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ELECTRON MICROSCOPE RESEARCH ON SUNFLOWER PROTEIN BODIES

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ABSTRACT Cereal Chem. 54(6): 1171-1181

Protein bodies in sunflower seeds were investigated primarily by means of several electron microscopic techniques. The cotyledon cells in sunflower seeds observed with a scanning electron microscope (SEM) or a transmission electron microscope (TEM) contained numerous protein bodies and spherosomes, as in other oilseeds. Protein bodies were isolated by sucrose gradient centrifugation; their surfaces were markedly uneven, as revealed in SEM-images. The results of energy-dispersive analysis of X-rays combined with a SEM suggested that the projecting points of protein bodies were richer in phosphorus than the depressed points, and that they might correspond to crystaloid-type inclusions (or globoids) observed in a TEM-image.

In the past decade, research on reserve protein in subcellular structures, protein bodies, or aleurone grains has become well established (1–14). Enzyme activities associated with these particles (2,3) and their changes during germination (4,5) and development (6,7) have been reported. Electron microscopic techniques have elucidated their morphological and biochemical characteristics.

Protein bodies of oilseeds such as peanut (8), cottonseed (9), and hempseed (10) contain additional substructures—crystaloids or globoids—within the membrane. Protein bodies of the soybean (11,12) (one of the oilseeds), however, have no such crystaloid-type substances, whereas those of cereal grains such as rice (13) and barley (14) show fine or faint lamellar structures which are electron-dense and are parallel to the protein membrane.

As far as the use of sunflower protein is concerned, several European workers have successfully studied composition (15,16), nutritional value (17, 18), and preparation of protein concentrate and isolate (19,20), but little on fine structure of sunflower protein bodies.

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In the present paper, protein bodies in sunflower seeds were investigated primarily by means of several electron microscopic techniques.

MATERIALS AND METHODS

Seed Source

Sunflower seeds harvested in 1973 (variety Peredovick) were a gift from the Plant Breeding Station, INRA, Clermont-Ferrand, France.

Observation of Protein Bodies in Tissue

A sunflower seed was removed from the seed coat, cut in thick slices, and put on a specimen stub. The specimen was observed under a scanning electron microscope (SEM) type JSM 50-A, after vacuum-coating with carbon and then gold.

Another sunflower seed was removed from the seed coat, cut into pieces, fixed with 5% glutaraldehyde and then with 2% osmium tetraoxide in phosphate buffer (pH 6.7). The specimen was then dehydrated by acetone and embedded in Epon resin. The blocks prepared were cut into ultrathin slices by an LKB Ultratome, stained with saturated uranyl acetate for 20 min, and examined in a transmission electron microscope (TEM) type JEM 100-B.

Isolation and Observation of Protein Bodies

Seeds were removed from seed coats by hand, ground, and defatted with n-hexane by reflux-extraction for 16 hr at room temperature and dried. One hundred milligrams of the resulting meal was suspended in 5 ml of 0.1 *M* citrate buffer solution (pH 5.0) containing 20% sucrose. The suspension was sonicated

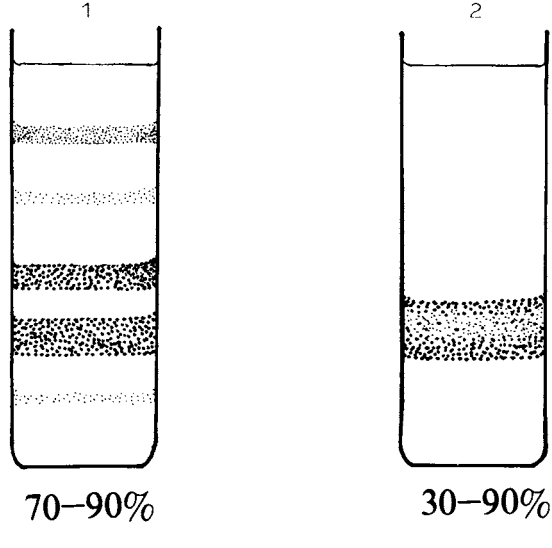

Fig. 1. Fractionation of protein bodies by sucrose gradient centrifugation.

