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Chemical Composition, Functional and Antioxidant Potential of Selected Chilli (*Capsicum annuum* L.) Seeds

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Abstract

Background: Chilly seeds are known for their pungency and oil content. However literature is scanty on their protein, functional properties and antioxidant activity. **Methods:** Defatted Seed Flour (DSF) of freshly harvested chilly varieties such as red (ripened, R), big green (bajji, B), small green (S) and green capsicum (C) were prepared by manual separation, tray drying, grinding and solvent extraction. Physico-chemical, functional characteristics, antioxidant activity and sorption behaviour of the DSF powder samples were evaluated using standard methods. **Results:** Drying, grinding and hexane extraction of seed powder yielded fat (16.56–27.35%) and DSF (69-78%). Sorption isotherms of the DSFs indicated that R, B, S and C chilli DSFs equilibrated between 50-52% RH indicating their non hygroscopic nature. The DSFs were found to be rich in mineral (3.8–5.3%), protein (19–26%), fiber (23–30%) and polyphenols (447–629 mg%). DSF exhibited water holding capacity (203–247g/100g) and oil holding capacity (127–146g/100g). DSF possessed a foam capacity (56–100ml/1g) and foam stability (22–93ml after 25 min). A concentration of 3.5-8 mg/ml and 0.7–1.1 mg/ml of DSF were required for 50% Inhibition of DPPH and ABTS radicals, respectively. The results showed that the red chilli DSF exhibited highest protein content, foam capacity and stability among the chilly seeds.

Keywords: Antioxidant Activity, Chemical Composition, Chilli, Defatted Seed Flour, Functional Properties

1. Introduction

Plant seeds are generally rich sources of protein, fat, fibre, minerals and functionally active components. Several vegetable seeds were explored for chemical composition, protein solubility, functional properties, fatty acids, amino acids and mineral[1–8]. Chilli (*Capsicum annuum* L.) belongs to the family of solanaceae, which is cultivated all over the world throughout the year. Chillies are potential source of active components such as polyphenols, ascorbic acid, pigments and fibre. Generally, fresh green, ripened and dehydrated red chilli are used in various culinary preparations. Different varieties of chillies were evaluated for chemical properties, mineral contents and fatty acid profile[9–14]. Literature on characterization of chilli seeds is limited and hence, the present study was undertaken to prepare defatted chilli seed powder and evaluate its chemical composition, minerals, sorption characteristics, functional properties and antioxidant activity. The whole seed fat was also analysed for fatty acid composition.

2. Materials and Methods

2.1 Materials and Chemicals

Freshly harvested chilly varieties namely, red (R) (Pusa Jwala), big green, bajji (Banana pepper) (B), small green (Pusa Sada Bahar) (S) and green capsicum (Priya F1

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hybrid) (C) were collected (10–15 kg each) during March – June, 2017 and 2018 at Hyderabad, Telangana, India.

Chemicals and reagents were procured form M/s Sd Fine Chem, Mumbai, India. Authentic standard fatty acids (fatty acid methylesters mixture C_4-C_{24}), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline, 6-sulphonic acid), gallic acid, Trolox, 4-*Amino*-3-hydroxy-1-naphthalenesulfonic acid and potassium dihydrogen ortho phosphate, were purchased from M/s Sigma Aldrich, Chemicals Pvt. Ltd. Bangalore, India.

2.2 Preparation of Defatted Seed Flour (DSF)

Freshly harvested chilly varieties namely, red (R) (Pusa Jwala), big green, bajji (Banana pepper) (B), small green (Pusa Sada Bahar) (S) and green capsicum (Priya F1 hybrid) (C) were collected (10–15 kg each) during March – June, 2017 and 2018 at Hyderabad, Telangana, India. Chillies were washed with water, soaked in hypochlorite solution (10 ppm) for 30 min. and air dried. The seeds were separated by cutting chillies longitudinally and dried in a cabinet tray drier (50–55°C) for 8 h. They were ground, defatted using hexane using soxhlet extraction, dried, ground to pass through 40 mesh sieve (420µ) to obtain Defatted Seed Powders (DSF). The DSFs were packed in Metallised Polyester Polyethylene (MPE) laminated pouches and stored at RT for further studies.

