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Production of Safflower Protein Isolates: Gel Filtration and Electrophoretic Patterns

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Research work was carried out to characterize safflower proteins by gel filtration chromatography (GFC) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), since little information is available on these components from this oilseed crop. Safflower meal defatted in the laboratory and safflower cake provided by an industrial plant were used for protein isolate production by micellization and isoelectric precipitation. GFC of all isolates and proteins from both defatted meals revealed two major peaks of about 490 and 200 kiloDaltons (kD). Micellization gave samples with more high molecular weight components than those from isoelectric precipitation. SDS-PAGE patterns of all isolates exhibited 14–16 similar subunits; one or two bands were faint or absent in some samples. A prominent band of 23 kD was common for all proteins. The preparation method of the protein isolate affected protein composition.

Introduction

Safflower (*Carthamus tinctorius* L.) is basically a crop which grows best in arid climates. Although one of the world's oldest crops, it has become a significant world-traded oilseed only in the past 35 years. Defatted meal, produced as a by-product after oil extraction, is a potential source of vegetable protein for food. However, little information is available on these proteins. Kohler (1) and Betschart (2) studied protein isolation from safflower meal. Latha and Prakash (3) reported some physicochemical properties of total proteins from safflower seed. These latter workers found that the proteins consisted mainly of two fractions, with sedimentation values of 12S and 2S (65 and 26% of the total, respectively), plus minor components with sedimentation values of 7S and 17S. We have also attempted to develop and adapt procedures to prepare safflower protein concentrates and isolates (4,5) by 'micellization' and isoelectric precipitation techniques. Some physicochemical and functional properties of these proteins have been assessed (6,7).

The present work characterizes safflower protein isolates, prepared by micellization and isoelectric precipitation, by gel filtration chromatography and by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Materials and Methods

Safflower samples

Safflower seeds (cv. Gila) were dehulled and extracted with hexane (1:10 w/v) at 65°C using a semipilot extraction plant (5). Defatted samples were air-dried and milled (UD Cyclone Sample Mill, UD Corp., Boulder, CO) using an 80-mesh sieve; the product was termed laboratory-prepared safflower meal (SML). Industrially produced safflower cake was provided by Grasas Mexicanas, S.A., Guadalajara. This cake was sieved to remove most of the fiber; it is termed commercially-prepared safflower meal (SMI) (7).

Preparation of protein isolates

An aqueous suspension of SML or SMI (10%, w/v) was pre-extracted for 30 min at 25°C and pH 5.5 which was adjusted with 0.1 M HCl. This suspension was centrifuged and supernatants discarded (7). Protein isolates were obtained by micellization (MP) and isoelectric precipitation (IP) from pre-extracted SML and SMI as reported elsewhere (7,8). Sodium chloride (pH 7, 0.8 M) was used to extract proteins. This extract was concentrated by ultrafiltration in a Pellicon apparatus (Millipore Corp., Bedford, MA). MP was flocculated by adding cold water at pH 7. IP was extracted with alkali (0.1 M NaOH) at pH 9 and precipitated by adding acid (0.1 M HCl) until the isoelectric point was reached. All isolates were freeze-dried.

Gel filtration chromatography

Gel filtration was used to characterize proteins from defatted safflower meals and protein isolates. About 1 g meal or 0.2 g protein isolates were suspended in 10 ml phosphate buffer (pH 7.5, 0.01 M) containing 1.0 M NaCl and 0.02% sodium azide (3,9). The suspension was magnetically stirred for 3 h at room temperature, centrifuged at 6000 g for 10 min, and passed through filter paper (Whatman no. 1). Then, 0.1 g polyvinylpyrolidone was added to remove phenolic components (10) from the filtered sample, which was magnetically stirred for 30 min and centrifuged at 2000 ***g*** for 10 min.

