Dextran has a highly branched network structure. On the other hand, pectin has a single-stranded chain structure. This structural difference probably affects the formation of the emulsion of the conjugates. The macromolecule branched structure may be efficient to wrap the oil drops to result in the excellent emulsifying properties.

The emulsifying properties of the ovalbumin–dextran conjugate were comparable or superior to those of the commercial emulsifier. The emulsifying properties of the conjugate were almost the same as SunSoft SE-11 in water or in 0.1 M phosphate buffer, pH 7.0. Interestingly, the emulsifying properties of the conjugate were superior to those of the commercial emulsifier in the presence of salt and in acidic conditions. Another advantage is that the ovalbumin–dextran conjugate is soluble in both water and oil. In addition, the heat-stable property of this conjugate is suitable for heat treatment and sterilization in food systems.

As mentioned above, the ovalbumin–dextran conjugate can be used in the food industry as a new functional emulsifier that is soluble, macromolecular, and stable in acid or in the presence of high concentrations of salt.

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Composition, Solubility, and Electrophoretic Patterns of Proteins Isolated from Kerman Pistachio Nuts (*Pistacia vera* L.)

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Extraction of protein from two pistachio sources (Kerman and Turkish) was performed with different solvents. The results showed the maximum amount of protein extracted with 0.5 M NaCl solution. The other solvents in sequential extraction, e.g. water, 60% (v/v) *tert*-butyl alcohol, and 0.1 M sodium borate (pH 10)/1% (w/v) $NaDodSO_4$/1% (v/v) 2-mercaptoethanol (2-ME), extracted little or no protein. The Kjeldahl data indicated that 66% of the total protein was globulin (NaCl soluble) while albumins (H_2O soluble), glutelins (sodium borate soluble), and prolamins (*tert*-butyl alcohol soluble), respectively, contributed 25, 7, and 2% of the total protein. Fractionation of the protein by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric focusing (IEF) showed three of the four solubility fractions had complex polypeptide compositions with molecular weights of 12–68K. However the protein profiles of all fractions were very similar except for the alcohol extract which did not produce any distinct bands. Two-dimensional electrophoretic analyses revealed about 100 protein spots when the meal was extracted directly with 6 M urea and 1% 2-ME, and the two pistachio sources showed similar polypeptide patterns.

The pistachio is one of the favorite tree nuts of the world. It is native to the Middle East region and was introduced to most of the Mediterranean countries and recently (1960) to the United States. Several species of the genus *Pistacia* are referred to as pistachio, but only the fruits of *Pistacia vera* attain sufficiently large size to be acceptable to consumers as edible nuts. Pistachio imports to the United States annually were in the vicinity of 15000 metric tons and came mainly from Iran (10–12000 metric tons) while the rest came from other countries (e.g., Turkey) (Woodroof, 1979). However, in recent years, an increase in local production has made the United States the world's second largest producer of pistachio nuts. The USDA reported in 1985 that the total U.S. production had reached 15500 metric tons, only 8600 metric tons being imported and almost exclusively (98.1%) from Iran. The

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famous Iranian pistachio from the Kerman province was introduced to California first in 1890, but much cultivation was not begun until 1960. Presently there are over 30 000 acres of Kerman pistachio orchards in California; this acreage is predicted to meet the demand for the pistachio in the United States before the end of the century (Woodroof, 1979).

There is not much information on the chemical composition of the pistachio, although it is popular and is considered to be a highly nutritious nut. The few reports include one by Shokraii (1977) and two by others (Kamangar et al., 1975; Kamangar and Farsam, 1977). These reports show that pistachio has a well-balanced composition of essential amino acids and fatty acids. There are two papers dealing with the chemical composition of pistachio in relation to the genotype and stages of maturation (Labavitch et al., 1982; Kader et al., 1982) and two involving the protein bodies and their course of development during embryogenesis and breakdown during seed germination (Buttrose and Lott, 1978; Shokraii and Zadeh, 1981). However, no detailed study has so far been reported about the protein composition of the pistachio. In this paper we report results on the isolation and fractionation of pistachio protein and also the heterogeneity of its components as revealed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), isoelectric focusing (IEF), and two-dimensional electrophoresis (2D). In addition, the protein content and profile of the Kerman pistachio were compared with those of the Turkish pistachio since Iran and Turkey are the world's major pistachio producers and exporters.

