Extraction of protein concentrates from faba beans

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Abstract

Faba bean (Vicia faba) is a major food and feed legume with high protein content (20-41g/100g) with potential for extraction of protein concentrates and isolates for use in speciality foods. The study aims to develop process parameter to extract protein concentrates from faba beans for diverse food applications. Protein concentrates from faba beans were extracted by alkaline extraction process followed by isoelectric precipitation. The faba bean flour was suspended in water 1:10 ratio at pH 10 using 1N NaOH, continuously stirred at room temperature for 60 minutes and decanting at 4°C for 4 hours followed by centrifugation at 5100 rpm for 15 min. The supernatant was collected and adjusted to the pH 4.5 (isoelectric pH) using 50% citric acid solution, followed by centrifugation of the precipitated solution at 5100 rpm for 15 min. The pellets were collected and neutralized to the pH 7, spray dried (moisture content - 5.05%) and packed in polyethylene bags. The yield of protein concentrates was 15.4g/100 g of faba bean flour (60%) and the protein content of the extracted faba bean protein concentrate was 84.80%. The proximate analysis (moisture, protein, fat, carbohydrate and ash), solubility test, amino acid profile and SDS PAGE were analyzed. The major solubility of protein concentrates were at pH 2 and 10. The faba bean protein concentrates can be utilised in functional food product development, contributing to fortified or enriched protein content which finds wide application in speciality foods.

Keywords: Faba bean, alkaline extraction, isoelectric precipitation, protein concentrates

Introduction

Pulses have gained much interest of late with respect to their unique nutritional and functional benefits. Increasingly, pulses are being regarded as healthier choice of protein source compared to animal proteins. Further, the present day consumer demand is towards innovative foods which offer better nutrition and convenience. The preference for plant based proteins such as pulses have increased. Currently, there has been a surging interest towards utilizing pulse protein ingredients in novel food product development because of their cheaper price, accessibility, nutritional value, desired functional properties, innovativeness and health benefits.

Hence the prospects for utilisation of novel protein sources like faba bean (Vicia faba) for extraction of protein concentrates needs to be explored as a healthy alternative to existing soya bean concentrates which may pose allergenicity problems. Faba bean also known as fava bean or broad bean is a good protein source for extraction of proteins because of its low cost, less allergenicity and high protein content. Globulins and albumins are the major proteins present in pulses. Globulins represent totally 70 per cent of pulse seed proteins and consist mainly of the 7S, 11S and 15S proteins. The molecular weight cut off of these proteins ranges from 8000 to 600 000 Da (Freitas et al., 2004) with minimum solubility of proteins at pH 4.0-5.0 (isoelectric point).

The extraction of protein fractions from pulses depends on several factors such as pH, particle size, Solvent to flour ratio, type of salt used, ionic strength and temperature. Various extraction methods have been studied to improve the protein yield and purity without altering the functionality of protein concentrates or isolate. The techniques employed for extraction of pulse protein fractions (concentrate/isolate) with more than 70% protein are air classification and wet protein extraction methods (acid/alkaline extraction, micellization, alkaline extraction followed by isoelectric precipitation). The appropriate selection of protein extraction techniques and extraction conditions for protein is important as it has significant influence on nutritional and functional properties of the end products.

Functional and nutritional properties of food proteins which are most are important in food processing include gel formation, thickening, water and oil holding capacities, foaming and
emulsifying properties and solubility. These functional properties may influence the texture of the food and sensory characteristics which are essential in the development of desired food products such as beverages, confectionaries, dressings, meat analogues etc.

Faba beans are good sources of nutrients which contain 20–28 per cent of crude protein and the amino acids are moderately balanced especially with high lysine content which can necessarily supplement other protein sources like cereals. Faba beans (snowdrop variety), (released by the Saskatchewan Pulse Growers, Canada) was selected for the study since it has low tannin, low vicine and convicine content. Hence the present study aimed to extract protein concentrates from faba beans (snowdrop variety) and to study its functional characteristics for further food applications.