Sephacryl S-300 gel (Pharmacia Biotechnology Int., Uppsala, Sweden) was packed into a column, 2.6 × 86 cm. Approximately 15 mg of protein in phosphate buffer were loaded on the column and eluted with the same buffer at 30 ml/h. Fractions of 4.2 ml were collected using an LKB Ultrarac II fraction collector (LKB Produkter AB, Bromma, Sweden). Protein content of each collected fraction was determined by the Bradford method (11) and the amount of eluted protein

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was calculated gravimetrically. To estimate protein recovery, this amount was related to total protein loaded on the column. The column void volume was assessed with blue dextran (MW 2000 kD). The following standard reference proteins from Sigma (St. Louis, MO) were used for calibration: carbonic anhydrase, 29.0; bovine serum albumin, 66.0; alcohol dehydrogenase, 150.0; and thyroglobulin, 669.0 kD. These experiments were run in duplicate. The molecular weight of the protein fractions was calculated from a regression equation derived by plotting values of elution volume/void volume (V_e/V_o) versus log molecular weight of standard proteins. This equation was:

$$\log MW = -1.7154\,(V_e/V_o) + 7.9142\;(r = 0.9959)$$

Electrophoresis

SDS-PAGE was conducted in a Dual Vertical Slab Gel Electrophoresis Cell (Bio-Rad, Richmond, CA) on 1.5-mm gels. Running gels were made with 12% total monomer (acrylamide and bisacrylamide), of which 2% was bisacrylamide, in pH 6.8, 1.0 M Tris-HCl buffer containing 0.1% SDS (12). The stacking gel was pH 6.8, 1.0 M Tris buffer containing 0.1% SDS and 5% total monomer (of which 2% was bisacrylamide). Approximately 4 mg protein were dissolved in 2 ml buffer containing 2% SDS and 0.5% 2-mercaptoethanol and heated 2 min at 95°C; 50 μl were applied to each well. A constant current of 20 mA/gel for 6 h was applied. The gel was stained in 50% methanol, containing 10% acetic acid and 0.25% Coomassie blue R-250 (Bio-Rad Laboratories, Richmond, CA) for 10 h and destained in methanol–acetic acid–water (30:7.5:62.5, v/v/v) solution. Standard reference proteins from Bio-Rad were: lysozyme, 14.4; soybean trypsin inhibitor, 21.5; carbonic anhydrase, 31.0; ovalbumin, 45.0; and bovine serum albumin, 66.2 kD.

Results and Discussion

Figure 1 illustrates elution patterns of defatted meal proteins and MP and IP samples. Proteins extracted from SML (**Fig. 1a**)

