to 0.1M, and further increase in NaCl concentration (0.2 and 0.5M) decreased the solubility (Fig 3). At the isoelectric point, the solubility was 34.68% in 0.1M NaCl at pH 4 and the maximum solubility was 72.83% at pH 12 (Fig 3).

a.



b.



c.

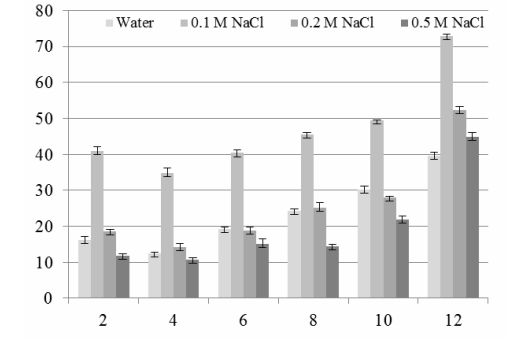


Fig 3. Protein solubility studies

a. Effect of sample to water ratio on protein solubility of SLP; b. Effect of extraction time on protein solubility of SLP; c. Effect of pH and ionic sodium chloride on protein solubility of SLP

Minimum protein solubility of alfalfa leaf protein occurred around pH 3.5- 4 and sodium chloride 0.05–0.2M had little effect on the solubility (Wang & Kinsella 1976).

The extracted protein could be precipitated to a maximum extent (78.65%) at pH 4. (Fig 3). On either side of the pH 4.0, precipitability of SLP decreased gradually. The protein precipitation was observed to be lower (50.66%) at pH 5.5.

Buffer capacity of SLP is presented in Fig 5. The study is useful for bulk preparation of protein isolates. The pH of dispersions in distilled water for SLP was found to be 6.61 at RT. Higher buffer capacity values were observed in alkaline pH than in acid pH, which indicates the requirement of larger volumes of alkali for changing the pH. Quantity of alkali required per gram SLP to bring about a pH of 12 was 4.71 mM. It was observed that in *Sterculia urens* seed protein concentrate, 0.44 mM of NaOH/g was required to bring about a change in pH from 6.83 to 10 (Narsing Rao & Govardhan Rao 2010).

3.4. Amino acid content

Amino acid composition of the dehydrated spinach leaf is presented as g/100 g protein in Table 2. The protein content was found to an extent of 28.23%. Glutamic acid (13.05 g/100 g), leucine (11.87%) and valine (9.24%) were found to be major amino acids. The ratio of essential to non-essential amino acids was found to be 1.05 indicating equal quantities of essential and non-essential amino acids. FAO/WHO (2007) recommended daily intake values for essential amino acids (mg/kg body weight per day) for adult humans as isoleucine, 20 mg; leucine, 39 mg; lysine, 30 mg; methionine and cysteine, 15 mg; phenylalanine and tyrosine, 25 mg; threonine, 15 mg; tryptophan, 4 mg; valine, 26 mg. The leaf powder is found to be a good source of essential amino acids, which can be supplemented in processed food products as a nutraceutical additive.

Table 2. Amino acid [b] composition of spinach leaf powder

Amino Acid	Composition, g/100g protein
Alanine	7.68 ± 0.21
Arginine	5.65 ± 0.21
Aspartic acid[c]	9.09 ± 0.09
Cysteine	1.73 ± 0.03
Glutamic acid[d]	13.05 ± 0.32
Glycine	6.64 ± 0.19
Histidine	2.01 ± 0.07
Isoleucine	4.38 ± 0.13
Leucine	11.87 ± 0.27
Lysine	5.56 ± 0.13
Methionine	1.62 ± 0.03
Phenylalanine	5.90 ± 0.18
Proline	5.24 ± 0.16
Serine	4.72 ± 0.13
Threonine	5.57 ± 0.16
Tyrosine	0.04 ± 0.01
Valine	9.24 ± 0.05

3.5. Total lipid profile

The yield of total lipid from dehydrated spinach powder (DSP) was 12.98%. The fatty acid composition of total lipid is presented in Table 3. The SFA contributed an amount of ~24% and monounsaturated accounted 8.1% of total lipid. Polyunsaturated fatty acids (PUFA) were the major component (68%) of the total lipid. The fatty acid composition of DSP was characterized by substantial amounts of palmitic (19.7%), linoleic (22.9%), linolenic (44.5%) and oleic (5.4%) acids. Polyunsaturated fatty acids consisted of linoleic (22.9%) and linolenic (44.5%) acids. Earlier, it was reported that amaranthus leaves yielded a total lipid of 10.6% with hexadecenoic acid (16:1, 12.3%) as the major fatty acid (Lakshminarayana *et al.*, 1984).