MATERIALS AND METHODS

Plant Materials. The pistachio nuts of Kerman used in this study were provided by Dr. J. M. Labavitch of the Pomology Department of the University of California, Davis, and represent the 1983 crop from a California pistachio orchard. The pistachio samples from Turkey were a 1982 crop obtained from a local market by A.E.

Sample Preparation. The nuts were ground to a fine paste in a prechilled mortar and pestle, and the resulting meal was defatted 3× with cold ethyl ether with a 10:1 ratio of solvent volume to meal weight at room temperature (23–25 °C) for 12–16 h. The defatted meal was then air-dried and stored in a freezer until needed.

Protein Extraction. The protein extraction procedure used was a modified version of the Osborn procedure (Landry and Moureaux, 1970). A weighed sample of defatted meal was extracted exhaustively in sequence with cold deionized distilled water, 0.5 M NaCl, 60% (v/v) *tert*-butyl alcohol, and finally an alkali buffer-detergent solution (0.1 M sodium borate, pH 10, containing 1% (w/v) SDS and 1% (v/v) 2-ME (2-mercaptoethanol)) using a solvent volume to meal weight ratio of 10:1 at 4 °C. Extractions were repeated up to eight times (2 h each), and the progress and completion of the extraction were monitored by the Coomassie Blue R-250 dye binding method of Esen (1978). The protein extracted with each solvent was quantitatively collected. All solvents contained 0.02% of NaN_3 and 2 mM PMSF (phenylmethylsulfonyl fluoride) to protect the samples against the possibility of bacterial infection and proteolysis. The NaCl extract was dialyzed against cold, distilled water, while the sodium borate extract was dialyzed against a solution of 50% (v/v) ethanol overnight, with at least three changes of dialysate to remove SDS. The protein extract of each fraction was then precipitated with 10% (w/v) trichloroacetic acid (TCA) in the cold, and the precipitate was recovered by centrifugation (10000*g*, 15 min). The pellet was quantitatively collected, freeze-dried, and stored in a freezer. The TCA supernatants lacked any soluble protein and were thus discarded. Total protein was isolated by extracting directly 10 mg of the defatted meal with either 1 mL of 6 M urea and 1% 2-ME or 1 mL of the Laemmli sample buffer (0.125 M Tris-HCl, pH 6.8, containing 2% SDS, 5% 2-ME) overnight. The latter was heated at 70 °C for 15 min and centrifuged before use.

Protein Determination. The protein content of each fraction was determined in duplicate on fat-free meals by the standard micro–Kjeldahl method according to the procedure recommended by the American Association of Cereal Chemists (1962). Nitrogen was converted to protein by the factor 6.25.

Protein Fractionation. Proteins were separated by SDS-PAGE (Laemmli, 1970) and IEF techniques (Hu and Esen, 1982) in one dimension. Lyophilized protein samples (10 mg) of each defatted metal fraction were dissolved in 1 mL of sample buffer (0.1 M Tris-HCl, pH 6.8, containing 2% SDS, 5% 2-ME, 10% glycerol, and 6 M urea) for SDS-PAGE and in a 6 M urea + 5% 2-ME solution for IEF analysis. The solubilized protein samples were stored at 4 °C in a refrigerator, and those to be used for SDS-PAGE were heated at 70 °C for 15 min and then centrifuged in a microfuge before use.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed in homogeneous (12%) or gradient (8–15%) polyacrylamide gel slabs. Isoelectric focusing (IEF) was performed in (5%) polyacrylamide gel slabs with a horizontal apparatus (Pharmacia) at 5 °C. The range of the pH gradient used for IEF analysis was 4–10.5 and was obtained by mixing equal volumes of four Pharmalytes (Pharmacia Fine Chemicals) of pH ranges 4–6.5, 5–8, 6.5–9, and 8–10.5. Two-dimensional electrophoresis was performed according to the procedure of O'Farrell et al. (1975), Basha (1979), and Bashha and Pancholy (1982).

Gel Filtration. The protein sample for gel filtration studies was prepared by extracting 0.5 g of defatted meal with a 10-mL solution of 10 mM Tris-HCl (pH 7.5) containing 1.5 M NaCl, 0.02% NaN_3, and 2 mM PMSF overnight and at 4 °C. It was then centrifuged at 10000*g* for 15 min, the pellet was discarded, and ammonium sulfate to 60% final concentration was added to the supernatant. The ammonium sulfate precipitate was then recovered by centrifugation, and the pellet was dissolved in 5 mL of 5 mM potassium phosphate buffer (pH 7.5). The partially purified protein was fractionated by gel filtration on a Sepharose Cl 6B column (3.0 × 80 cm) equilibrated with a 5 mM potassium phosphate buffer (pH 7.5). Immediately before application to the column, 10 mg of Dextran Blue was added to the sample to serve as a molecular weight and sample front marker. Fractions of 5 mL were collected with an automatic fraction collector (ISCO), and absorption was monitored at 280 nm with a UV monitor (LKB).

