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# SOME PROPERTIES OF THE PROTEIN FRACTIONS OF TOMATO SEEDS

**G. A. Piyakina, V. V. Maksimov, and T. S. Yunusov**

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*The solubilities and rheological properties of interphase adsorption layers (IALs) of the protein fractions of tomato seeds have been studied. Regions of minimum solubility have been determined. It has been established that the globulin fraction exhibits pronounced surface properties, forming strong IALs at liquid phase-separation boundaries. A correlation has been found between the rheology of IALs and the stability of emulsions of the oil-in-water type.*

The isolation of food protein from secondary plant raw material and its use in the food industry enables various food products with improved properties to be obtained and decreases the polluting influence of the industry on the environment. There is no information in the literature [1-3] on the fractional composition of tomato seed proteins or on the structure of the quantitatively most important protein components and their functional properties. In this connection, we have studied the properties of the protein components of tomato seeds, since, in the final account, the properties of the individual protein components determine the properties of isolates and concentrates as a whole.

We have previously [4] investigated the chemical composition of the proteins of tomato seeds and skin and have given a general characterization of them. It was established from the results of electrophoresis in PAAG (polyacrylamide gel) that the albumin fraction consisted basically of a single component.

We have investigated the seeds of tomatoes of the Volgogradskii variety. The total yield of protein from a flour of the seeds was 39.4%, and the amount of extractable protein was 34%, or 86.3% on its amount in the flour. The yield of protein from the extract on isoelectric precipitation was 16.1%. The yields of the main protein fractions — albumins, globulins, and readily-soluble globulins — were 9.21, 12.3, and 3.2%, respectively. In the purification process (dialysis in distilled water) the albumin fraction separated into a precipitate and a supernatant liquid, which were analyzed separately. As shown in [4], according to the results of gel electrophoresis, the precipitate from the albumin fraction coincided in electrophoretic mobility with the proteins of the globulin fraction, i.e., during extraction part of the globulins had passed into the water-extracted fraction.

The quantitatively main component of the albumin fraction was isolated from the supernatant liquid by fractional precipitation in the cold with ethyl alcohol (50% by volume). Two fractions were obtained — a precipitate (protein content — (N 10.1% × 6.25) = 63%) and a supernatant liquid ((N 9.5% × 6.25) = 59.4%) — with yields of 38 and 44%, respectively. Both fractions gave a qualitative reaction for carbohydrates (phenol–sulfuric acid reagent).

The purity of the protein fractions obtained after precipitation with alcohol was confirmed by TLC on plates with cellulose in the butanol–acetic acid–pyridine–water (15:3:10:12) system (Fig. 1).

By electrophoresis in a block of 15% PAAG in the presence of 0.02% Na DDS we determined the fractional composition of the albumin fraction and showed its inhomogeneity (Fig. 2, track 1). As can be seen from Fig. 2 (track 4), the quantitatively main component of the albumin fraction was shown by a single broad band. The molecular mass of the component under investigation ranged between 6 and 13 kDa. As marker proteins we used insulin and ribonuclease.

Then a fraction (supernatant liquid after precipitation with alcohol) was separated on a column of Sephadex G-50f in 30% acetic acid. As can be seen from Fig. 3, three fractions were obtained, which were analyzed from their absorption at $\lambda$ = 280 nm. According to TLC (Fig. 1) fractions 1 and 3 were heterogeneous. Fraction 2 was the main one quantitatively.

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Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 62 73 48. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 537-540, July-August, 1998. Original article submitted January 16, 1998.



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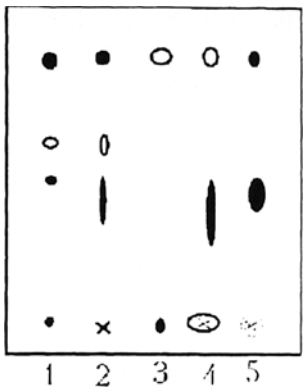


Fig. 1

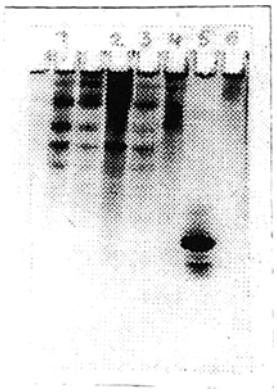

Fig. 2

Fig. 1. Thin-layer chromatography of the protein fractions of tomato seeds: 1) albumin fraction; 2) supernatant liquid (precipitation with alcohol); 3) fraction 1 from a Sephadex G-50f column; 4) fraction 2 from the same column; 5) fraction 3 from the same column.

Fig. 2. Electrophoregrams of protein fraction of tomato seeds in 15% PAAG, 0.02% Na DDS, pH 8.9; 1) albumin fraction; 2) readily soluble globulins; 3) precipitate from the albumin fraction (alcohol precipitation); 4) supernatant liquid from the albumin fraction (alcohol precipitation); 5) insulin; 6) ribonuclease.

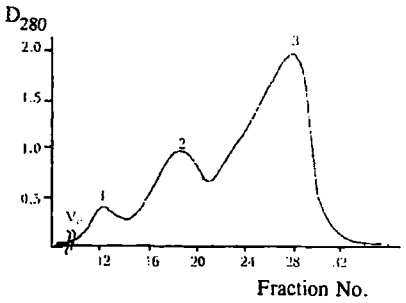


Fig. 3

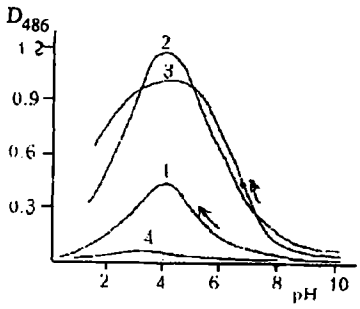


Fig. 4

Fig. 3. Gel chromatography of the supernatant liquid (alcohol precipitation, Sephadex G-50f, 30% acetic acid).

