SOLUBILITY OF SUNFLOWER PROTEINS AND GEL FILTRATION CHROMATOGRAPHY OF THEIR WATER-SOLUBLE FRACTIONS

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Summary

Effect of pH on the solubility of sunflower proteins was studied. The isoelectric points were found to be at pH 4.1, 5.4 and 5.6 for water, salt-soluble proteins and the protein isolate, respectively. The water-soluble sunflower proteins (albumins) were fractionated into seven components on Sephadex G-100 column (phosphate buffer pH = 7.8).

The spectrophotometric analyses revealed the contamination of all fractions with varying amounts of nucleic acids and the association of chlorogenic acid with the last protein fraction emerged from the Sephadex G-100 column.

Introduction

It has been established, by the Food and Agriculture Organization of the United Nations [1, 2], that at least 500 millions people suffer from severe protein-calorie malnutrition. Protein deficiency is common among large sections of the world population, due to shortage of protein and therefore, it is imperative that new sources of potential foodstuffs from currently unexploited resources would be investigated.

Study of sunflower seed protein fractions extracted by water and salt solution was reported by Tyurina and Klimenko [3]. This study showed that the saltsoluble fraction contained mainly components of seed storage material, while the maximum amount of electrophoretic components was revealed in the water soluble fraction. They used DEAE-cellulose chromatography, as well as paper and polyacrylamide electrophoresis in their study.

Baudet and Mossé [4] fractionated the salt soluble proteins into three groups. Their proportions were roughly: 20% for light (molecular weight,

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14.000) albumins, 5—10% for heavy (molecular weight; 48.000) albumins, and 70—80% for globulins.

The purpose of this work was to study the effect of pH on the solubility of the proteins of sunflower cultivated in Egypt. Further, fractionation and some physico-chemical properties of water-soluble proteins (albumins) were also investigated.

Materials and methods

The sunflower (Helianthus annus variety, Giza 1) seed used in this study were obtained from the Ministry of Agriculture Experimental Farm at Giza, Egypt.

The sunflower meal

After dehulling the seeds, the lipids were extracted from the ground sunflower kernels with cold acetone in a blender. The defatted meal was air-dried and stored in a closed container at 0 °C until used.

Removal of colour forming phenols from sunflower meal

Colourless protein concentrate was obtained from defatted sunflower meal by means of five consecutive extractions of the meal with acidic 1-butanol (1 : 20, w/v) for 15 min at room temperature [5].

Extraction of proteins for solubility studies

Defatted sunflower free of chlorogenic acid meal (SFFCM) (10 g) was extracted twice with 100 ml of distilled water for 1 hr. The extracts were pooled, centrifuged at 5000 rpm for 30 min and filtered through Whatman No. 1 paper. The clear supernatant contained the water-soluble albumins. The insoluble materials were collected, washed twice with distilled water and suspended in 200 ml of 1 *M* sodium chloride solution. It was stirred for 1 hr, centrifuged at 5000 rpm for 30 min and filtered. The clear filtrate contained the salt-soluble globulins.

Preparation of protein isolate

Defatted sunflower meal (10 g) was extracted with 150 ml of distilled water for 1 hr at room temperature under magnetic stirring. The pH was kept constant at 9.0 by the addition of 0.5 *N* sodium hydroxide solution. After

centrifugation at 15,000 rpm for 20 min the pH of the clear supernatant was adjusted to pH 5.5 by adding 0.5 *N* HCl. The resulting precipitate was collected by centrifugation and freeze dried. The protein fraction obtained was coded protein isolate (PI).

Effect of pH on solubility

Solubilities of water-, salt-extractable proteins and protein isolate (PI) at different pH values were determined using protein solutions (1 mg/ml). The aliquots of 10 ml were adjusted to the desired pH by the addition of either 1 *N* NaOH or HCl and magnetically stirred for 5 min. After leaving overnight at 4 °C, the solutions were centrifuged at 5000 rpm for 30 min. The protein concentration in the supernatant was determined by Lowry method [6].

Extraction of protein for fractionation

The fat-free sunflower meal (15 g) was homogenized in 0.1 *M* Tris buffer (pH 7.6) containing 0.5 *M* NaCl and 10 m*M* mercaptoethanol (225 ml). The mixture was stirred for 2 hrs at room temperature (25 °C). Insoluble materials were removed by centrifugation at 15,000 rpm for 30 min. The clear supernatant was dialyzed against distilled water for 3 days at 4 °C. The precipitate formed during dialysis was removed from the supernatant by centrifugation, washed twice with distilled water and freeze dried (globulin fraction). The supernatant was freeze dehydrated without further treatment (albumin fraction).

