

## The Identification of Main Proteins Fractions from Pea Protein Isolates

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**Abstract.** Pea's proteins represent a valuable source of edible proteins which are well tolerating by human body, and contain all essential amino acids. Mature pea's seeds are very rich in proteins that can be extracted in order to be used to improve the nutritional value of other foods.

Pea's proteins contain several fractions of albumins and globulins. We have used electrophoresis to separate and identify these fractions, to establish the molecular weight of each fraction, and also the proportion between them. The electrophoresis was conducted following the SDS-PAGE protocol.

Using pea protein isolate as sample, we have found a number of 12 protein fractions with molecular weights ranging between 12500 and 140000 Daltons. From these, six fractions are prevalent.

**Keywords:** pea proteins, proteins fractions, electrophoresis, SDS-PAGE

### INTRODUCTION

The animal origin proteins which are the most valuable for human nutrition are expensive because are obtained with low efficiency. Also the world population is continuously increasing and the food resources become limited. For this reasons there are many attempts to find other sources of edible proteins that can be used directly or after processing in alimentation. (Hamilton, 1991; Schaafsma, 2000)

Soy is 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. (Erdman, 1989; Messina, 1999; Montgomery, 2003) The disadvantage of soy proteins is that these proteins can produce many allergic reactions at some peoples due to various antinutritive factors. (Christopher, 2004)

Pea (*Pisum Sativum*) 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's proteins can be extracted and used to improve the nutritional value of other foods. (Savage, 1989; Slinkard, 1990)

In order to be used for food fortification proteins should be purified. First proteins are extracted using specific solvents, than are separated from extracts and then dried.

In order to determine the protein fractions, in this study we have used pea protein isolate produced by My Protein Co. from UK.

For determining the molecular mass of pea protein fractions we used sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) protocol, which is most suitable for this purpose. (Hames, 1990)

## MATERIALS AND METHODS

In order to determine pea protein fractions we have used pea proteins isolate supplied by My Protein Co. from UK. From this isolate we have prepared a stock solution with a proteins content of 10mg/ml. Because pea proteins have the maximum solubility in alkaline medium, for better dissolving we have adjust the pH to 8, by adding drop by drop 0,1% Sodium hydroxide solution, using a pH meter.

The obtained solution was then used to determine the main protein fractions by electrophoresis. For electrophoresis we used the Mini Vertical Gel System, (EC120) from Thermo Electron Corporation with Power supply for electrophoresis, model Consort EV265.

Also we used the specific reagents provided by Amresco Inc. which includes:

- 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 we have followed the steps described in product technical support provided by Amresco Inc. (Amresco, 2008) Since the gel supplied have special gradient like properties, it is not necessary to prepare stacking gel as in classical SDS-PAGE protocol.

For sample preparation, prior to electrophoresis, the stock protein solution was diluted with distilled water in Eppendorf tubes, and than used for electrophoresis assay according to procedure described in table1.

In parallel we have prepared a sample from K494 protein molecular weight marker provided by Amresco inc. (Tab.1)

Tab. 1

Samples preparation for electrophoresis

	MW marker k494	Pea protein isolate
Stock solution	30µl	10µl
Distilled water	-	40 µl
Sample loading buffer	10 µl	17 µl
Boiling on water bath	3minutes	3minutes
Transfer on plates	10 µl	10µl
Electrophoresis parameters	200V, 40minutes (SPRINT NEXT GEL10%)	

After the electrophoresis was performed, the gel was stained overnight in a solution prepared from 40mg Coomassie Brilliant Blue R250, 230ml water, 220ml methanol and 50ml acetic acid, according to one step protein staining method. (Chen, 1993)

The protein fractions appear on gel as blue bands on light background. The gel was washed with water and then scanned for results interpretation.

By measuring the migration distances for each fraction from K494 MW marker with known molecular mass we have determined the equation between the molecular weight and migration. Using this equation we have established the molecular weight for each band from sample.

In order to determine the proportion between proteins fractions from pea proteins we have used specialized software (UN-SCAN-IT gel) that measures and quantify the optical density of gel digital images.

## RESULTS AND DISCUSSION

Protein fractions contained by the sample, and molecular weight marker, separated in accordance with their molecular weight, are shown in Fig. 1. Concordantly to the gel image, we have also realised the diagrammatic disposal of bands corresponding to protein fractions.