Fig. 4. Turbidimetric titration curves (concentration of protein in the solution 0.06%): 1) albumin fraction; 2) readily soluble globulins; 3) globulin fraction; 4) supernatant liquid from the albumin fraction (alcohol precipitation).

We studied some functional properties of the protein fractions from tomato seeds. Solubilities were evaluated by turbidimetric titration up to the pH of maximum precipitation. Figure 4 gives curves of the turbidimetric titration of the albumin and globulin fractions. It can be seen that the pH of maximum precipitation of the albumin fraction was between 3.5 and 4.0, while for the globulin fraction the range of the precipitation pH was broader (pH 3.8-6.2) and the readily soluble globulin precipitated at pH 3.5-4.6. The main component of the albumin fraction (after precipitation with alcohol) gave practically no clear peak.

One of the most important functional characteristics determining the consumer properties of the finished products is its emulsifying capacity. The rheological properties of the IALs of tomato proteins at liquid phase separation boundaries have

TABLE 1. Influence of the Protein Concentration on the Rheological Properties of Tomato Protein IALs at a Benzene–Water Phase Separation Boundary

| Protein concentration, % | Albumins | | Globulins | |
|---|---|---|---|---|
| | $P_{sr}$, mN/m | $P_{ss}$, mN/m | $P_{sr}$, mN/m | $P_{ss}$, mN/m |
| 0.02 | 0.033 | | 3.5 | 1.85 |
| 0.1 | 0.495 | 0.33 | 4.3 | 2.7 |
| 0.5 | 0.39 | 0.35 | | |

**Note.** (T = 293 K; pH = 7.0; I = 0; W = 0.01 rad/s; time of formation of the layer 5 h).

TABLE 2. Stability of Benzene Emulsions Stabilized by Tomato Seed Proteins

| Protein concentration, % | Globulin fraction $\tau_{1/2}$, s | Albumin fraction $\tau_{1/2}$, s |
|---|---|---|
| 0.1 | Stab. | Stab. |
| 0.02 | Stab. | Stab. |
| 0.01 | Stab. | 5400 |
| 0.004 | Stab. | 2000 |
| $8 \times 10^{-4}$ | 100 | 180 |
| $1.6 \times 10^{-4}$ | 30 | 60 |
| $3.2 \times 10^{-5}$ | 10 | 30 |
| $6.4 \times 10^{-6}$ | 10 | 10 |
| $1.3 \times 10^{-6}$ | 10 | 10 |

**Note.** (pH = 7.0; T = 293K; speed of rotation of the disperser 3000 rpm; time of emulsification 2 min; ratio of the phases, benzene-water (1:4, v/v).

been studied by the disk torsion method [5]. Table 1 gives results concerning the influence of protein concentration on the rheological properties of IALs of the albumin and globulin fractions at a water–benzene phase separation boundary.

It follows from Table 1 that the globulin fraction of tomato seeds exhibited pronounced surface properties, forming strong IALs at liquid phase separation boundaries with a value of the interphase strength of 4.3 mN/m ($C_p \doteq 0.1\%$; 5 h). On the other hand, for the albumin fraction the IALs formed possessed a low strength (0.495 mN/m) under the same conditions.

The stability of the emulsions obtained from the tomato proteins was evaluated from the rate of separation of 1/2 of the nonpolar phase (benzene) from the emulsion [6]. Table 2 gives the results of a determination of the stability with time. For the globulin fraction, emulsions of unlimited stability were formed at a concentration of emulsifying agent in the solution of 0.004% and above, and for the albumin fraction at a concentration of 0.02%.

Thus, the main component, quantitatively, of the albumin fraction of tomato seeds has been isolated and has been characterized by electrophoresis in PAAG, gel filtration, and TLC. Some functional properties of the albumin and globulin fractions have been studied. A correlation has been found between the rheological properties of tomato proteins and the stability of emulsions. The proteins of the globulin fraction possess high emulsifying properties.

## EXPERIMENTAL

A flour of tomato seeds of the Volgogradskii variety was defatted with hexane and petroleum ether. A weighed sample (300 g) of seeds ground in a mill was covered with hexane in a ratio of 1:20 (50) weight/volume and stirred on a magnetic stirrer for an hour, the extraction being repeated five times.

The proteins were separated into fractions as described in [4]. Emulsion stability was determined as in [6]. The solubilities of the protein fractions were determined as described in [7]. The albumin fraction was separated on a column (1.7 × 68 cm) of Sephadex G-50f in 30% acetic acid. Rate of elution 9 ml/h, 4-ml fractions being collected.

The electrophoretic investigations of the total proteins and their fractions were conducted in 7.5 and 15% PAAGs in a basic buffer (pH 8.9) in the presence of 0.02% NaDDS. An AVGÉ-2 apparatus for vertical gel electrophoresis was used.

Electrophoresis was conducted in tris-glycine buffer, pH 8.3, for 2 h at I = 250 V and U = 33 mA per plate. The plates were fixed in 10% TCA for 20 min and were stained for an hour in 0.2% Coomassie Blue R-250. The excess of dye was washed out with 7% acetic acid until the plate background was colorless.

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