Influence of working methods in the extraction, separation and drying of pea proteins on protein fractions

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Abstract

The aim of this study was to compare extraction methods (Mechanical and ultrasound assisted extraction), and drying methods (dehydration with organic solvents and freeze drying) in order to evaluate the influence of tested methods on pea proteins structure. The comparative determination of protein fractions from samples was conducted by electrophoresis following SDS-PAGE protocol. Using electrophoresis were separated 12 protein fractions, (6 prevalent and 6 less prevalent), for every sample. The migration distances of protein fractions were the same for all samples, which prove that if correct parameters are used, the protein structure is not damaged by extraction and drying.

Key words: proteins extraction, pea protein isolates, electrophoresis, SDS-PAGE

Introduction

Soybeans are recognized as a rich source of vegetable proteins, and these proteins are widely used after extraction for producing different foods that can substitute different meat or milk products. The disadvantage of soybeans proteins is that these proteins can produce many allergic reactions for some people due to various antinutritive factors (Cordle, 2004). Pea (Pisum Sativum L.) is a species related with soy, which is also rich in proteins, but these proteins are better tolerating by human body, and produce less allergic reactions. Pea proteins can be extracted and used to improve the nutritional value of other foods.

The ultrasound assisted extraction is a modern extraction technique which is based on the acoustic cavitation phenomenon that takes place when a solution is placed in an ultrasonic field. This creates powerful currents in solution, which improve the mass transfer and increase the extraction yield (Knorr et al., 2004) (Mäntysalo and Mäntysalo, 2000). However, high intensity ultrasounds can produce changes in proteins structure, especially by breaking the large molecular chains (Gulseren et al., 2007). So, it is necessary to determine the structure of proteins extracted in the ultrasonic field, by comparing them with those extracted by mechanical stirring.

Also in order to obtain protein isolates, it is necessary to separate and dry the extracted proteins. The drying methods can also change the proteins structure.

Material and methods

In this study were extracted proteins from mature pea seeds, using the ultrasound assisted extraction and mechanical stirring extraction procedures. The extracted proteins have been dried by cryodessication and organic solvents dehydration.

1. Samples preparation; For protein extraction were used pea seeds from Kelvedon Wonder variety, harvested at complete maturity, produced by Agrosem company from Romania.
Pea seeds were grounded, and the flour obtained was strained through the sieve with 1mm mesh for uniform samples. From the pea flour, were prepared four samples of 1g each.

2. Proteins extraction; Every sample was treated by 19ml of 0.1% sodium hydroxide solution as solvent. Samples were left resting for 30 minutes at room temperature for hydration, and then extraction was performed by mechanical stirring, respectively by ultrasound assisted extraction for 15 minutes.

In all cases was used the 0.1% sodium hydroxide solution as the extraction solvent, because we have proved in our previous studies that it gave the best results for the pea protein extraction yield (Ianchici et al., 2008).

Mechanical stirring was achieved with a magnetic stirrer at 1200 rpm, and the ultrasonic extraction was done with an ultrasonic bath type Bandelin Sonorex with 80W power at the frequency of 35 KHz.

3. Proteins separation and drying; The protein extracts were separated from the plant residue by centrifugation and decanting. For the protein precipitation, 0.1N hydrochloric acid was added until reaching pH 5, which correspond to minimum solubility for pea proteins. The protein precipitates were separated from the aqueous phase by centrifugation and decanting, and then dried.

The major steps of pea proteins isolates preparation are shown in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Pea proteins isolates preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operation/material</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Mixing</td>
</tr>
<tr>
<td>Hydration</td>
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<tr>
<td>Extraction</td>
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<tr>
<td>method</td>
</tr>
<tr>
<td>Precipitation</td>
</tr>
<tr>
<td>Centrifugation</td>
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<tr>
<td>Re dissolving</td>
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<tr>
<td>Drying</td>
</tr>
</tbody>
</table>

Beside the four pea proteins isolates that were obtained (PMLRS, PULRS, PMSPI, PUSPI), it was also used as reference a commercial proteins isolate supplied by My Protein Co, from UK. This sample was named PMAUK. In a previous study, we have determined the molecular weight of each protein fraction from this product (Ianchici et al., 2009) The molecular weight of pea proteins is ranging between 12500 to 140000 Daltons.

