Asian Journal of Chemistry Vol. 22, No. 3 (2010), 2027-2030

Effect of pH Variation on Solubility of Seed Protein of *Pisum sativum*

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Increased utilization of legume protein by the food industry has increased research in the utilization of legume or seed proteins in foods. Solubility is a critical functional property, since a protein generally has to be in solution in order to exert its other desirable functional characteristics. Nitrogen solubility index and protein dispersibility index (PDI) are the two most common methods of evaluating solubility characteristics. Hence in this study new variety breeder seeds (Arkel, Pusa pragati, IPF-99-25, JP-885, MM-15 and JM-6) of *Pisum sativum* are studied for protein solubility at wide range of pH from 0.5 to13.5. The knowledge of protein structure and size of different legumes seeds, of different varieties, will bring an understanding of the protein properties. This will permit manipulation of these properties for food product development.

Key Words: Protein solubility, Protein dispersibility index, Nitrogen solubility.

INTRODUCTION

Nutritional and functional qualities of protein are largely determined by its amino acid content and nitrogen solubility[1]. Nitrogen solubility is one of the aspects of hydration which is the most important characteristics in evaluating protein quality. Since many functional properties of protein depend upon their capacity to go into solution initially. Solubility is affected by many factors such as pH during extraction or solubilization, size of meal particle, temperature, meal solvent ratio, composition of solvent and character of protein[2-10].

Present study aims to determine solubility behavior of seed protein for new variety breeder seeds (Arkel, Pusa pragati, IPF-99-25, JP-885, MM-15 and JM-6) of *Pisum sativum* at different pH that may help in future understanding of functional properties of seed protein and is necessary for their successful extraction and purification in large quantities, so that these could be used as additives in the cereal diets of food product commonly marketed as supplementary protein to enrich their nutritive value. The new variety, healthy and matured legume seed (Arkel, Pusa pragati, IPF-99-25, JP-885, MM-15 and JM-6) of *Pisum sativum* under consideration are collected from Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, India were studied for their protein solubility at considerable wide pH range (0.5-13.5).

EXPERIMENTAL

The solublized proteins content was determined by semi-micro Kjeldahl method[11]. 1 g of seed powder was suspended in 20 mL extractant (aqueous medium) which had been already brought to the desired pH by adding either few drops of dilute hydrochloric acid or sodium hydroxide solution or by dilution of the contents as was found necessary. The contents were shaken in an electric shaker for about 2 h at room temp. (30 °C) and centrifuged for 20 min at 2000 rpm in a centrifuge. Nitrogen thus solubilized was determined by taking 10 mL aliquot of diluted digest was poured into the distilling flask of Kjeldahl apparatus. The silver condenser of distillation unit was dipped inside 20 mL of boric acid solution (aqueous and saturated) containing 2 drops of mixed indicator (consisting of 5 mL of 0.1 per cent bromocresol green and 2 mL of 0.1 % methyl red solution in ethanol, w/v) and in the mean time steaming was started to avoid back suction. About 20 mL sodium hydroxide (40 %, w/v) were then poured into the distilling flask to release ammonia. Steam distillation was carried out vigorously for 15 min. During this period, ammonia was completely released from the digest and absorbed into the boric acid solution. The absorption of ammonia was indicated by the change of colour from pink to bluish-green. Ammonia thus absorbed was titrated against 0.025 N hydrochloric acid till the indicator turned from bluish-green to light pink colour. This indicated end point.

A blank estimation was also carried out under the same conditions, using the same reagents and glucose in place of nitrogenous material in order to determine the impurities of ammonia if any in the reagents. Nitrogen content was calculated as follows:

$$\text{Gram nitrogen} = \frac{(V_2 - V_1) \times 0.025 \times 14}{100 \times W}$$

where, V_2 = Volume in mL of standard acid used in titration, V_1 = Volume in mL of standard acid used in blank titration, W = Weight of the sample in grams.

The soluble crude protein content of the seeds could be calculated by multiplying the total nitrogen content by factor 6.25.

RESULTS AND DISCUSSION

The seeds of *Pisum sativum* new variety Arkel, Pusa pragati, IPF-99-25, JP-885, MM-15 and JM-6 were studied for their protein solubility behaviours in considerable wide pH range from 0.5-13.5. The results are given in Table-1.

The determination of per cent of total proteins (21.6095 %) of *Pisum sativum* variety Arkel solublized showed the maximum solubility of 19.7415 % at pH 13.0. While it was minimum (2.6109 %) at pH, 3.5, (3.2478) at pH 5.0 and (4.0969) at pH 10.0. At the remaining pH, solubility of the proteins was found to vary between 5.2219-18.9561 %.