2.3 Equilibrium Relative Humidity

As per the method of Ranganna (1986)[15] Equilibrium relative humidity studies of DSFs were carried out. In order to determine their sorption behavior Defatted seed flours (2–3g) were exposed to Relative Humidity (RH) of 10, 30, 50, 70, 90 and 100% prepared using dilute sulphuric acid solutions in glass desiccators at room temperature. The DSFs were weighed at regular intervals to obtain constant weight. Moisture sorption isotherms of DSFs were drawn by plotting RH versus EMC values to determine the packaging requirements.

2.4 Physico-chemical and Mineral Content

Physico-chemical composition and mineral content of DSFs were determined using methods reported by Ranganna (1986)[15]. Colour characteristics such as L* (lightness index) a* (red-green) and b* (yellow-blue) were measured using a Hunter Labscan colorimeter[16].

2.5 Fatty Acid Composition

The whole seed fat obtained by hexane was converted into fatty acid methyl esters (FAME) by refluxing with methanol containing 2% sulphuric acid for 8 h. The MEs were extracted into ethyl acetate, concentrated and analysed by using GC-FID–MS capillary column and maintaining 160°C for 2 min increasing to 230°C at 6°C/min[17,18]. Fatty acids present in the chilly seed fat were identified by comparing the retention times and fragmentation patterns of authentic standard fatty acids (fatty acid methylesters mixture C_4–C_{24}, Sigma Aldrich, Chemicals Pvt. Ltd., Bangalore, India) and further confirmed by data from the Wiley and NIST libraries.

2.6 Protein Solubility and Buffer Capacity

The protein solubility and buffer capacity of DSFs was evaluated using a method reported by Narsing Rao and Govardhana Rao (2010)[19]. One gram DSF was dispersed in pH between 2–12 using 0.5 M HCl and NaOH and magnetic stirring for 20 min. The suspensions were centrifuged at 4000 × g at RT for 9 min, and the supernatants were collected. Protein content in supernatant was determined using micro Kjeldahl method. Buffer capacity was determined by dispersing one gram DSF sample in 50 ml water and changes in pH between 2 to 12 were recorded on addition of 0.5 M HCl or NaOH. In each range buffer capacity was expressed as average mmol of NaOH or HCl/g of DSF required for changing the pH value by one unit.

2.7 Functional Properties

Water holding capacity (WHC), Oil Holding Capacity (OHC), Foam Capacity (FC) and Foam Stability (FS) of DSF samples were determined according to the method reported. One gram of DSF was dispersed in 10 g water or oil in a centrifuge tubes, vortexed for 2 min, centrifuged at 4000 × g at RT for 9 min, weight was noted after water or oil decanted. Water or oil holding capacities were calculated and expressed as grams of water or oil for 100g DSF.

2.8 Total Polyphenol Content

Total polyphenol content (TPC) of DSF was measured in ethanolic extract[20]. The extract was added with Folin–Ciocalteau reagent and purple-blue colour was developed by addition of saturated sodium carbonate solution. The optical density of samples was measured at 675 nm in a UV-Visible spectrophotometer (Shimadzu UV-1800, Kyoto Japan). A calibration curve of gallic acid standard (Sigma Aldrich Chemicals Pvt. Ltd., Bangalore, India) using concentrations in the range of 19–76 µg/ml was plotted and the concentration of test samples were determined and expressed as mg of gallic acid per 100g sample. The total polyphenol content (mg/100g) was calculated as per the formula:

$$(mg/100g) = \frac{\text{µg of polyphenols from Total Volume Totla polyphenols calibration curve in the aliquot} \times \text{of Solution (50 ml)}}{1000 \times \text{Weight of the DSF}} \times 100$$

2.9 Antioxidant Activity

2,2-Diphenyl-1picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline, 6-sulphonic acid) radical scavenging activity of methanol extracts from DSFs were determined following a reported method by Nanjo *et al.* (1999)[21] and Yildrim *et al.* (2001)[22]. The IC_{50} values were computed as micromoles of Trolox Equivalents (TE) per gram of sample and presented.