RESULTS AND DISCUSSION

Extraction. Comparison of the amount of protein extracted with each of the four different solvents showed that most of the protein was extracted with 0.5 M NaCl solution while the smallest amount was extracted with 60% *tert*-butyl alcohol. The overall extraction resulted in about a 38.5% loss in the weight of the original defatted meal sample (8% by water, 19% by NaCl solution, about 7% by butanol, and finally 5% by sodium borate). The dye-binding test revealed the lowest amount of protein in the alcohol extract in spite of its higher percentage by weight than the sodium borate extractable fraction. This was

Table I. Composition of Protein in the Pistachio Nut[a]

protein fraction	% of total	protein fraction	% of total
albumin	25.0	glutelin	7.3
globulin	66.0	prolamin	2.0

[a] The results are the averages of three determinations and with two replicates in each experiment.

probably due to the presence of a variety of alcohol-soluble, nonprotein materials in this presumptive prolamin fraction. Determination of the total protein in the meal by the micro-Kjeldahl method showed 18.2% of the weight of the meal was protein, which is about 50% of the total extractable dry weight (38.5% of the meal). The percent contribution of each of the four solubility fractions to the total protein content in the pistachio nut is presented in Table I. It is obvious that the globulin fraction (NaCl soluble) is the major storage protein in the pistachio, contributing about two-thirds of the total protein. Albumins are second in predominance to globulins, contributing 25% of the total protein, followed by glutelins (7.3%) and prolamins (2%). Since there are no other published reports on fractionation of pistachio protein, it was not possible to compare these results with pistachio nuts of other localities or with those from other tree nuts.

Electrophoretic Fractionation. Fractionation of proteins by various electrophoretic techniques revealed the presence of a highly heterogeneous group of polypeptides ranging in molecular weight from 12 to 100K in the seed extract. Gradient gels yielded better resolution of the polypeptides in all fractions than homogeneous gels. Even though one could detect as many as 32 bands after SDS-PAGE and about 48 after IEF separation, only about 10 predominant bands (mol wt 17–40K) compose most of the total protein (Figure 1). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis of each fraction was carried out separately, and the results are shown in Figure 1a (lanes 1–4) along with the profiles of the total protein extract from California and Turkish samples (Figure 1a, lanes 5 and 6). The minor differences between the Kerman and Turkish samples are indicated in Figure 1b (arrows). In spite of solubility differences, the various fractions of the reserve protein exhibit extensive similarity in the general pattern and size of the polypeptides they contain. Of the four fractions, the butanol-extractable fraction did not yield any distinct protein band (Figure 1a, lane 3) suggesting that the nitrogen this fraction contained was mainly from nonprotein and nitrogenous substances soluble in alcohol. In a separate experiment, we attempted to extract alcohol-soluble protein (prolamin) from the meal using 60% butanol as the first solvent. Again the results failed to show any specific alcohol-soluble protein upon electrophoresis and confirmed the results we obtained earlier by the conventional Osborn protocol. The degree of similarity was much greater between the NaCl and sodium borate fractions (Figure 1a, lanes 2 and 4). It was also observed that the water-soluble fraction exhibited a larger number of protein bands and thus greater heterogeneity, as compared with the other fractions, which may have included mainly the water-soluble enzymes. The occurrence of similar polypeptides in the SDS-PAGE profiles of different fractions indicated that these three solubility fractions did not differ much in terms of polypeptide composition. Extraction with each solvent was repeated several times with a high solvent to meal ratio, monitoring the completion of extraction in each step before the next solvent in the sequence was introduced. Under these conditions the second solvent was added only after the preceding solvent was no longer extracting any detectable amount of protein. Thus, the apparent homology between different protein fractions must be due to the presence of oligomers and polymers of different sizes and/or isoelectric points (p*I*) resulting from association of similar monomeric groups of polypeptides in all fractions. Apparently, these oligomers and polymers of dif-