TABLE-1
EFFECT OF pH VARIATION ON THE SOLUBILITY OF SEED PROTEINS OF SIX NEW VARIETIES OF LEGUMINOUS BREEDER SEEDS OF *Pisum sativum*

pH Value	Arkel	Pusa pragati	IPF-99-25	JP-885	MM-15	JM-6
0.5	17.4065	16.1329	16.7697	15.7720	15.9843	14.7106
1.0	17.8947	15.5597	17.4702	16.5574	16.7697	17.1093
1.5	11.5902	11.5265	11.6751	13.2247	9.4037	10.9533
2.0	8.7032	4.2455	5.0946	3.9483	4.7974	4.8823
2.5	10.2528	10.4014	5.0946	4.9460	7.4933	10.8897
3.0	6.3682	4.7974	5.8587	6.6442	4.9460	5.7951
3.5	2.6109	4.0332	3.1841	4.2455	4.3728	3.5237
4.0	7.5569	2.3350	2.8869	2.6746	3.5237	3.0355
4.5	5.2219	4.3728	2.4623	2.8232	2.0378	2.3350
5.0	3.2478	3.6086	3.7360	3.0355	2.6109	3.0992
5.5	7.3447	9.6160	8.3424	14.0100	9.4674	15.7720
6.0	5.7951	6.3682	6.5805	4.2455	5.3068	5.4342
6.5	8.6183	6.9201	4.0969	5.7314	6.8564	12.3119
7.0	9.6160	8.2787	4.5214	4.5851	6.1559	10.4014
7.5	11.4628	10.8260	3.1841	4.2455	5.7314	5.5191
8.0	8.9155	8.0664	4.8823	5.5191	5.5191	6.1559
8.5	7.6419	4.0332	8.4910	4.2455	5.5191	14.8592
9.0	6.3682	4.8823	3,8209	6.5805	15.0715	11.8874
9.5	15.0715	10.4014	6.7923	7.6419	10.8260	7.2173
10.0	4.0969	8.4060	5.0946	3.6723	4.3091	3.9483
10.5	7.2810	16.6848	3.2478	17.9584	8.4910	19.6566
11.0	18.7438	19.6566	19.5293	18.1707	8.8306	12.8638
11.5	9.8283	18.5316	16.1965	6.2833	6.1559	5.8587
12.0	3.3964	13.3733	3.6723	4.3091	3.1841	3.4600
12.5	18.9561	17.6188	17.4702	17.0456	16.9820	16.4725
13.0	19.7415	20.5057	19.5293	16.9820	17.3216	19.3807
13.5	15.6234	13.6492	11.3142	13.0124	14.6469	11.1656

The solubility of total seed proteins (21.4713 %) of variety Pusa pragati was minimum (2.3350 %) at pH 4.0 and (3.6086) at pH 5.9 solubility of seed proteins was observe to be maximum (20.5057 %) at pH 13.0 and (19.6566) at pH 11.0. The solubility of the proteins was found to fluctuate between 4.0332-18.5316 % at the remaining pH.

The determination of per cent of total proteins (20.0953 %) of IPF-99-25 variety solublized showed the maximum solubility of 19.5293 % at pH 11.0. and pH 13.0. While it was minimum (2.4623 %) at pH 4.5, (2.8869 %) at pH 4.0. At the remaining pH, solubility of the proteins was found to vary in between 3.1841-17.4702 %.

The percentage of total proteins (18.1383 %) of JP-885 variety, solubility was found to be the maximum (18.1707 %) at pH 11.0. While it was minimum (2.6746 %) at pH 4.0 and (2.8232) at pH 4.5. At the remaining pH, solubility of the proteins was found to fluctuate between 3.0355-17.0456 %.

The solubility of total seed proteins (20.2454 %) of MM-15 was found to vary in between 5.17-27.59 %. Solubility of the proteins was observed to be maximum (17.3216 %) at pH 13.0 and minimum (2.0378 %) at pH 4.5. At the remaining pH, solubility of the proteins was found to vary between 3.5237-16.9820 %.

The solubility of total seed proteins (19.3878 %) of JM-6. Solubility of the proteins was observed to be maximum (19.6566 %) at pH 10.5 and (19.3807 %) at pH 13.0. Minimum (2.3350 %) at pH 4.5. At the remaining pH, solubility of the proteins was found to vary between 3.5237-16.9820 %.

It was found that alkaline medium were more effective in extraction of protein from food legume. As the acidity was increased, solubility drastically reduced rapidly and minimum is observed. This is isoelectric region. Almost similar solubility behaviour has been observed towards their protein solublization at different pH in early reported for other varieties of legume seed proteins[3-5,7].

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(*Received*: 14 May 2009; *Accepted*: 25 November 2009) AJC-8090