3. Results and Discussions

3.1 Yield of Defatted Seed Flour (DSF)

Photographs of chilli varieties, seeds and defatted seed flour of Red chilli (R), Bajji chilli (B), Small chilli (S) and Capsicum (C) are presented in Figure 1. Fresh chillies yielded seeds after separation and tray drying yielded 3.3, 1.8, 1.9 and 0.4% R, B, S and C respectively. The yields of defatted seed flours are presented in Table 1. The yields of

Table 1. Composition of defatted chilli seed flour (DSF)

Parameter, %	R	B	S	C
Yield	66.92	75.12	77.95	72.95
Moisture	8.18	8.30	8.00	7.96
Total ash	4.01	4.30	3.87	5.36
Protein	25.90	20.09	19.61	24.09
Fiber	23.62	29.89	24.14	28.71
Carbohydrates	38.29	37.42	44.38	33.88
Energy kCal/100g	257	230	255	231
Minerals mg/100g				
Iron	8.95	11.42	6.26	7.08
Calcium	182	206	172	179
Phosphorous	627	587	398	640
T P C mg/100g	476	629	602	447
Hunter Colour Units				
L*	87.90	81.76	84.25	87.26
a*	1.55	1.93	1.51	0.78
b*	18.99	15.54	14.18	12.46

*Values are average of duplicate analysis for 2 consecutive years; R: Red chilli seed; B: Bajji chilli seed; S: Small chilli seed; C: Capsicum seed.

Figure 1. Photographs of chilli varieties, seeds and defatted seed flour; R: Red chilli; B: Bajji chilli; S: Small chilli; C: Capsicum chilli.

DSFs were 67-78% after de-fatting, drying and grinding of dried chilli seeds.

3.2 Moisture Sorption Isotherms

The equilibrium moisture content and relative humidity (EMC-RH) data of moisture sorption isotherms are presented in Figure 2. The DSFs of R, B, S and C chilli DSFs respectively had Initial Moisture Contents (IMC) of 8.8, 8.30, 8.0 and 7.96%, which equilibrated between 50-52% RH. Similarly, their critical moisture contents of 9.29, 9.15, 9.24 and 15.05%, which equilibrated at 85, 65, 65 and 85% RH respectively. Results indicated

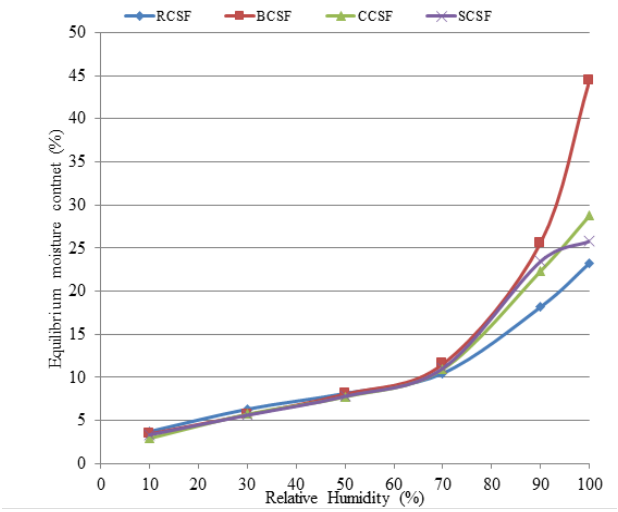

Figure 2. Sorption isotherms of defatted chilli seed flours.

that the DSFs are non-hygroscopic in nature. Hence, an economical flexible packaging material such polyethylene can be recommended for storage at room temperature.

3.3 Physico-chemical and Mineral Composition of DSFs

Hunter colour and chemical characteristics of DSFs of R, B, S and C are presented in Table 1. Defatted seed flours of R, B, S and C are rich in protein, minerals and fiber respectively. Hunter colour units such as L* (81.76–87.90), a* (0.78–1.93) and b* (12.46–18.99)were shown in Table 1. The visual colour of the seed powder was woody with a fibery feel. The DSFs are rich in protein (19.61–25.90%) and fiber (23.62–29.89%). Among the four DSFs, R was found to be rich in protein (25.90%) and B in fibre (29.89%). The DSFs were rich sources of mineral matter in which phosphorous (398-640mg/100g), calcium (172, 206mg/100g) and iron (6.2–11.4 mg/100g) were observed (Table 1). The total polyphenolic content was observed to be high (629 mg/100g) in B DSF. It was reported that grape seed contained 33.9 mg/g total polyphenolic content Miriana *et al.* (2017)[23].

3.4 Functional Properties

Functional properties such as WAC, OAC, FC and FS of DSFs are presented in Table 2. The DSFs exhibited higher water absorption capacities between 203–247g/100g than oil absorption capacities between 127–146g/100g. The phenomena can be explained based on the capacity of hydrophilic molecules with high hydrogen bonding. The foam capacity of DSFs were found to be between 56–100 ml, stable foam was observed in R (55%) and C (84%) DSF when compared to B and S DSFs respectively, which may be due to the presence of higher hydrophilic components.