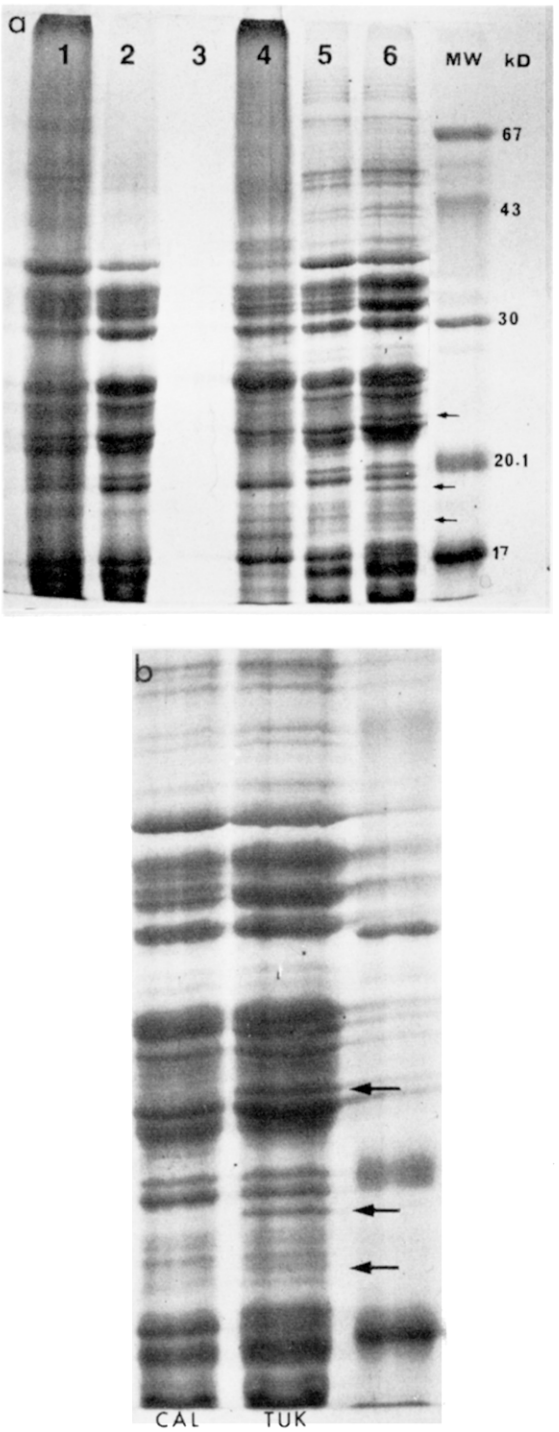

Figure 1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) profiles of storage proteins from Kerman pistachio nut meal. (a) Protein pattern of different solubility fractions (12% homogeneous gel): 1, water soluble; 2, NaCl soluble; 3, *tert*-butyl alcohol soluble; 4, sodium borate soluble; 5 and 6, whole-meal extract of Kerman and Turkish pistachio samples, respectively; unmarked, molecular weight markers. Note the absence of any distinct protein bands in the *tert*-butyl alcohol soluble fraction. (b) Close-up view of lanes 5 and 6 from Figure 1a showing the three polypeptides (arrows) unique to the pistachio sample from Turkey but absent in the one from Kerman.