Gel filtration of sunflower albumins

The freeze-dried sunflower albumins (152 mg) were dissolved in 4 ml of 46 m*M* phosphate buffer, pH 7.8, and loaded onto the top of Sephadex G-100 column (2.5 × 79 cm) previously equilibrated with the same buffer. Using Blue Dextran-2000 the void volume (V_0) was found to be 144 ml. The proteins were eluted from the column by the above buffer and fractions each of 5 ml were collected at a flow rate of 20 ml/hr. The absorbance of each fraction was monitored at 280 and 328 nm for protein and chlorogenic acid content, respectively. A portion of some fractions were analysed by Lowry [6] method at 750 nm. Absorbance readings were plotted against tube number.

Ultraviolet spectrum

The spectra of the protein fractions were recorded in the range of 240 to 340 nm with Unicam SP 1800 doublebeam recording spectrophotometer.

Results and discussion

Protein solubility relative to pH

The solubility profile of a protein provides some insight into the extent of denaturation or irreversible aggregation and precipitation which may have occurred. It also gives an indication of the types of foods or beverages into which the protein might be incorporated. Factors such as concentration, pH, ionic strength, and the presence of other substances influence the solubility of a protein. Solubility properties relative to pH of sunflower proteins prepared with different extract media are presented in Fig. 1. Proteins from extraction media containing 1.0 *M* sodium chloride had isoelectric point at pH 4.1, while those of water and protein isolate were at pH 5.4 and 5.6, respectively.

As shown in Fig. 1, the solubility of water extractable proteins is greater than 85% at pH 2.0, decreasing to 50% at pH 3 and to 3% at pH 5.4 (isoelectric point). Upon comparing the solubility profile of water extractable proteins with that of protein isolate, it is apparent that the solubility of the protein is reduced as a result of the isoelectric precipitation. At pH 2.0, as well as, at pH 10 and above the solubility of the sunflower water extractable proteins is higher than that of its protein isolate. This change in solubility can be attributed to some irreversible transformations in the tertiary and possibly in the secondary

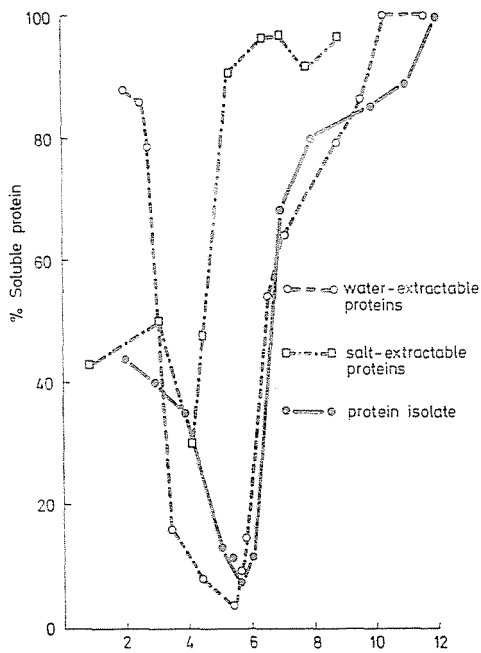

Fig. 1. Solubility profiles of sunflower proteins

structure of the protein during the preparation of the protein isolate. The solubility profile of salt extractable proteins (globulins) showed that the solubility at the isoelectric point was about 30% which is greater than that of water extractable (about 3%) and that of protein isolate (about 8%). The high solubility of the extract containing sodium chloride, at the isoelectric point, may be related to the activity coefficient of protein charged groups or to salting [7]. Further on, salt concentration decreases the influence of pH on protein solubility [8, 9]. At pH 2.5 and 0.9, protein solubility of globulins increased above that at the isoelectric point, and then decreased, respectively as a result of acid denaturation. In contrast to our results, a sharp solubility minimum, for sunflower proteins, was not observed by several investigators [10, 11]. However, Rahma et al. [19] reported that chlorogenic acid-free sunflower meal and the protein isolate gave fairly sharp solubility minimum around pH 6.0.

Fractionation of sunflower albumins on Sephadex G-100 column

Gel filtration chromatography was used to determine the components of sunflower water-soluble proteins. The elution profile of these proteins from the Sephadex column is presented in Fig. 2. It is apparent that water-soluble albumins can be separated into seven fractions—four large and three small—labelled a—g in the order of their emergence from the column. The first fraction was eluted immediately after the void volume (Table 1).

On the other hand, the smallest protein was eluted under the lower fractionation range of the column (below molecular weight 5000). The seventh fraction (Fraction g) showed far greater absorption at 280 nm than the higher molecular weight fractions. However, the results of protein examination at 750 nm by Lowry method indicated that fraction g contains smaller amount of protein than fractions a, b and e (Fig. 2).