3.5 Protein Solubility and Buffer Capacity

The protein solubility of DSFs in the pH range between 2–12 are presented in Table 2. Protein solubility was minimal over a wide pH range with 5.15–6.15% solubility of DSFs between ranges of 4–8 pH 4. The lower solubility might be attributed to the difference in the isoelectric points of the proteins. The highest solubility of protein in DSFs was found to be 34.58% at pH 12 in B and 17.72% in S.

Buffer capacity of DSFs was presented in Table 2. Higher buffer capacity values were observed in alkaline pH than in acid pH. Quantity of average alkali required per gram DSFs are 0.37–2.91 mmols to bring about a change of one pH unit. Similarly, average acid required to change of one pH unit are 0.97–2.17 mmols.

3.6 Fatty Acid Composition

The dried chilli seeds on hexane soxhlet extraction yielded fat content in the range of 16.56-27.35%. The fatty acid composition of chilli seed oil evaluated by GC and GC–MS data is presented in Table 3. The major fatty acids were 18:2 and 16:0 followed by 18:1. The total saturates were in the range 15.3-17.3%, monounsaturates were 8.6–10.6% and polyunsaturates were 72.2–74.3%. Similar observations (18:2, 70.59%) were reported[24]. Tomato and grape seeds possessed 44.8 and 46.5% linoleic acid (18:2) respectively[23]. The ratio of polyunsaturated to saturated fatty acids (PUFA/SFA) and the ratio of polyunsaturated to monounsaturated fatty acids (PUFA/MUFA) were found to be in the range 4.17–4.84 and 6.89–8.64 respectively in R, B, S and C oils, respectively. In the present study, low MUFA/SFA (0.50–0.69) ratio and very high PUFA + MUFA/SFA (4.78–5.54) ratio of chilly seed oils were observed. Similarly highly nutritious foxtail millet bran oils were found to be rich in polyunsaturated fatty acids

Table 2. Functional properties, protein solubility and buffer capacity of DSFs*

Parameter	R	B	S	C
Functional Properties				
Water Holding Capacity, %	227	247	226	203
Oil Holding Capacity, %	129	146	142	127
Foam Capacity, ml	56	70	92	100
Foam Stability ml (25 min.)	31 (55%)	22 (32%)	86 (NSF)	93 (NSF)
Protein solubility, %				
pH 2	8.46	12.50	6.68	32.00
4	7.30	5.83	6.14	17.00
6	6.15	6.75	5.15	7.50
8	7.69	16.66	13.19	17.20
10	18.46	33.33	15.16	24.00
12	23.84	34.58	17.72	24.69
Buffer Capacity mmols/100g				
2-6	0.98	1.75	1.60	2.17
6-12	2.22	2.26	2.58	2.91

*Values are average of duplicate analysis for 2 consecutive years; R: Red chilli seed; B: Bajji chilli seed; C: Capsicum chilli seed; S: Small chilli seed; NSF: Not stable foam

(83.47%) with very low saturated fatty acids[25]. "Higher the ratio of (n-3):(n-6) PUFA in the Western diet, incidence of chronic inflammatory diseases such as nonalcoholic fatty liver disease, cardiovascular disease, obesity, inflammatory bowel disease, rheumatoid arthritis, and Alzheimer's disease may be reduced"[26].

3.7 Antioxidant Activity

"The IC_{50} of a sample is inversely proportional to antioxidant capacity, as it expresses the amount of antioxidant required to decrease the DPPH and ABTS concentration by 50%. A lower IC_{50} indicates a higher antioxidant activity of a compound. Phenolics were the main antioxidant components, and their total contents were directly proportional to their antioxidant activity"[27].

