SUNFLOWER SEED PROTEINS

by

F. J. JOUBERT

National Chemical Research Laboratory, South African Council for Scientific and Industrial Research Pretoria (South Africa)

The earliest published work on the proteins of sunflower seed is that of RITTHAUSEN[1]. OSBORNE AND CAMPBELL[2] considered that the protein extracted from oil-free sunflower seed meal with salt solution contained a single globulin. Their first preparations were dark in colour owing to the presence of a substance known at that time as helianthotannic acid. Later GORTER[3] identified this compound as chlorogenic acid. To remove the chlorogenic acid, OSBORNE AND CAMPBELL extracted the meal with ethanol of 0.820 specific gravity at 65–75° C. Similarly, SMITH AND JOHNSEN[4] used hot 70% ethanol or near absolute methanol to remove the chlorogenic acid but they found that the protein was severely denaturated. The present communication is concerned (i) with the removal of chlorogenic acid from sunflower seed meal (Sunrise type) under conditions such that the protein is not denaturated, and (ii) the examination of the proteins of sunflower seed by ultracentrifugal methods.

Complete removal of chlorogenic acid from sunflower meal was achieved by the following method:

Dehulled and defatted sunflower seed meal was repeatedly extracted with 50–50 ethanol-water mixture at room temperature and then with cold water by using a Waring blendor. The meal was next repeatedly washed with acetone at room temperature and left in air to dry. After this purification the meal was completely free from chlorogenic acid, when tested for by the method described by HOEPFNER[5]. As 80% of the proteins present in this meal could be extracted with salt solution, probably very little denaturation of the protein occurred.

The proteins of purified sunflower seed meal were extracted with 10% sodium chloride and then precipitated by adding ammonium sulphate to 80% saturation. The precipitated proteins resemble edestin[6] in being soluble only in buffers of relatively high salt concentration. Measurements on a Spinco electrically driven ultracentrifuge, revealed (Fig. 1(a)) two major components of sedimentation constants 11.9 and 1.7 Svedberg Units (S.U.) and minor quantities of components of 7.8 and 18.1 S.U., for this preparation in buffer of ionic strength 1.0 and pH 8.8.

The proteins present in sunflower seed meal were fractionated as outlined in Fig. 2.

When the 10% sodium chloride protein extract was saturated to 40% with ammonium sulphate, about 70% of the proteins were precipitated (Precipitate A). On the addition of more ammonium sulphate to the supernatant of precipitate A the residual proteins were precipitated (Precipitate B). The proteins extracted with 10% sodium

References p. 523.

chloride were partially precipitated by adding five volumes of water at 4° C to each volume of the extract (Precipitate C). When the supernatant of precipitate C was saturated with carbon dioxide a further amount of protein was obtained (Precipitate D.)

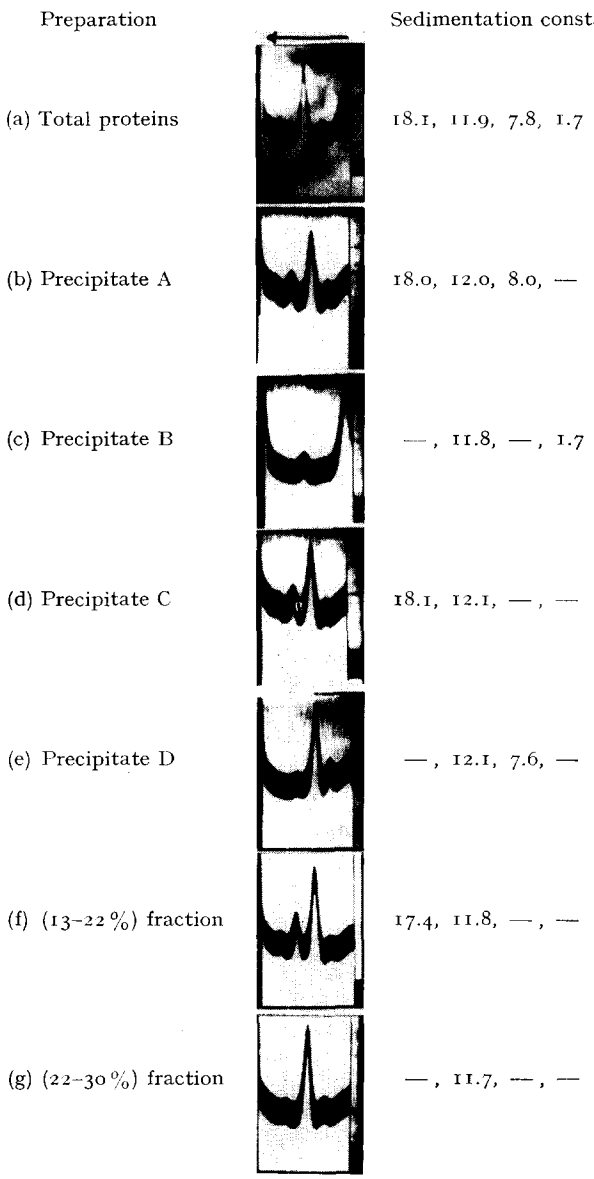

Fig. 1. Sedimentation diagrams of sunflower seed proteins.