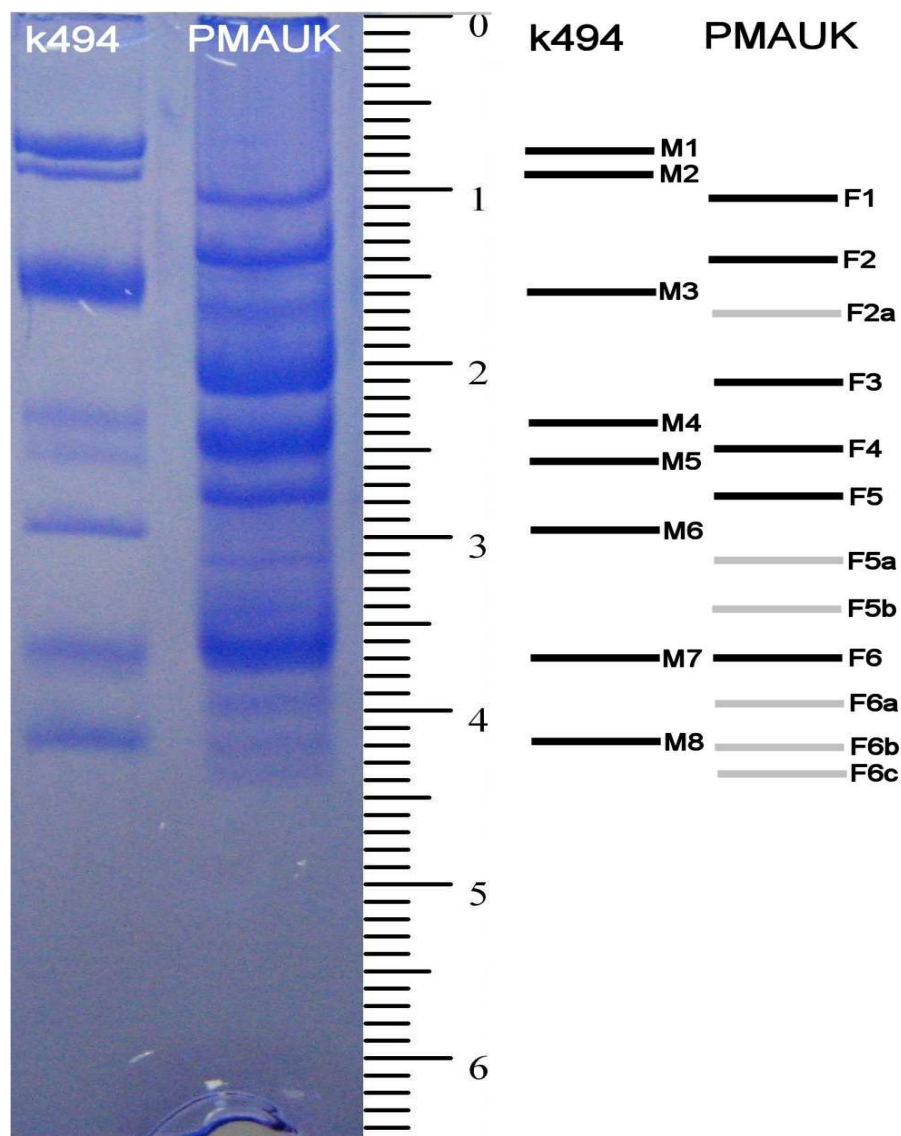


Fig. 1. Electrophoresis for pea protein and K494 MW marker (k494 – protein MW marker; PMAUK – pea protein sample)

For estimating molecular weights, first step was measuring the migration distance from the start for every protein fraction, both from juice sample and MW markers too. A second phase consisted of  $R_f$  calculation as a ratio of movement distance from the start and

the buffer movement. In accordance with Tab. 2 data, a calibration curve was outlined (Fig. 2).