Materials and Methods
Raw ingredients
Faba beans (Snowdrop variety) were sourced from Protein Oil and Starch Biosciences, University of Saskatchewan, Saskatoon, Canada. The chemicals used for the analysis were purchased from Sigma-Aldrich.

Preparation of protein isolate
Faba beans were cleaned and ground into flour and suspended in water at 1:10 ratio. The suspension was stirred for uniform mixing in magnetic stirrer. The pH of the suspension was adjusted to 10 using 1 N NaOH and stirred for 60 min. at room temperature. The alkaline suspension was kept for decanting at 4°C for 4 hours. After 4 hours, the suspension was centrifuged at 5200 rpm for 30 min. The supernatant (protein solution) was collected and adjusted the pH to 4.5 using 50% citric acid to facilitate isoelectric precipitation of proteins. The precipitated protein solution was allowed for decanting for 30 min. The decanted solution was centrifuged at 5200 rpm for 30 min. and the protein pellets were collected and further neutralized to pH 7. The neutralized protein solution was spray dried and the spray dried powder (moisture content - 5.05%) was stored for further analysis.

Proximate composition analysis
Proximate composition of faba bean protein concentrates viz., moisture, protein, fat, carbohydrate and ash was analyzed using standard AOCS method.

Amino acid analysis
Amino acid analysis was carried out on faba bean protein concentrates at POS Bio-Sciences Corp. (Saskatoon, SK, Canada) utilizing acid/heat hydrolysis followed by quantification using chromatographic techniques. In brief, 20 mg of each protein concentrate sample were weighed into separate 20’150 mm screw cap Pyrex® tubes containing 15 mL of 6 M HCl. Each tube was then flushed with N2 gas. The tubes were then capped and placed into an oven at 110°C for 20 h. After acid hydrolysis, the individual amino acids were quantified by high-performance liquid chromatography using the Pico Tag amino acid analysis system (Waters Corporation, Milford, MA, USA).

Amino acid score
The amino acid profile of faba beans samples were used for determination of Amino acid score (chemical score). Amino acid score (FAO/WHO/UNU, 2008) [5] was calculated as:

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\text{Amino acid score} = \frac{\text{% essential amino acids in sample}}{\text{% essential amino acid recommended by FAO}}
\]

Gel Electrophoresis (SDS PAGE)
Prepared faba bean protein concentrates were solubilized in sample buffer (250µL). The electrophoresis was performed using 12% separating and 4% stacking gel on Bio-Rad Mini Protean 3 System (Bio-Rad Laboratories, Hercules, CA, USA). The loaded gels were run at constant voltage (60V) for 2.5h till the front dye shifted thoroughly far down the gel. Staining of gels was carried out by using Coomassie Brilliant Blue (CBB) and de-stained with methanol-water mixture (Tang and Sun, 2011) [15].

Functionality test (Solubility)
Protein solubility was determined using the method of Boye et al., (2010) [3] with slight modification. Dissolve 1% (w/w) protein solution (disperse 0.2g of protein concentrates – corrected on a weight basis for protein content) in 18g of 0.1 N NaCl. Adjust to the desired pH (pH 2, 4, 5, 7, 10) using 0.1 M NaOH or 0.1 M HCl, then bring the weight of the solution up to 20g with 0.1 N NaCl followed by stirring at 500 rpm for 1 hr at room temperature. Centrifuge the solution at 3500 rpm for 10 min. at room temperature. Determine the nitrogen content within the supernatant using a micro-Kjeldahl digestion and distillation unit (Labconco Corp., Kansas City, MO, USA). Percent protein solubility is calculated by dividing the nitrogen content of the supernatant by the total nitrogen in the sample (×100%).