two to three times for 30-sec intervals at 20 kcps in ice water and then passed through several layers of gauze. The filtrate was first fractionated by 70–90% of sucrose gradient centrifugation, which gave 5 bands as shown in Fig. 1 (left). The bands were easily recognized when Fast Green was added in the suspension before centrifugation. To make it easier to fractionate protein bodies from the upper smaller substances, 30–90% of sucrose gradient centrifugation was used throughout the present experiment. It resulted in a single broad band as shown in Fig. 1 (right). The fraction containing protein bodies was collected, rinsed with distilled water with the aid of centrifugation, and submitted to observation under a SEM. Fixations of protein bodies with Fast Green or osmium tetraoxide or a vapor of osmium tetraoxide were tried in order to get clear images under a SEM. But these fixations only resulted in SEM-images in which protein bodies often were aggregated or clotted. Consequently, isolated protein bodies were kept in the suspension in sucrose solution and rinsed with distilled water just before observation, as described above. The observation by a SEM was carried out after coating with carbon and then gold.

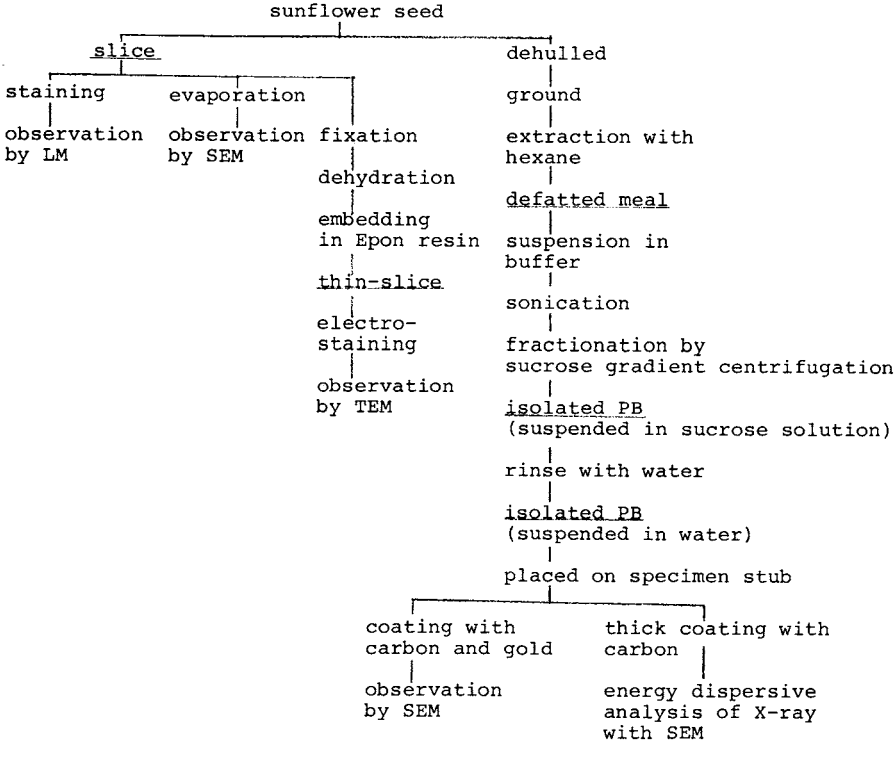

Fig. 2. Flowsheet of experiments to observe protein bodies in sunflower seeds. LM = Light microscope, SEM = scanning electron microscope, TEM = transmission electron microscope, and PB = protein bodies.