Higher saturated (23.9%) and polyunsaturated (68%) and lower monounsaturated (8.1%) fatty acids were noticed in spinach lipid. The ratio of polyunsaturated to saturated fatty acids (PUFA/SFA)

Table 3. Fatty acid[a] composition of spinach leaf powder

Fatty acid	Composition (wt. %)
Palmitic acid	19.7
Stearic acid	1.5
Eicosanoic acid	0.4
Docosanoic acid	0.8
Arachidonic acid	1.5
Saturated	23.9
Palmitoleic acid	2.3
Oleic acid	5.4
Eicosamonoenoic acid	0.4
Monounsaturated	8.1
Linoleic acid	22.9
Eicosadienoic acid	0.2
Docosadienoic acid	0.4
Linolenic acid	44.5
Polyunsaturated	68.0

[a]Values are mean of duplicate analyses

Table 4. Functional properties of spinach leaf powder (SLP)[a]

Parameter	SLP
Water absorption capacity (g/g)	2.91± 0.13
Fat absorption capacity (g/g)	1.43 ± 0.10
Emulsifying capacity (ml/g)	8.83 ± 1.04 (53 ml/g BSA)
Foam capacity (ml/g)	78.33 ± 2.89 (148 ml/g BSA)
Foam stability (ml), after 15 min	69.00 ± 2.65
Foam stability (ml), after 30 min	52.67 ± 4.16
Foam stability (ml), after 45 min	36.00 ± 3.46
Foam stability (ml), after 60 min	28.67 ± 0.58

[a] Values are average of triplicate analysis with ± SD; DSLP: Defatted spinach powder; Yield: Based on fresh spinach 100g after drying and defatting; BSA: Bovine serum albumin

Table 5. Antioxidant activity of aqueous extract of SLP [a]

Concentration (mg/ml)	SLP		
	DPPH (Inhibition, %)	ABTS Assay	FRP (OD)
0.3	35.17 ± 1.02	45.52 ± 0.62	0.069 ± 0.002
0.6	36.86 ±1.18	48.81 ±1.13	0.079 ± 0.006
0.9	37.27 ± 0.46	60.07 ± 1.20	0.096 ± 0.003
1.2	38.76 ± 0.56	62.81 ± 0.67	0.116 ± 0.005
1.5	39.92 ± 0.55	64.13 ± 1.24	0.125 ± 0.003
1.8	42.63 ± 0.60	69.55 ± 0.80	0.166 ± 0.005

[a] Values are average of triplicate analysis with ± SD; SLP: Defatted spinach leaf powder; DPPH: 2,2-diphenyl-1-picrylhydrazyl 1,1-diphenyl-2-picrylhydrazyl radical; ABTS: 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid
FRP: Ferric reducing power; OD: Optical density

was found to be 2.84; the ratio of polyunsaturated to monounsaturated fatty acids (PUFA/MUFA) was 8.39 in total lipid. PUFA + MUFA/SFA ratio was found to be 3.18. Apart from these fatty acids considerable amounts of higher fatty acids such as 24:0 (1.5%), 22:0 (0.8%) and very lower quantities of 20:1 (0.4%), 22:2 (0.4%) were also observed in the total lipid. Lignoceric acid (1.5%), eicosenoic acid (0.4%) and eicosadienoic acid (0.2%) were also observed in minor quantities in the total lipid. The presence of stearic acid (1.49%), oleic acid (5.03%), linoleic acid (16.34%) and linolenic (18.16%) in *Spirulina maxima* lipid (Otles and Pire, 2001) is comparable with the present work. The ratio of PUFA/SFA is used to evaluate the nutritional value of lipid. The effects PUFA + MUFA/SFA on plasma and liver lipid concentrations in rats were reported (Chang & Huang 1998).