Statistical analysis
The statistical design, completely randomized design (CRD) was applied for functional properties of faba bean proteins. Data were analyzed statistically by analysis of variance (ANOVA) using AGRES for Window version 10.0. Means with a significant difference (P<0.01) were compared by Least Significant Difference.

Results and Discussion
Extraction of Faba bean protein concentrates (FPC)
Protein concentrates from faba beans were extracted by alkaline extraction process followed by isoelectric precipitation. The optimized extraction conditions for faba bean protein concentrate were flour: water ratio – 1:10, stirring time – 1 hour, decanting time – 4 hours at 4°C and isoelectric pH – 4.5. The spray dried faba bean protein concentrate was packed in zip lock cover for further analysis.

Proximate composition of FPC
The proximate composition of the faba bean flour and faba bean protein concentrates were illustrated in Table 1. The moisture content of faba bean flour was decreased from 7.59 to 5.05 per cent when subjected to protein extraction process, since during extraction process the spray drier was used to dry the protein solution samples. Protein purity of the protein concentrates was increased (84.80%) fourfold times than in the faba bean flour (25.80%). Boye et al., (2010) [3] made similar observation on processing of pulse flours by both isoelectric precipitation and ultrafiltration methods wherein the concentration of proteins was increased 4-fold times which results in protein concentrates with the protein content in the range of 63.9- 88.6% (w/w). The carbohydrate and ash

\[
\text{Amino acid score} = \frac{\text{% essential amino acids in sample}}{\text{% essential amino acid recommended by FAO}}
\]
content also decreased during protein extraction process due to acid and alkali treatment. There was no significant change in fat content among the flour and protein concentrates because fat are alkali and acid resistant.

### Table 1: Proximate composition of faba bean flour and its protein concentrates

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatments</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>CHO (%)</th>
<th>Fat (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Faba bean flour</td>
<td>7.59±0.02</td>
<td>25.80±0.08</td>
<td>58.80±0.01</td>
<td>1.54±0.3</td>
<td>6.27±0.03</td>
</tr>
<tr>
<td>2.</td>
<td>Faba bean protein concentrate</td>
<td>5.05±0.01</td>
<td>84.80±0.68</td>
<td>3.37±0.009</td>
<td>1.50±0.05</td>
<td>5.24±0.004</td>
</tr>
<tr>
<td></td>
<td>SED</td>
<td>0.0158</td>
<td>0.4003</td>
<td>0.2105</td>
<td>0.0111</td>
<td>0.0198</td>
</tr>
<tr>
<td></td>
<td>CD (0.01)</td>
<td>0.0729**</td>
<td>1.8433**</td>
<td>0.9694**</td>
<td>0.0511*</td>
<td>0.0911**</td>
</tr>
</tbody>
</table>

**Protein yield and purity**
The protein content of faba beans of the selected variety was 25.80%. The yield of protein concentrates (% based on protein content) from 100g of faba bean flour was 15.4g (60%). The protein recovery from alkaline extraction followed by isoelectric precipitation was 60 per cent which is similar to the results reported for other plant proteins processed with similar method (Papalamprou et al., 2009) [3]. The purity of the extracted faba bean protein concentrates was 84.80 per cent.

### Amino acid profile
Amino acid profile of faba bean seeds revealed the amino acid composition of the protein (Table 2) which was similar to the amino acid composition of other legumes and it has a better nutritional quality except that it was deficient in sulphur amino acids and tryptophan when compared to FAO recommendations (FAO, 2008) [5]. This limitation of sulphur containing amino acids (methionine and cysteine content) of faba bean protein fractions indicated, it may be combined with cereals which are rich in sulphur containing amino acids and hence they complement each other to achieve good amino acid profile and to form more complete protein.