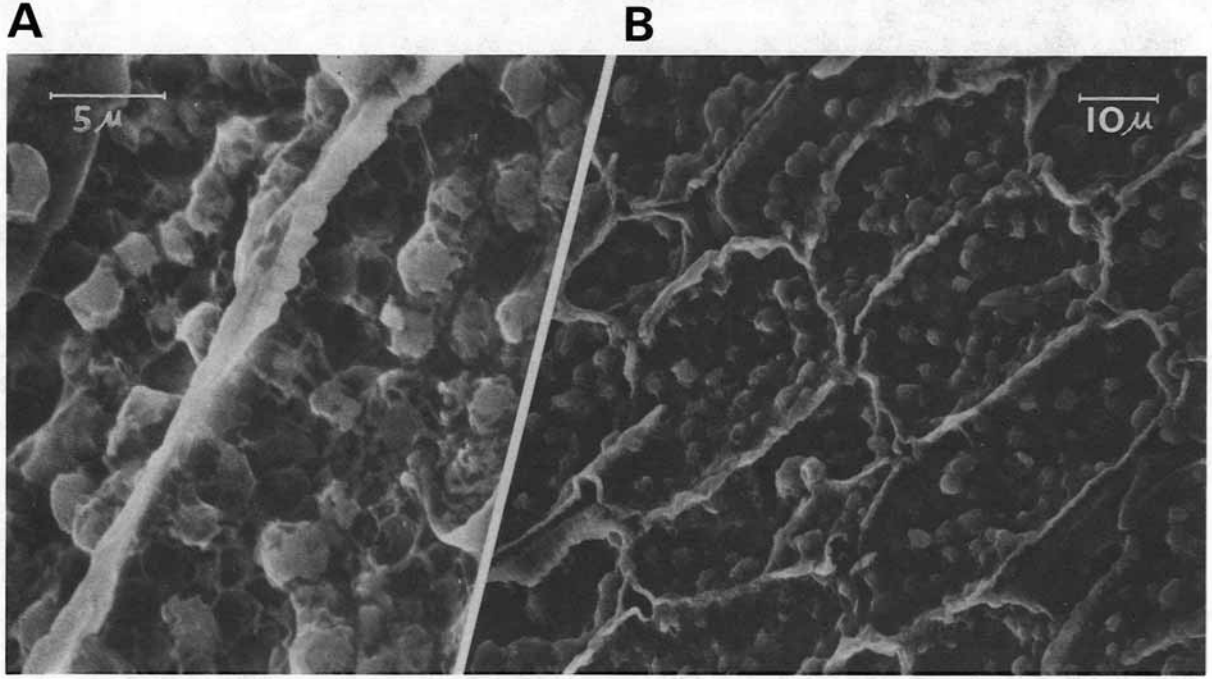

Fig. 3. SEM-image of cotyledon cells of sunflower seed. A, 3000×; B, 1000×.

Microanalysis of Isolated Protein Bodies

Isolated protein bodies put on a specimen stub were very thickly vacuum-coated with carbon only and submitted to energy-dispersive analysis of X-rays (Edax-system) combined with a SEM.

Figure 2 summarizes the experimental approaches used.

RESULTS

Figure 3 shows the SEM-image of cotyledon cells in sunflower seeds. The surfaces of the protein bodies were uneven and numerous hollows, where some subcellular structures seemed to be stripped away, were recognized in the cell. With the help of a TEM, such hollows appeared as stripped spherosomes or lipid granules containing reserve fatty substances (see Fig. 4). Moreover, protein bodies of sunflower seeds contained a crystalloid-type inclusion within a membrane.

In the isolation of protein bodies, the fractionation by 70–90% of sucrose gradient centrifugation, which was first carried out on trial, gave 5 bands as shown in Fig. 1 (left). Protein bodies observed through a SEM were mainly located in the 3rd and 4th bands from the top of the tube. In the 4th band, many clumped protein bodies were observed as shown in Fig. 5, but there existed no morphological difference in protein bodies between the two bands. In the case of 30–90% of sucrose gradient centrifugation, a single broad band suggests partial separation, sketched in Fig. 1 (right).

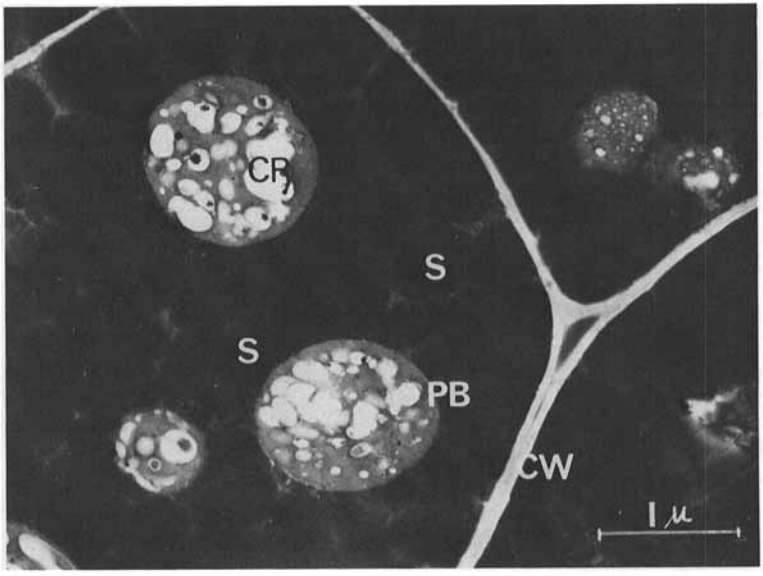

Fig. 4. TEM-image of cotyledon cells of sunflower seed; about 5000×. PB = Protein body, CR = crystaloid, S = spherosome, CW = cell wall.