Fig. 1 gives sedimentation diagrams of the different precipitates outlined in Fig. 2. Whereas practically identical sedimentation diagrams were obtained for precipitates C and D, the diagrams of precipitates A and B were very different. Thus, two entirely different protein fractions were obtained with ammonium sulphate fractionation. The

References p. 523.

sedimentation diagrams of precipitates A, C and D are identical in that they all contain a major component of sedimentation constant of 11.9 S.U. and minor quantities of components of sedimentation constant 7.8 and 18.1 S.U.

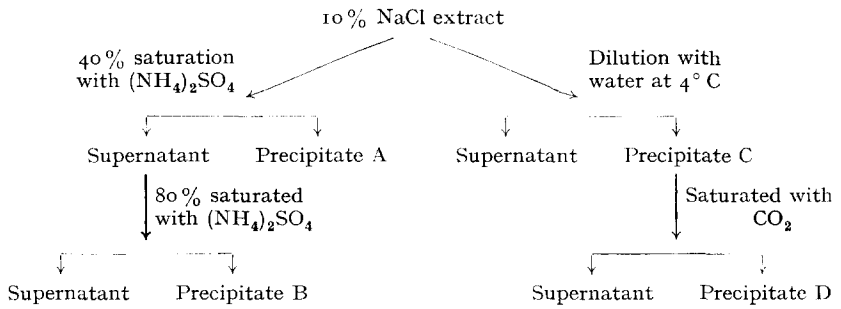

Fig. 2. Scheme for the fractionation of sunflower seed proteins.

In a further attempt to fractionate the impurities of sedimentation constant 7.8 and 18.1 S.U. from the main component, successive amounts of saturated ammonium sulphate were added to a 10% sodium chloride sunflower seed protein extract. The precipitate obtained at 13% saturation with ammonium sulphate was centrifuged off and discarded. To the supernatant more ammonium sulphate was added to 22% saturation. As shown in Fig. 1f, this fraction (13–22%) still contained some of the faster sedimenting component. However, the precipitate obtained at 30% saturation (i.e. the 22–30% fraction) was almost free from the other components, as shown in Fig. 1g and was used for the molecular weight determinations.

Diffusion coefficients have been determined at 20° C by the free boundary method using the Tiselius U-tube for boundary formation, and the results were calculated by the maximum height and standard deviation methods. The values found for the 22–30% fraction and precipitate B are shown in Table I. Using these diffusion coefficients and the average values of the sedimentation constants of the two fractions, the molecular weights and frictional ratios of Table I were found.

TABLE I

MOLECULAR WEIGHTS OF THE MAJOR SUNFLOWER SEED PROTEINS

	22–30% fraction	*Precipitate B*
D^0_{20} cm^2 sec^{-1} (max. height)	3.19×10^{-7}	7.59×10^{-7}
D^0_{20} cm^2 sec^{-1} (standard deviation)	3.24×10^{-7}	8.29×10^{-7}
S^0_{20} Svedberg Units	11.9 ± 0.2	1.67 ± 0.04
Partial specific volumes (assumed)	0.74	0.74
Molecular weight	343,000	19,000
Frictional ratio	1.42	1.45

Although only one peak was observed in the ultracentrifuge for precipitate B, the different values found when the diffusion coefficient was calculated by the two methods, suggests a certain degree of heterogeneity[7].

References p. 523.

ACKNOWLEDGEMENT

This communication is published with the permission of the South African Council for Scientific and Industrial Research.

SUMMARY

Complete removal of chlorogenic acid from sunflower seed was achieved by repeated extraction with 50–50 ethanol-water mixture at room temperature followed by extraction with cold water. The proteins of the purified meal were separated by ammonium sulphate fractionation. Using sedimentation and diffusion measurements, molecular weights of 343,000 and 19,000 were found for the two major components.

RÉSUMÉ

L'élimination complète de l'acide chlorogénique de la graine de soleil a été réalisée par extractions répétées à l'aide du mélange éthanol-eau 50–50 à la température ordinaire suivies d'une extraction à l'eau froide. Les protéines de la farine purifiée ont été séparées par fractionnement au sulfate d'ammonium. Les poids moléculaires des deux constituants principaux, déterminés par sédimentation et par diffusion, sont 343,000 et 19,000.

ZUSAMMENFASSUNG

Zum vollkommenen Eliminieren von Chlorogensäure aus Sonnenblumensamen wurden diese wiederholt bei Zimmertemperatur mit einem Gemisch Ethanol-Wasser 50–50 und darauffolgend mit kaltem Wasser extrahiert. Die Proteine des so gereinigten Mehls wurden durch Fraktionieren mit Ammoniumsulfat getrennt. Als Molekulargewichte der beiden Hauptkomponenten wurden mit Hilfe von Sedimentations- und Diffusionsbestimmungen 343,000 und 19,000 festgestellt.

REFERENCES

1 H. RITTHAUSEN, *Pflüger's Arch. ges. Physiol.*, 21 (1880) 89.
2 T. B. OSBORNE AND G. F. CAMPBELL, *J. Am. Chem. Soc.*, 19 (1897) 487.
3 K. GORTER, *Arch. Pharm.*, 247 (1909) 436.
4 A. K. SMITH AND V. L. JOHNSEN, *Cereal Chem.*, 25 (1948) 399.
5 W. HOEPFNER, *Chem. Ztg.*, 100 (1932) 991.
6 T. SVEDBERG AND A. J. STAMM, *J. Am. Chem. Soc.*, 51 (1929) 2170.
7 P. A. CHARLWOOD, *J. Phys. Chem.*, 57 (1953) 125.

Received August 28th, 1954