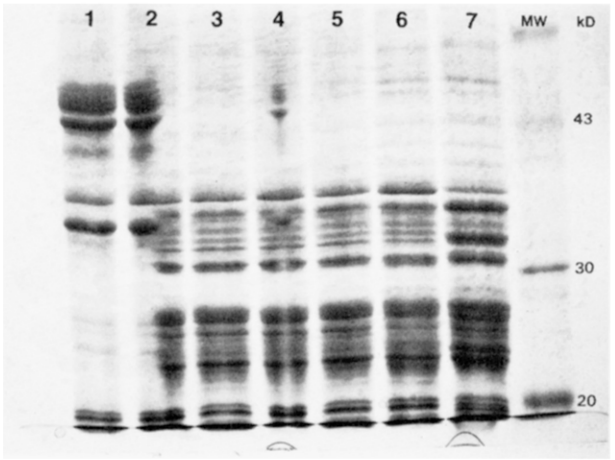

Figure 2. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) patterns of the total seed protein from Kerman pistachio showing the effect of urea and 2-mercaptoethanol (2-ME) on the mobility of protein bands: lane 1, total protein extracted with 10 mM Tris-HCl, pH 7.5, containing 1.5 M NaCl and mixed with the laemmli sample buffer lacking 2-ME before electrophoresis, –6 M urea, and –2-ME; lane 2, +6 M urea and –2-ME, showing that urea did not cause any shifts in the mobility of the protein bands as evident in the left half of the lane while 2-ME diffusing from lane 3 resulted in the disappearance of high molecular weight components and appearance of numerous low molecular weight ones as evident in the right half of the lane; lane 3, –6 M urea and +2-ME, showing the effect of a reducing agent on shifting the size of protein components from high molecular weight to low molecular weight; lane 4, +6 M urea and –2-ME, showing the presence of high molecular weight unreduced components in the middle flanked by reduced components on both sides due to the diffusion of 2-ME from lanes 3 and 5; lane 5, +6 M urea and +2-ME, yielding a pattern identical with that in lane 3; lanes 6 and 7, Kerman and Turkish samples, respectively, in the presence of 6 M urea and 2-ME; unmarked lane, molecular weight standards. These results clearly show that the pistachio storage protein occurs as large disulfide-linked oligomers and polymers in its native state.

ferent sizes and/or p*I* values exhibit differences with respect to solubility in various solvents.

Attempts to fractionate storage proteins of the pistachio under nondenaturing conditions at various gel concentrations and in cold did not result in resolution of any distinct protein bands. At first this was thought to be due to a high degree of aggregation through various protein–protein interactions among different proteins. However, the addition of urea, dithiothreithol (DTT), 2-ME, or a combination of these to the sample medium did not improve resolution. Similarly, the addition of 6 M urea to the SDS-treated samples did not produce any changes in the protein profile. In contrast, the addition of 2-ME resulted in drastic changes in the number and mobility of the protein bands as shown in Figure 2. In fact, the effect of reducing agents could be seen dramatically in an unreduced sample when it was electrophoresed in a lane next to that of a reduced sample. In this case, mobility shift was evident in half of the profile (Figure 2, lane 2) due to the diffusion of the reducing agent from the adjacent lane. These results indicate the presence of extensive S–S linkages between constituent polypeptides to form oligomeric associations that are easily disrupted by 2-ME but remain intact in the presence of urea.

The isoelectric focusing studies of the proteins under nondenaturing conditions also did not yield satisfactory separation of the protein components present in the different solubility fractions. When IEF studies under nondenaturing conditions were repeated using agarose gels instead of polyacrylamide, there was no improvement in

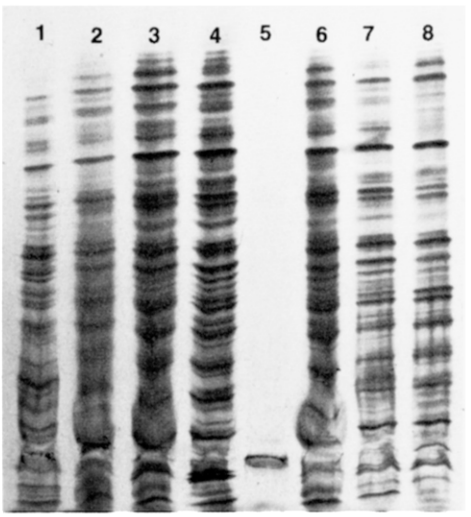

Figure 3. Isoelectric focusing (IEF) patterns of the various pistachio (Kerman) protein solubility fractions in the pH gradient 4.0–10.5, showing extensive charge heterogeneity within each fraction and also very few qualitative differences among fractions: lane 1, H_2O-soluble fraction; lane 2, same fraction as in lane 1, but after precipitation with 10% trichloroacetic acid (TCA); lane 3, NaCl-soluble fraction; lane 4, same fraction as in lane 3, but after TCA precipitation; lane 5, butanol-soluble fraction showing no detectable protein components; lane 6, sodium borate/SDS-soluble fraction; lanes 7 and 8, total protein profiles obtained by extracting the meal of Kerman and Turkish samples directly with 6 M urea + 1% 2-ME. Note that there are no major qualitative differences between Turkish and Kerman samples.

the resolution of native proteins. The number of bands resolved in each fraction by IEF under denaturing conditions was greater than that observed after SDS-PAGE. The apparent p*I* for the components resolved ranged from 4 to 10.6 (Figure 3). Again, there were no discernible protein bands in the IEF-profile of butanol extract, and there was extensive similarity among the three solubility fractions as was observed after SDS-PAGE. Moreover, precipitation of the proteins by TCA during isolation did not have any discernible effect on IEF banding patterns (Figure 3, lanes 2 and 4).