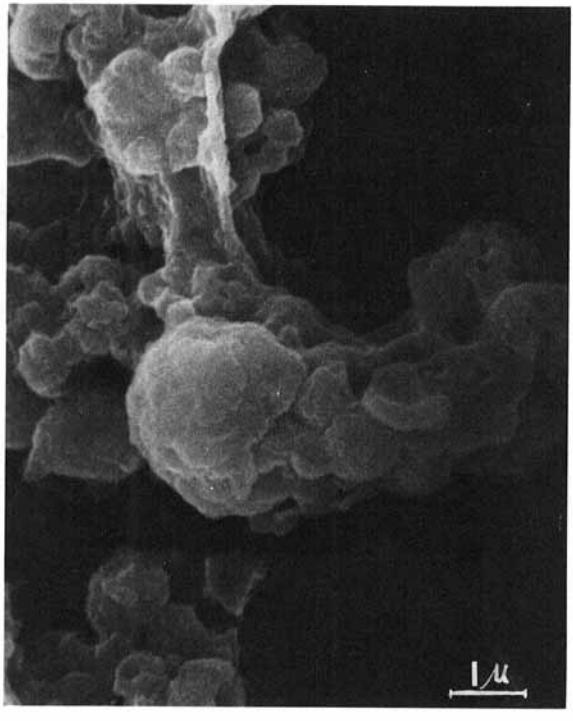

Fig. 5. SEM-image showing protein bodies clumped together; 10,000×.

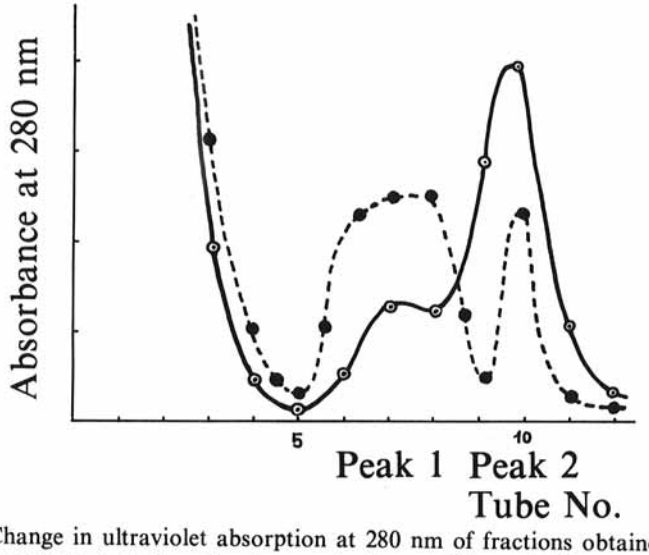

Fig. 6. Change in ultraviolet absorption at 280 nm of fractions obtained by 30–90% sucrose gradient centrifugation by sonication. With sonication o——o; without sonication •- - - -•.