4. Electrophoresis; For determining the protein fractions from protein isolates, we have used sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) protocol, which is most suitable for this purpose. (Hames, 1990)

The electrophoresis, was performed with the Mini Vertical Gel System, (EC120) from Thermo Electron Corporation with Power supply for electrophoresis, model Consort EV265.

Also there were used the specific reagents provided by Amresco Inc. which includes:
- SPRINT NEXT GEL 10% solution with acrylamide
- APS/TEMED polymerization tablets
- Sample loading buffer 4X
- NEXT GEL running buffer 20X
- K494 wide range protein molecular weight marker (8 bands from 14.0 to 212.0 kDa)
In order to prepare the gel plates were followed the steps described in the product technical support provided by Amresco Inc. (Amresco, 2008) Since the gel supplied had special gradient like properties, it was not necessary to prepare a stacking gel as in the conventional SDS-PAGE protocol.

For the sample preparation, prior to the electrophoresis, the protein solutions were diluted with distilled water in Eppendorf tubes. The resulted solutions were used for the electrophoresis assay as it is described in Table 2.

Table 2. Samples preparation for electrophoresis

<table>
<thead>
<tr>
<th>Sample</th>
<th>K494</th>
<th>PMAUK</th>
<th>PMLRS</th>
<th>PULRS</th>
<th>PMSPI</th>
<th>PUSPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock solution</td>
<td>30μl</td>
<td>10μl</td>
<td>10μl</td>
<td>10μl</td>
<td>10μl</td>
<td>10μl</td>
</tr>
<tr>
<td>Distilled water</td>
<td>-</td>
<td>40 μl</td>
<td>40 μl</td>
<td>40 μl</td>
<td>40 μl</td>
<td>40 μl</td>
</tr>
<tr>
<td>Sample loading buffer</td>
<td>10 μl</td>
<td>17 μl</td>
<td>17 μl</td>
<td>17 μl</td>
<td>17 μl</td>
<td>17 μl</td>
</tr>
<tr>
<td>Boiling on water bath</td>
<td>3min.</td>
<td>3min.</td>
<td>3min.</td>
<td>3min.</td>
<td>3min.</td>
<td>3min.</td>
</tr>
<tr>
<td>Transfer on plates</td>
<td>10μl</td>
<td>10μl</td>
<td>10μl</td>
<td>10μl</td>
<td>10μl</td>
<td>10μl</td>
</tr>
<tr>
<td>Electrophoresis parameters</td>
<td>200V, 40 min (SPRINT NEXT GEL 10%)</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

5. Visualization. After the electrophoresis was performed, the gel was stained overnight in a solution prepared from 40mg Coomassie Brillant Blue R250, 230ml water, 220ml methanol and 50ml acetic acid, according to a step protein staining method. (Chen et al., 1993). The protein fractions appear on the gel as blue bands on a light background. The gel was washed with water and then scanned for the results interpretation.

Results and discussion
The electrophoregram resulted from the gel scanned image is shown in Picture 1.

In the picture above, k494 is the protein molecular weight marker supplied by Amresco inc. (lane 1 and 7), PMAUK is the commercial pea protein isolate supplied by My Protein Co. (lane 2), and PMLRS, PULRS, PMSPI, PUSPI (lanes 3 to 6), are pea protein isolates that we have obtained according to procedure described previously.
Also we have noted M1 to M8 protein fractions from k494 MW marker, and F1, F2, F2a, F3, F4, F5, F5a, F5b, F6, F6a, F6b, F6c, protein fractions from pea protein isolates. According to picture 1, six predominant protein fractions and six secondary fractions were identified for these electrophoresis parameters. As seen from electrophoregram obtained from the protein isolates samples, (picture 1), for all samples are found the same protein fractions which have migrated at the same distance on the gel plate. The protein fractions from our samples correspond with the fractions from commercial protein isolate, which means they have the same molecular weight. In all cases were identified six fractions that are prevalent (F1, F2, F3, F4, F5, F6) and six fractions that are less prevalent (F2a, F5a, F5b, F6a, F6b and F6c)

Conclusions

For working parameters that were studied, any method of extraction (mechanical stirring or ultrasound assisted extraction) or any method of drying (cryodessication and organic solvents dehydration) did not alter primary structure, respectively the molecular weight of the pea proteins. Because ultrasound assisted extraction is more efficient than mechanical stirring this extraction method could be used for obtaining pea proteins isolates with better yield, and less energy consumption, without any changes in proteins structure.

References

***Amresco Inc. (2008). NEXT GEL™ Electrophoresis system, product technical support, USA.