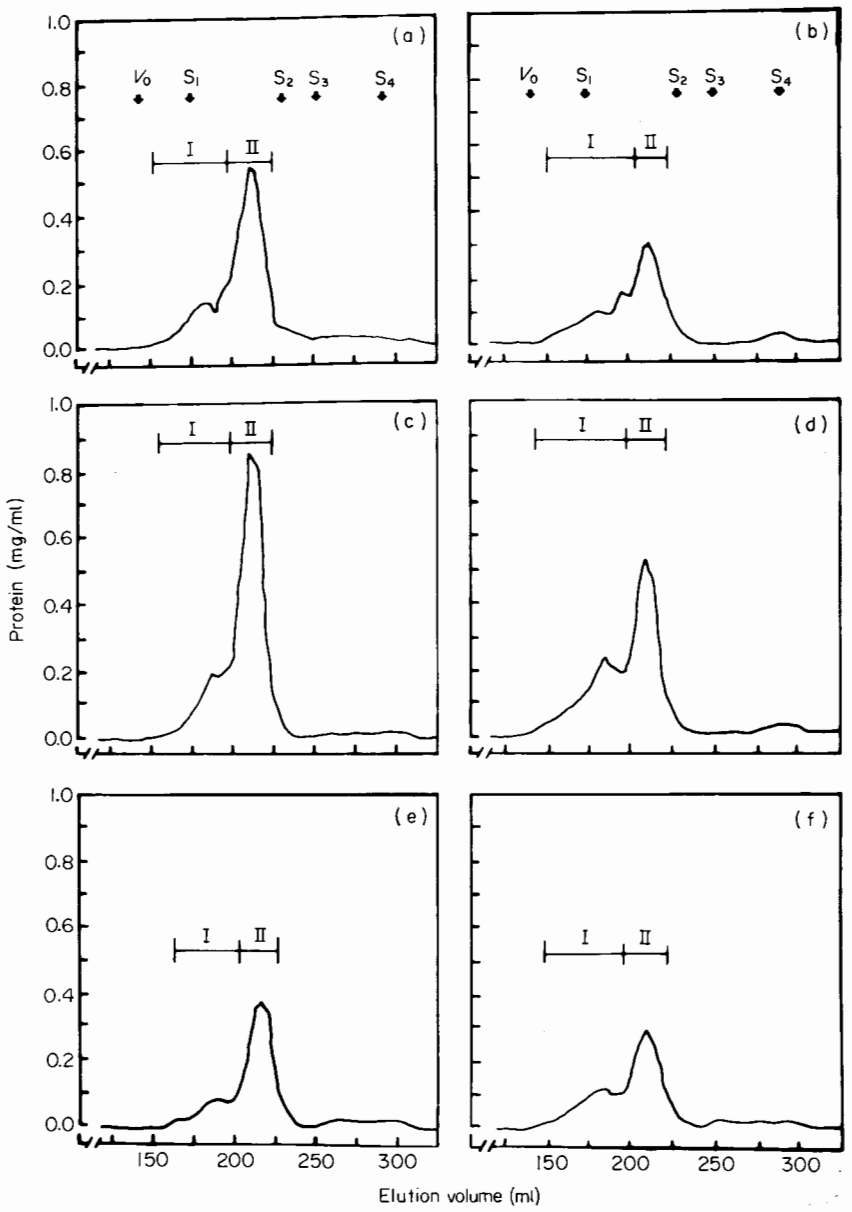

Fig. 1 Gel filtration patterns of safflower proteins. (a) Laboratory-prepared safflower meal (SML); (b) commerically-prepared safflower meal (SMI); (c) micelle protein isolate (MP) from SML; (d) micelle protein isolate (MP) from SMI: (e) isoelectric protein isolate (IP) from SML; (f) isoelectric protein isolate (IP) from SMI. Standard reference proteins: (V_0) void volume, blue dextran; (S_1) thyroglubulin, 699; (S_2) alcohol dehydrogenase, 150; (S_3) bovine serum albumin, 66; and (S_4) carbonic anhydrase, 29 kD

Table 1 Recovery of protein eluted from gel filtration column*

Protein sample	Recovered protein (%)†		
	Fraction I	Fraction II	Fractions I + II
SML	21	59	80
SMI	23	28	51
SML-MP	8	83	91
SMI-MP	26	47	73
SML-IP	10	36	46
SMI-IP	12	32	44

*Data were calculated from **Fig. 1** and represent mean of two runs.
†Fraction I includes recovered proteins with a MW higher than 340 kD. Fraction II proteins average 200 kD.
See **Fig. 1** for key to abbreviations used

showed two major and well-defined peaks of about 490 and 200 kD, whereas SMI proteins (**Fig. 1b**) exhibited another additional peak of 350 kD. This peak might be the result of degradation by harsh conditions for industrial extraction of oil. Interestingly, all isolates prepared by the MP and IP procedures from either SML or SMI (**Figs 1c–1f**) showed the two major peaks of nearly 490 and 200 kD found in the raw materials (**Figs 1a** and **1b**). However, isolates from SMI by MP (**Fig. 1d**) and IP (**Fig. 1f**) did not show the 350 kD peak (**Fig. 1b**). Another difference was the small peak of high molecular weight (approximately 820 kD) observed only in the SML-IP sample (**Fig. 1e**). This peak could be hidden in the other samples as suggested by peak shapes. In all samples the major peak corresponds to 200 kD.

Table 1 presents protein fraction distributions calculated from **Fig. 1**. Fraction I corresponds to proteins of MW higher than 340 kD and fraction II to proteins averaging 200 kD (see **Fig. 1**). Protein recovery (fractions I + II) for SML was much