Tab. 2

Migration data's for K494 MW marker fractions

Frac- tione	Fractions from K494	Molecular weight [Da]	Migration distance [mm]	Front migration [mm]	Rf	Log MW
M1	Miosin	212000	8	62	0,129	5,326
M2	$\beta$ -galactosidase	116000	9,2	62	0,148	5,064
M3	Phosphorylase $\beta$	94400	16	62	0,258	4,989
M4	BSA	66200	23,4	62	0,377	4,821
M5	Ovalbumine	45000	25,5	62	0,411	4,653
M6	Carbonic Anhydrase	31000	29,8	62	0,481	4,491
M7	Soybean Inhibitor	21000	37	62	0,597	4,322
M8	Lysosime	14400	41,8	62	0,674	4,158

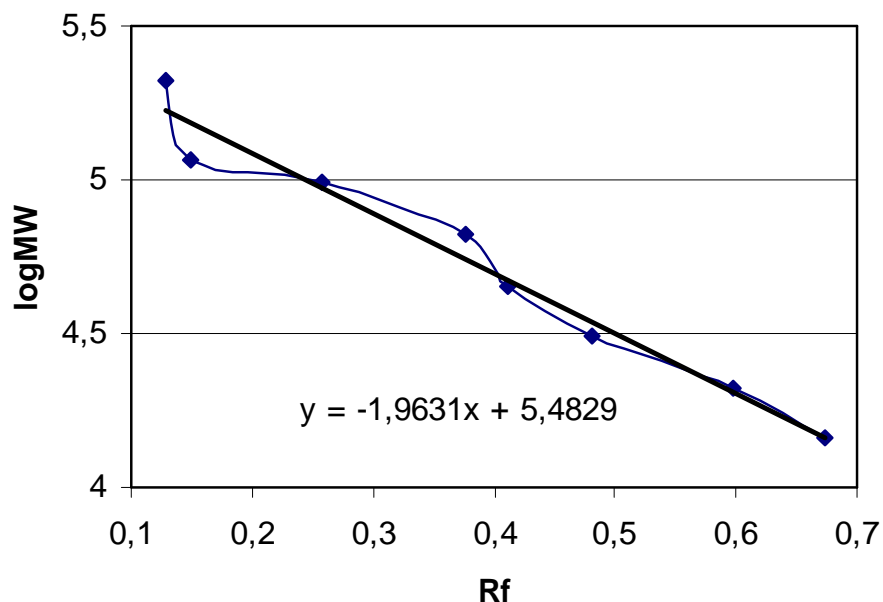


Fig. 2. Calibration curve for K494 MW marker fractions

Molecular weights of protein fractions contained by sample were established using the equation  $y = -1,9631x + 5,4829$  resulted from Fig. 2, and the migration distance for each band (Tab. 3).

Tab. 3

Determination of molecular weight for each fraction from pea protein

Fraction	Migration distance [mm]	Front migration [mm]	Rf	Log MW	Molecular weight [Da]
<b>F1</b>	10,6	62	0,171	5,147	<b>140000</b>
<b>F2</b>	14,1	62	0,227	5,036	<b>109000</b>

<b>F2a</b>	17,1	62	0,276	4,941	<b>87000</b>
<b>F3</b>	21,1	62	0,340	4,814	<b>65000</b>
<b>F4</b>	25	62	0,403	4,691	<b>49000</b>
<b>F5</b>	27,8	62	0,448	4,602	<b>40000</b>
<b>F5a</b>	31,4	62	0,506	4,489	<b>31000</b>
<b>F5b</b>	34,1	62	0,550	4,403	<b>25000</b>
<b>F6</b>	37	62	0,597	4,311	<b>20500</b>
<b>F6a</b>	39,7	62	0,640	4,226	<b>17000</b>
<b>F6b</b>	42,1	62	0,679	4,150	<b>14000</b>
<b>F6c</b>	43,8	62	0,706	4,096	<b>12500</b>

The density profile obtained after scanning using UN-SCAN-IT gel software is shown in Fig. 3.