### Table 2: Amino acid profile of faba bean protein concentrate

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Faba beans IEP</th>
<th>FAO a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>9.69±0.26</td>
<td>-</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>15.8±0.05</td>
<td>-</td>
</tr>
<tr>
<td>Serine</td>
<td>5.15±0.06</td>
<td>-</td>
</tr>
<tr>
<td>Glycine</td>
<td>3.32±0.002</td>
<td>-</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.39±0.02</td>
<td>1.90</td>
</tr>
<tr>
<td>Arginine</td>
<td>7.71±0.02</td>
<td>-</td>
</tr>
<tr>
<td>Threonine</td>
<td>2.34±0.03</td>
<td>1.40</td>
</tr>
<tr>
<td>Alanine</td>
<td>3.31±0.01</td>
<td>-</td>
</tr>
<tr>
<td>Proline</td>
<td>3.44±0.03</td>
<td>-</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.74±0.007</td>
<td>6.30 d</td>
</tr>
<tr>
<td>Valine</td>
<td>3.63±0.02</td>
<td>3.50</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.56±0.01</td>
<td>2.50 f</td>
</tr>
<tr>
<td>Cystine</td>
<td>1.03±0.007</td>
<td>-</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>3.56±0.006</td>
<td>2.80</td>
</tr>
<tr>
<td>Leucine</td>
<td>6.64±0.07</td>
<td>6.60</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3.66±0.009</td>
<td>-</td>
</tr>
<tr>
<td>Lysine</td>
<td>5.31±0.009</td>
<td>5.80</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.70±0.004</td>
<td>1.10</td>
</tr>
<tr>
<td>Total amino acids</td>
<td>81.06</td>
<td>-</td>
</tr>
<tr>
<td>%EAA/TAA b</td>
<td>35.52</td>
<td>-</td>
</tr>
<tr>
<td>AAS c</td>
<td>102</td>
<td>-</td>
</tr>
</tbody>
</table>

*FAO (2008) [5]; a % Essential amino acids/total amino acids; b Amino acid score; c Tyr + Phe; d – Essential amino acids, FAO/WHO (children), and FAO reference are not available for non essential amino acid; e Met + Cys.

**SDS page**
The electrophorogram of SDS-PAGE for faba bean protein concentrates along with standard has been presented in Figure 1. The SDS PAGE profile of faba bean protein concentrate exhibited a wide variety of polypeptide subunits of molecular weight (Mw) between 10 and 150 kDa. The molecular weight of conglycinin components were between 48 to 75 kDa. The glycinin components with molecular weights of 30 kDa and 20 kDa approximately indicated that they are acidic and basic subunits, respectively. This electrophoretic protocol shows only subunits, because it was carried out in the presence of mercaptoethanol (reducing agent). The results obtained in faba bean protein concentrates SDS-PAGE profiles were in accordance with the results of Medrano and Del Castillo (2011) [12]; Souza (2000) [14]; Fassini (2010) [6] and Bittencourt et al., (2007) [2] for glycinin and conglycinin.
Solubility

Solubility is one of the important functional property which gives useful information regarding the efficient utilization of faba bean protein concentrates in wide range of food applications. The solubility of the protein concentrates was examined at pH of 2, 4, 5, 7 and 10. Graph 2 represents the results for the solubility of faba bean protein concentrates. The highest solubility was seen in low acidic (pH 2) and high alkaline pH (10). The solubility of faba bean protein concentrates was minimum at pH 4 and 5 (30%) because most legume proteins precipitated at pH 4-5. Studies conducted by various workers have also revealed that the protein solubility of pulses such as pea, faba bean and chickpea were lowest at the pH of 4-6 and highest at pH 8 and 9 (Fuhrmeister and Meuser, 2003) [8].

Conclusion

Faba beans are good sources of protein and hence can be gainfully utilized for the extraction of protein fractions. The extracted faba bean protein concentrates had 84.8 g/100g of protein purity with good amino acid profile. Hence it can be used as substitute for soy protein isolate in the development of protein enriched foods. The major solubility of protein concentrates were at the pH 2 and 10 which indicates that the faba bean protein concentrates can be utilized for the development of acid based protein enriched beverages and other food applications.

Acknowledgement

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References