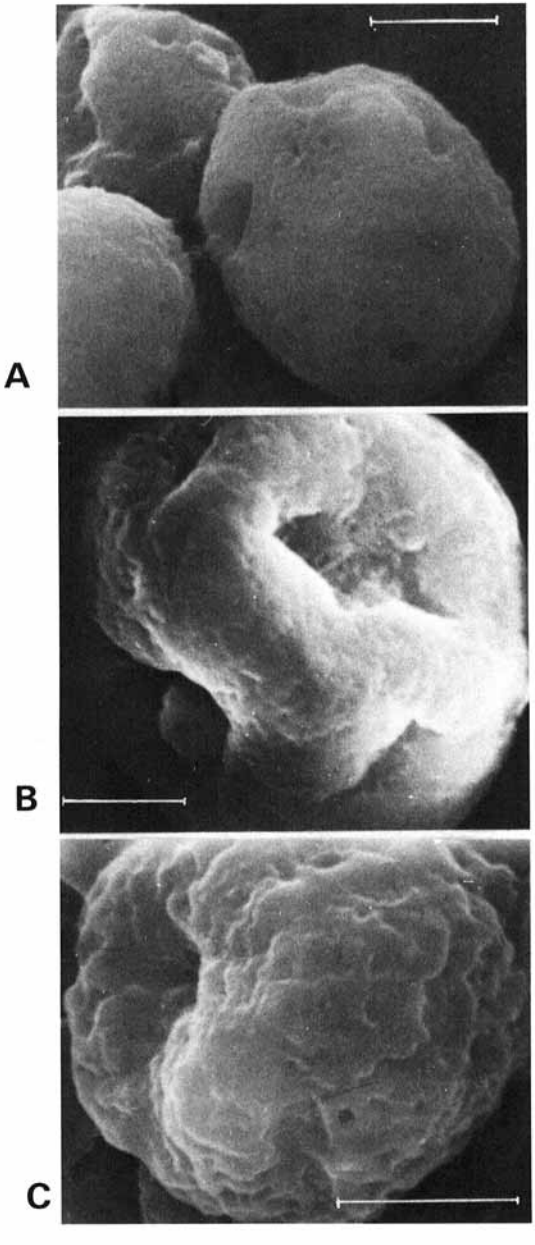

Fig. 7. SEM-images of isolated protein bodies. A, 21,000× at 15 kV; 2.1 cm = 1 μ. B, 20,000× at 25 kV; 2 cm = 1 μ. C, 30,000× at 20 kV; 3 cm = 1 μ. (Scale bars in each photograph = 1 μ.)

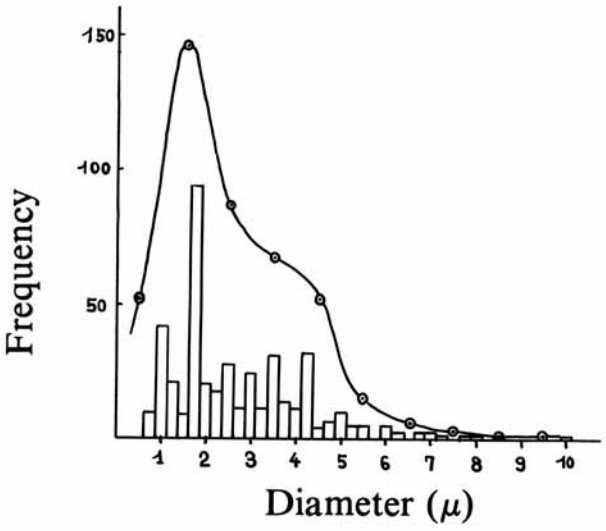

Fig. 8. Distribution of diameter of protein bodies. The curve shows the sum of frequency at intervals of 1 μ.

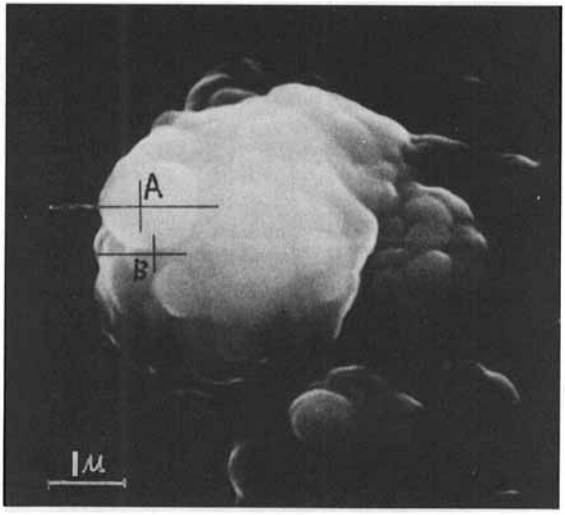

Fig. 9. SEM-image of isolated protein bodies thickly vacuum-coated with carbon; 10,000×. A, protuberance; B, depression. 1 cm = 1 μ.

Ultraviolet absorption, at 280 nm of each fraction (2.5 ml) from the top to the bottom of centrifugal tube, was measured by a spectrophotometer immediately after centrifugation. The turbidity of each fraction was eliminated by adding drops of dilute NaOH. Figure 6 shows that without sonication, the absorption was higher in peak 2 (which corresponded with the 4th band) than in peak 1 (which corresponded with 3rd band) whereas, with sonication, peak 1 was higher than peak 2. Excessive sonication resulted in a broad and obscure band in the region of peak 1.