Data on DPPH and ABTS radical scavenging activity are presented in Figures 3 and 4. Inhibition concentration of DPPH radical scavenging activity by 50% (IC_{50}) ranged between 4.1–8.0 mg/ml (14.61–7.49 µmol TE/g) of DSFs. Similarly, the IC_{50} for ABTS inhibition values were in the range 0.71–1.09 mg/ml (24.6–16.14 µmol TE/g). Presence of higher polyphenol, lower molecular weight proteins, and free amino acid content in the chill DSF samples might be responsible for the activity with both DPPH and ABTS radicals among which radical inhibition was higher with ABTS radical. It was reported that TEAC (Trolox equivalent antioxidant capacity) for pomegranate, tomato and grape seeds were 19.8, 9.8 and 178.2 µmol TE/g whereas oleoresins extracted using supercritical carbon dioxide showed 19.1, 4.5 and 3.4 µmol TE/g respectively

Table 3. Fatty acid composition of chilly seed varieties*

	% Composition			
Fatty acid	R	B	S	C
12:0, Lauric	-	0.4	-	0.2
14:0, Myristic	0.1	0.3	0.2	0.2
16:0, palmitic	14.2	13.0	14.1	11.7
16:1, palmitoleic	0.3	0.2	0.3	0.2
18:0, Stearic	2.2	2.2	2.3	2.6
18:1, Oleic	10.2	7.8	8.5	10.2
18:2, Linoleic	71.9	73.7	73.4	73.8
18:3, linolenic	0.3	0.6	0.4	0.3
20:0, dicosanoic	0.3	0.3	0.2	0.2
20:1, docosenoic	-	0.6	0.1	0.2
22:0, behenic	0.3	0.6	0.3	0.2
24:0, arachidic	0.2	0.3	0.2	0.2
Total Saturates	17.3	17.1	17.3	15.3
Monounsaturates	10.5	8.6	8.9	10.6
Polyunsaturates	72.2	74.3	73.8	74.1

*Values are average of duplicate analysis for 2 consecutive years; R: Red chilli seed;
B: Bajji chilli seed; C: Capsicum chilli seed; S: Small chilli seed

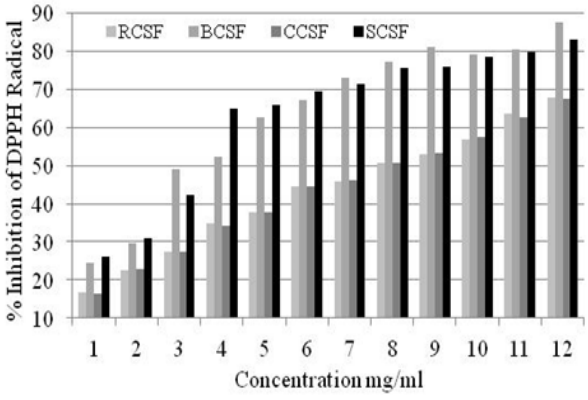

Figure 3. DPPH antioxidant activity of defatted chilli seed flours.

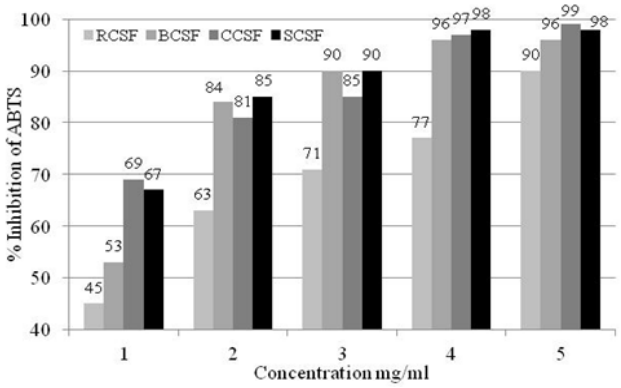

Figure 4. ABTS antioxidant activity of defatted chilli seed flours.

for pomegranate, tomato and grape seeds respectively[23]. "The total phenolic content in red chilli seed was reported in the range of 7.95–26.15 gallic acid equivalents (GAE mg/g). Antioxidant activity *via* DPPH assay of *n*-hexane and chloroform extracts showed 26.9% and 30.9% free radical scavenging abilities, at a concentration of 1 mg/mL"[28].

4. Conclusion

Chilli DSFs are good source of protein, fat, fiber and minerals specially iron 6.2-11.41 mg/100g DSF. Seeds yielded 16-27% fat, in which linoleic (~70%) and palmitice (~25%) are the major fatty acids. Highest protein solubility was observed at pH 2 and 12. DSFs are stable, non-hygrocopic in nature, which picked up moisture rapidly above 70% RH, hence, PE pouches can be recommended for storage at RT. Higher TPC was noticed in DSF of B and S chilli, which further conformed by DPPH and ABTS activity. This study indicated interesting antioxidant activity of defatted chilly seed powders for its use in functional foods and pharmaceutical application.

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