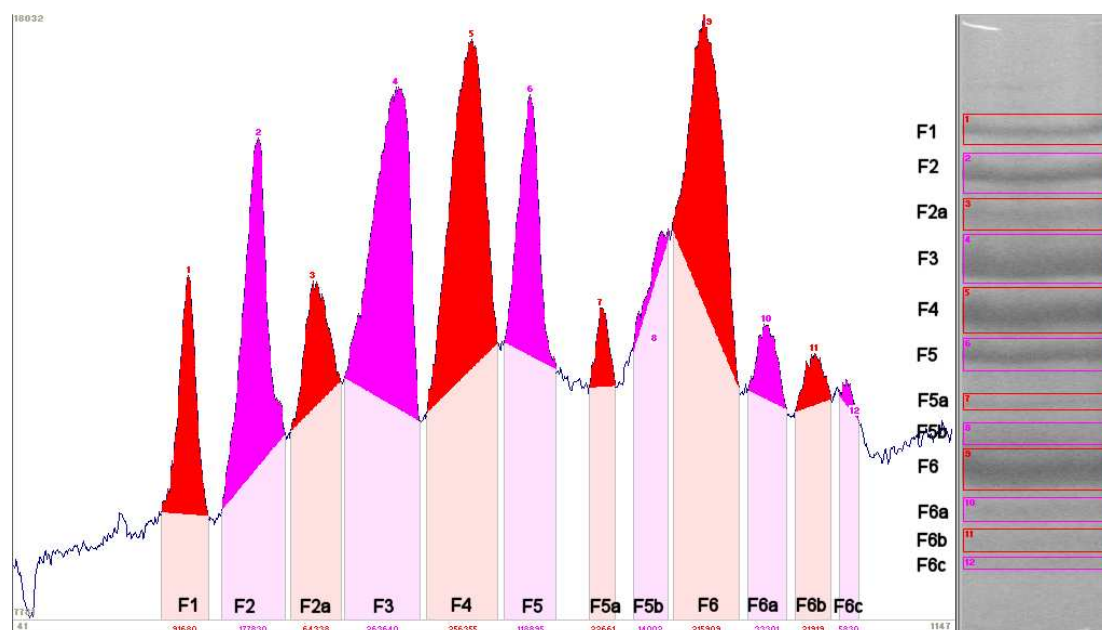


Fig. 3. Density profile for pea proteins fractions

From the data's resulted by quantification of the chart from figure 3, using UN-SCAN-ITgel software we have obtained the proportion between protein fractions. We have represented this proportion in the chart from Fig. 4.

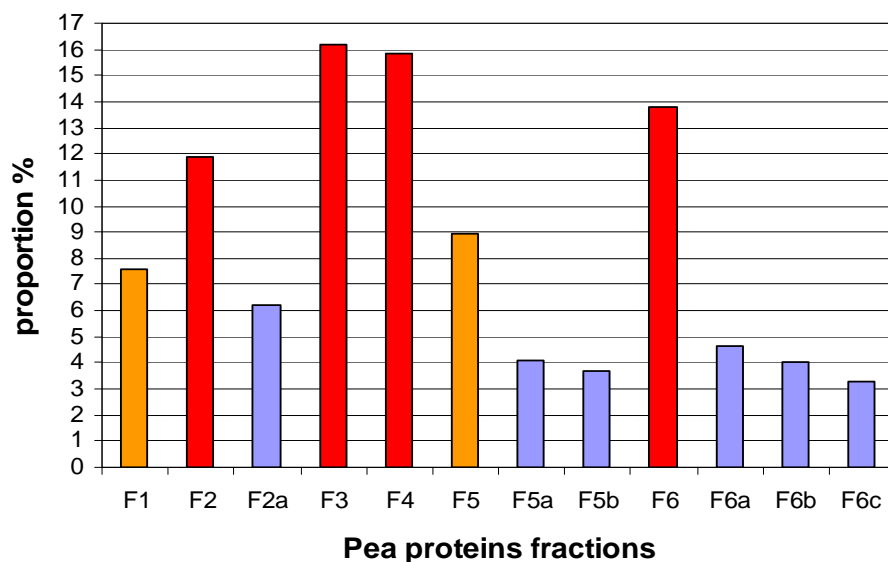


Fig. 4. Proportion between pea protein fractions

## CONCLUSIONS

According to the electrophoregram from Fig. 1, we have separated 12 protein fractions from pea isolate. Six fractions are prevalent noted with F1 to F6, and the others less prevalent, noted with F2a, F5a, F5b, F6a, F6b, F6c. The molecular weight of these fractions is ranging from 12500 to 140000 Daltons. (Tab. 3). The proportion between these fractions is shown in the chart from figure 4. Albumins fractions F3, F4 and F6 with molecular weights of 65000, 49000 and 20500 Da respectively, have the biggest concentration. Also globulin fraction F2 with a molecular weight of 109000 Da have a significant proportion.

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