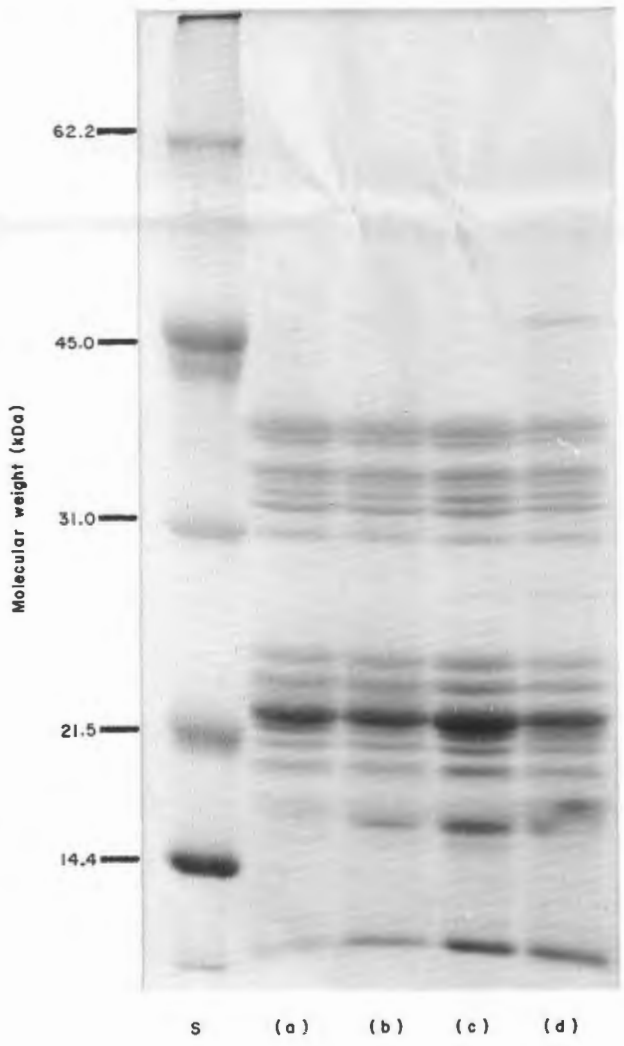

Fig. 2 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of safflower protein isolates. (a) SML-MP; (b) SML-IP; (c) SMI-MP; (d) SMI-IP; (S) Standards. Standard reference proteins: bovine serum albumin, 66.2; ovalbumin, 45; carbonic anhydrase, 31; soybean trypsin inhibitor, 21.5; and lysozyme 14.4 kD (see **Fig. 1** for keys to abbreviations used)

higher than for SMI. Also, MP from both sources exhibited a higher recovery (73–91%) than IP (44–46%). For all samples, recovery of fraction II was higher than that of fraction I and total recovery ranged from 89 to 95% in relation to the loaded sample. For some samples (e.g. SML-IP, SMI-IP), recovery of fractions I + II was rather low. In this case, after elution of fractions I + II more protein of low MW components under undefined small peaks eluted from the column. **Table 1** also shows that MP procedure tended to produce isolates with a higher percentage of high MW components as compared to the IP technique. These results suggest that differences in distribution of solubilized protein loaded on the column are related to the sample source and method of isolate preparation. Using gel filtration chromatography, Matsudomi *et al.* (13) also observed formation of low MW components by thermal treatments of soybean proteins.

SDS-PAGE patterns of protein isolates from both meals are illustrated in **Fig. 2**. These patterns having 14–16 subunits (48–13 kD) were similar, but had some slight differences. For example, the band of 48 kD was well defined in SMI-IP (**Fig. 2d**), but faint or absent in other samples. The band of nearly 23 kD was prominent in all isolates. The subunit of 13 kD was faint and that of 15.4 kD may be absent in SML-MP (**Fig. 2a**). Latha and Prakash (3,9) reported that major components of safflower seed proteins have MWs of 240–290 kD (12S) and 13.8–18.5 kD (2S), as assessed by analytical ultracentrifugation and SDS-PAGE, respectively. Our results generally agree with those findings. The major fraction we obtained by gel filtration had a MW of 200 kD; by SDS-PAGE, the most prominent band had 23 kD. However, there are some basic differences between the research work carried out by the two laboratories. Our study involves characterization of protein isolates from two different seed cakes (SML and SMI) and two preparation procedures (MP and IP), whereas that of the former workers characterizes proteins extracted by a single procedure based in a buffer system from seeds belonging to a different variety. In summary, both micellization and isoelectric precipitation isolates gave by gel filtration two major fractions common to all samples. Our results indicate protein breakdown by treatments such as heating during oil extraction. Also, conditions in the isoelectric procedure negatively affect the recovery of high MW proteins. In general, electrophoretic patterns of all isolates were similar. Studies are needed on molecular weight determinations of protein isolates components by other procedures, and on protein characterization by two-dimensional electrophoresis.

Acknowledgements

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