Chlorogenic acid, which absorbs at 328 nm, has been identified by numerous investigators as the principal phenolic acid in sunflower meal [12]. This acid was associated with low molecular weight fraction (Fraction g). A similar finding was reported by Sabir et al. [13, 14] but Rahma and Narasiga Roa [19] found that chlorogenic acid was associated with all three major peaks resulted by the gel filtration of salt extractable sunflower proteins. Dialysis removed a high proportion of the chlorogenic acid from the protein extract but a significant amount was still detected in the seventh fraction (Fig. 2). Apparently, this phenolic acid was bound to the proteins. It is known that phenolic acids are also capable of strong hydrogen bonding with the Sephadex dextran gels producing chlorogenic acid-protein complex that would be retarded in elution during the protein fractionation [15].

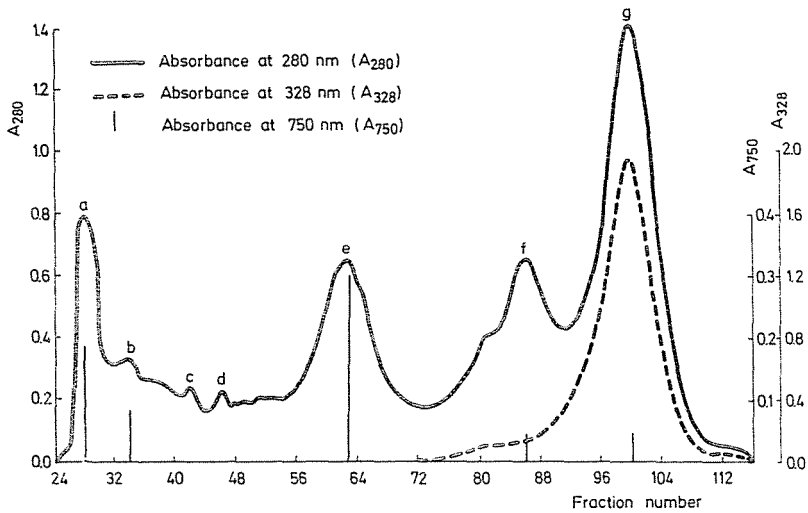

Fig. 2. Gelfiltration patterns of sunflower albumins (Sephadex G-100 column, phosphate buffer pH = 7.8)

Table 1
Ultraviolet absorption characteristics and Ve/Vo of the major protein fractions obtained from Sephadex G-100 column

Fraction	max. nm	280/260 nm	Ve/Vo
a	277	0.77	1.00
b	278	0.73	1.18
e	276	0.81	2.18
f	—	0.50	2.99
g	328	1.43	3.47

This would explain why proteins having failed to be dialysed from the salt extractable protein (over molecular weight of 12,000) were eluted under the lower fractionation range of the column (below molecular weight of 5000).

Ultraviolet spectrophotometric analyses demonstrated low 280/260 nm ratios, for most fractions, indicating varying degrees of contamination with nucleic acids (Table 1) [13, 14, 16, 17]. Pure nucleic acids absorb at 260—265 nm and fraction *f* with its lowest 280/260 nm ratio appeared to contain highest level of nucleic acid [18] and the ultraviolet absorption spectrum of this fraction (Fig. 3) supported that conclusion.

The absorption spectrum of fraction g (Fig. 3) shows a characteristic peak (at 328 nm) for chlorogenic acid which demonstrated the association of this

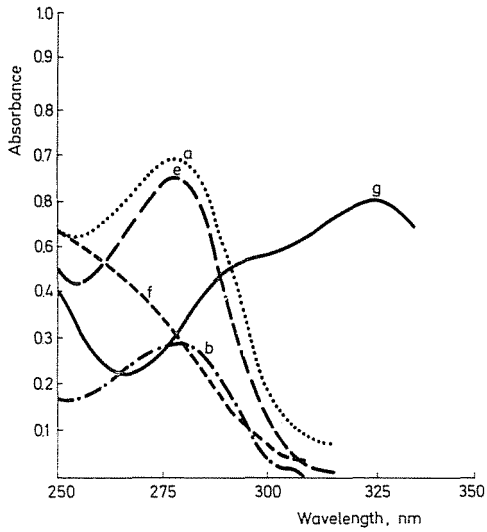

Fig. 3. Uv-absorption spectra of some fractions presented in Fig. 2

acid with the protein fraction. In contrast, other fractions exhibited essentially no spectrophotometric evidence of chlorogenic acid association.

The ultraviolet absorption spectra of fractions a, b and e were characterized by absorption maxima at 277,278 and 276 nm, respectively and minima at 250—255 nm (Fig. 3).

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