The two-dimensional electrophoresis of the total protein sample was also carried out in order to obtain a more accurate measure of the degree of heterogeneity of the protein in pistachio (results are not shown). In the two-dimensional comparison of the Kerman and Turkish samples, those minor differences observed after one-dimensional separations, as expected, were better resolved.

Gel Filtration. When the total protein extract was subjected to gel filtration on a Sepharose (Cl 6B column, three major protein peaks with apparent molecular weights of 398, 93, and 31.6K were resolved (Figure 4). No protein was detected in the effluent immediately following the void volume of the column, suggesting that none of the native proteins were excluded, and thus none had apparent molecular weight greater than 2000K, the exclusion limit of the matrix. The electrophoretic analysis of column fractions showed that fraction I (referred to as pistacin I fraction) had all the bands present in fractions II and III (pistacins II and III, respectively). In fact, pistacin I had a profile very similar to that of the whole meal, while there was only one major band in pistacin III and two bands in the pistacin II fractions. Gel filtration data suggest that pistachio protein in its native state occurs as large oligomers and polymers of limited size heterogeneity. This would account for our failure to resolve the native protein by electrophoresis under nondenaturing conditions.

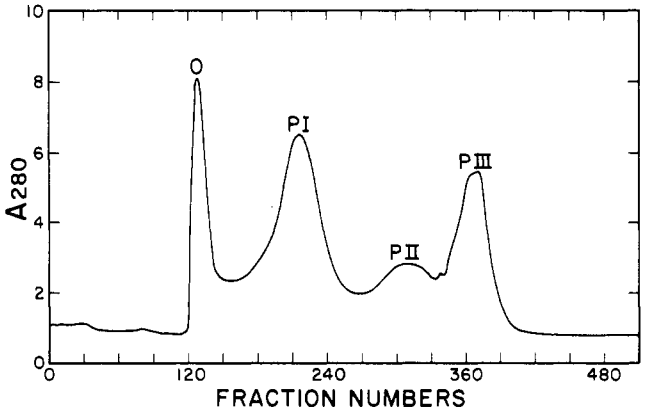

Figure 4. Gel filtration profile of pistachio protein fractionated on a Sepharose Cl 6B column (3 × 80 cm, equilibrated with 5 mM KPO_4 buffer, pH 7.5). The sample was 60% (final concentration) $(NH_4)_2SO_4$ precipitate, initially extracted from 0.5 g of defatted Kerman meal with 10 mM Tris-HCl/1.5 M NaCl, dissolved in 4 mL of 5 mM K_3PO_4 buffer before application to the column. Gel filtration was performed at room temperature, and 5-mL fractions were collected. The first peak (labeled 0) indicates the position of Dextran Blue (mol wt 2000K) followed by those of the major pistachia protein fractions, pistacin I (PI), pistacin II (PII), and pistacin III (PIII), respectively.

Comparison of SDS-PAGE and IEF profiles of the Kerman and Turkish pistachio samples shows a high degree of similarity between the two samples except for a few protein bands (Figure 1b, arrows). This may indicate that the pistachio of Kerman and Turkey shares a common origin in the plateaus of Iran where a good number of wild pistachio species grow naturally. An alternative explanation would be the presence of powerful selective constraints that maintain the size and net charge of the storage proteins in the pistachio. Although there were a few morphological differences when the two nuts were compared, the nuts from Turkey were also richer in total protein (about 1.3 times higher than Kerman nuts). This higher protein content was not surprising because the Turkish nuts used in this study were much smaller in size and lighter in weight and it is well-known that there is a negative correlation between seed size, crop yield, and protein content in most, if not all, plants (Esen, 1982). There are no similar studies on other tree nuts that our results can be compared with. An observation worth mentioning is the resemblance in the general pattern (molecular weight, isoelectric points) of proteins from pistachio to those of the common legume crops such as soybean and peanuts. In legumes (dicots) the storage proteins are mainly composed of a small group of globulins (7S and 11S proteins) given different names according to species. Thus, there is an overall similarity among the storage globulins in dicotyledonous plants. This indicates that the genes coding for storage proteins occur as a family and have homologous couterparts in other taxa, which explains the similarity of their products (proteins) in seeds of diverse plant species, and these genes are active only in the embryo and in cotyledons and not in any other organ (Bryant, 1985). This similarity is reflected in seeds of widely differing dicot taxa as a common storage protein architecture, which probably represents an optimum for efficient reserve nitrogen storage and utilization in this subclass of flowering plants.

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