High PUFAs/MUFAs ratio increases the level of very low density lipoprotein in plasma but reduces the effect of dietary cholesterol in elevating the triglyceride level in liver (Chang *et al.*, 2004). The presence of high

amounts of PUFA compared to SFAs, make spinach leaf oil suitable for nutritional applications.

3.5. Results of functional properties

Data pertaining to WAC, FAC, EC, FC and FS are presented in Table 4. The SLP exhibited higher water absorption (2.91g/g) than oil (1.43g/g). The phenomena can be explained based on the capacity of hydrophilic peptides binding to water molecules and high hydrogen bonding. The leaf proteins were found to be good emulsifying agents. Their ability to emulsify water-oil dispersion had good industrial applications. SLP exhibited marginal emulsification capacity of 8.83 ml/g, when compared to BSA (53 ml/g). The emulsification capacity data generated for BSA were also presented in Table 1 for comparison. EC is a very important property if the spinach leaf powder can be used in products such as salad dressings. Protein, carbohydrates and fat play an important role in WAC, FAC and EC. The higher value of WAC of SLP (2.91 g/g) indicates the presence of more hydrophilic sites in SLP. Fat absorption plays an important role in food preparations, because fat improve the flavor and mouth feel of products. High WAC and FAC of SLP may find useful applications in the preparation of bakery products such as cakes and biscuits.

The foam capacity of SLP was found to be 78.33 ml, when compared to 148 ml/g for BSA. SLP exhibited a foam stability of 28.67 ml over a period of 60 min. Foam stability depends on protein content with hydrophilic nature of peptides and these characteristics are desirable in preparation of beverage. The lower protein solubility and quantity of denatured proteins might be the causes for lower FC and FS values. Hence, further studies can be taken up to improve the protein solubility and functional characteristics of SLP by incorporation of additives such as ionic salts for its use as a functional food ingredient.

3.6. Results of antioxidant activity

Data on DPPH radical scavenging activity is presented in Table 5. DPPH radical scavenging activity in terms of inhibition was 35.17 and 42.63% with 0.3 and 1.8 mg respectively. Similarly, the percent ABTS inhibition values were 45.52 and 69.55% for 0.3 and 1.8 mg respectively. Optical density values ranged between 0.069-0.166 for 0.3 and 1.8 mg respectively. Under identical conditions, percent DPPH inhibition of 45.31 and 69% was noticed with BHT at 30 and 180 µg, respectively. Similarly percent inhibition values in ABTS assay for BHT were 35.79 and 70.36% for 30 and 180µg respectively. Optical density values ranged between 0.188-0.814 for 30 and 180µg respectively. Similarly, the percent DPPH inhibition of 37.12 and 84.62% was noticed with trolox at 5 and 30 µg, respectively and inhibition values were 56.88 and 99.14% for 5 and 25µg respectively in ABTS assay. Optical density values ranged between 0.047-0.152 for 5 and 30µg respectively. The activity of protein depends on solubility of protein, nature of peptides and free amino acids present in the sample. The results revealed that SLP possessed higher radical scavenging activity, which could be due to reaction of free radicals to form stable products in preventing oxidative degeneration. SLP rich in smaller peptides and free amino acids would have contributed to the activity. SLP showed very good antioxidant ability against DPPH free radical and ABTS assay. Overall antioxidant activity of proteins from curry leaf and jujuba fruits was reported to be dependent on the quantity of smaller peptides and free amino acids (Memarpoor *et al.*, 2013). The antioxidant activity of SLP can be correlated with the presence of free amino acids and shorter chain peptides similar to soy, canola seeds, curry leaf and jujuba fruits. Hence, further studies can be carried out for isolation, purification and identification of bioactive peptides useful against degenerative diseases.

4. Conclusions

The investigation revealed that spinach leaf powder has encouraging functional characteristics and antioxidant activity. Spinach leaf powder (SLP) with a protein content of 31.72% and rich in essential minerals like calcium, iron and phosphorous was non-hygroscopic in nature. Maximum and minimum solubility of protein was noticed at pH 12 and 4 respectively. The lipid was found to be rich in ω - 3 polyunsaturated fatty acids (linolenic acid). The study helps enthuse researchers for further investigation on spinach leaf powder for purification and identification of bioactive peptides for food and pharmacological applications.

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