Several SEM-images of isolated protein bodies are shown in Fig. 7. The images were variable, depending upon the conditions of isolation, metallization, or observation, while the marked unevenness on the surface of protein bodies was observed in every image.

Figure 8 shows the distribution in diameter of protein bodies measured with a SEM. The distribution ranged between 0.5 and 10 μ and the highest frequency was shown at about 2 μ in diameter. In the course of this experiment, crystalloid-type substances were often found in isolated protein body fractions, especially when sonicated excessively. Taking size of crystaloids into consideration, the average diameter of protein bodies might be bigger than the data indicated in Fig. 8, if only intact protein bodies could possibly be measured. But here, protein bodies of 5–6 μ in diameter were used when taken in a photograph such as Fig. 7.

When isolated protein bodies were thickly vacuum-coated with carbon for energy-dispersive analysis of X-rays, the unevenness on the surface was distinctly observed, as shown in Fig. 9. The image suggests that the protein bodies are constructed of many smaller granules.

Table I and Fig. 10 show the results of point analysis to projecting part A and depressed part B in Fig. 9. Energy-dispersive analysis of X-rays offers a qualitative or semiquantitative analysis of the element present. This can be readily obtained from the spectrum by rapid identification of the peaks and by rough comparison of the relative heights for the various elements. In these experiments, the projecting part was richer in phosphorus than the depressed part.

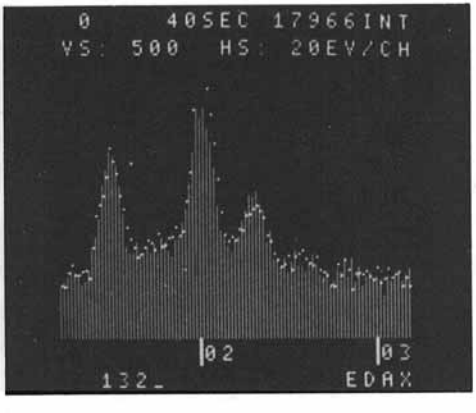

Fig. 10. Results of microanalysis of protuberance (dotted) and depression (shaded).

Table II shows the relative values of phosphorus and sulfur in small and large protein bodies. The analysis was carried out by SEM observation of protein bodies with different diameters. The amount of phosphorus was higher in small than in large protein bodies, and that of sulfur was the opposite.

DISCUSSION

The cotyledon cells in sunflower and other oilseeds contain numerous protein bodies and spherosomes. Spherosomes in sunflower cotyledon cells are much larger than those of soybean, but resemble those of cottonseed. The spherosomes were surrounded by layers of membrane. The protein bodies of sunflower seed included crystalloid-type substructures, or globoids, which seemed to be rich in phosphorus according to the results of energy-dispersive analysis combined with a SEM.

The investigation on isolated globoids from cottonseed by Lui and Altschul (9) revealed that these substructures in protein bodies contained a high amount of

TABLE I
Microanalysis of Protuberant or Depressed Sites in Protein Bodies

	Protuberance		Depression	
	1	2	1	2
Al[a]	1.0000	1.0000	1.0000	1.0000
Mg	0.0906	0.0679	0.1017	0.1009
P	1.1072	1.0804	0.8351	0.7863
S	0.2484	0.2180	0.3759	0.3672
Cl	0.0190	0.0266	0.0000	0.0027
K	0.0026	0.0045	0.0000	0.0000
Ca	0.1407	0.1541	0.0783	0.1580
Fe	0.0560	0.0610	0.0541	0.0359

[a]Comparative values when intensity of Al was equal to 1.0000.

TABLE II
Microanalysis of Phosphorus and Sulfur in Protein Bodies Varied in Diameter[a]

Experiment No.	Diameter μ	Phosphorus	Average	Sulfur	Average
1	1	99.022		0.977	
2	2	98.695	98.858	1.304	1.141
3	5	83.821		16.178	
4	6	92.212		7.787	
5	6	75.023	83.685	24.976	16.314

[a]Phosphorus + sulfur = 100.

phytic acid and metals and might serve as a site of storage for these constituents. Moreover, protein bodies from various seeds are also known to contain a high amount of phytate.

The results of an energy-dispersive analysis of X-rays suggest that the substructures in protein bodies of sunflower seeds might also be sites of phytate storage, though these data could not prove these results conclusively.

Acknowledgments

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