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Biochemical Study of Storage Proteins of Akbari and Kalehghoochi Pistachio Cultivars (*Pistacia vera*) in Rafsanjan, Iran

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ABSTRACT

Seeds are one of the richest sources of plant proteins. The storage proteins have no enzymatic activity and constitute a source of amino acids, nitrogen and carbon skeletons for the developing seedlings. Morphological study of aleurone grains indicated that pistachio nuts had crystalloid protein bodies but no globoid protein bodies. The storage proteins in aleurone grains of two pistachio cultivars (Akbari & Kalehghoochi) in Rafsanjan in Iran were extracted as dry, soaked and during germination. After SDS-PAGE electrophoresis, determination of molecular weight and amount of storage proteins, we found that all of these amounts were the highest in dry grains. These amounts decrease in other grains, except in aleurone of pistachio grains at 6 and 8 days and were similar for both cultivars.

Key Word: Storage protein; Pistachio; Seed; Protein bodies; SDS-PAGE

INTRODUCTION

Seeds are one of the richest sources of plant proteins. Among major crop plants, cereals contain 10-15% and some legumes (e.g., Soybean) have 40-50% protein content on dry weight basis. Of the total proteins, about 85% are storage in nature. The storage proteins have no enzymatic activity and simply provide a source of amino acids, nitrogen and carbon skeletons for development of seedlings. These proteins are stored in the seed in an insoluble form in protein bodies in the endosperm in cereal and cotyledons in legume seeds and survive desiccated for long periods of time (Shotwell & Larkins, 1989).

Because seeds provide an important source of protein for human and livestock nutrition, much research has been devoted to increasing the content of the essential amino acids and improving the nutritional quality of seed proteins (Nelson, 1979). Osborn (1859-1929) developed a classification of plant proteins based on their solubility in a series of solvents, to albumins and globulins (Shewry & Halford, 2002). Rost (1972) describes three structural types of storage proteins: (a) those consisting of amorphous protein without inclusions (e.g., those of peanut, soybean & corn), (b) those with phytin globoids in the protein matrix (cotton) and (c) those with both globoids and protein crystalloids (cannabis, cucurbita & yucca). Tully and Beevers (1975) on Castorbean, Youle and Huang (1976) on castorbean and Tabatabaee-panah *et al*. (2003) on Ohadi pistachio cultivar, extracted storage protein and prepared SDS-PAGE pattern in dry, water soaked and germinated samples and found that amount of storage protein was highest in dry grain, decreased in other grains, except in aleurone of pistachio grains 6 and 8 days.

The pistachio nut is one of the most important agricultural products, which is exported as well as being largely consumed locally. In this paper we report electrophoretic pattern of the aleurone storage protein of the nuts of Akbari and Kalehghoochi pistachio cultivars.

MATERIALS AND METHODS

Akbari and Kalleghoochi pistachio (*Pistacia vera* L.) nuts were collected from Iran's Pistachio Research Institute collection orchard in Rafsanjan, soaked overnight in running tap water and germinated in moist vermiculite in darkness. Germinated nuts at various ages (2, 4, 6, 8, 10, 12, 14 & 16 days) were withdrawn and lyophiled. Protein bodies were isolated by successive non-aqueous linear density gradient centrifugation. A 1.5 gr of deshelled sample nuts (dry, soaked & germinated nuts) were ground in 20 mL of hexane at low speed in a VirTis 45 homogenizer for 5 min. The homogenate was filtered through a Nitex cloth of 35 µm pore size. Three mL of the filtrate were layered on to a 32 mL linear gradient of hexane and carbon tetrachloride from density 1.20 to 1.50 g cm^3. The gradient was centrifuged at 12000 rpm for 2 h in a Beckman L2-65 B ultracentrifuge

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using a SW 27 rotor. After centrifugation, the gradient was fractionated. Each fraction was assayed for absorbance at 280 nm. Most of the protein bodies were present at density around 1.36 g cm^3. The fractions with the protein bodies were pooled and made to 1.50 g cm^3 with CCl_4. The preparation was then put in a centrifuge tube and a similar gradient of hexane-CCl_4 was layered upon it. Centrifugation was performed as before and the protein bodies again floated to their equilibrium density at 1.36 g cm^3. After centrifugation, the gradient was fractionated (Youle & Huang, 1976). And the fractions were observed under the light microscope photograph prepared by zeiss Axio plan 2 microscope and digital camera, in 1000 magnifying power. Also each fraction was assayed for absorbance at 280 nm. Using excel program we drew OD/tube number curve.

Total protein was measured according to Bradford assay (Rost, 1972). The extracted protein were loaded on SDS-PAGE gel (Resolving gel: 12% & Stacking gel: 4%) and determined storage protein band on the gel along with their molecular weight (Altschul *et al*., 1966; Basha & Beevers, 1975).

RESULTS

Morphological study of aleurone grains indicated the presence crystalloid protein bodies but no globoid protein bodies (Fig. 1). Regarding (OD) per tube number curves, the first extraction had two picks, of which second pick was related to chloroplast, the second extraction was relatively pure because of had just one pick (Fig. 2). All of the sample diagrams showed that pick of curve in dry sample was highest and began to decrease in water soaked, 2 and 4 days, increase in 6 and 8 days and decrease again. The amount of storage proteins according Bradford assay were the highest in dry grains. This amounts decreased in other grains, except in aleurone of pistachio grains at 6 and 8 days (Table I).

Table I. Amount of storage proteins according Bradford assay.(µg ml^{-1})

	dry	Water Soaked	2 days	4 days	6 days	8 days	10 days	12 days	14 days	16 days
Akbari	750	700	590	570	700	680	650	565	552	531
kalleghoochi	730	670	560	550	670	682	581	545	541	529

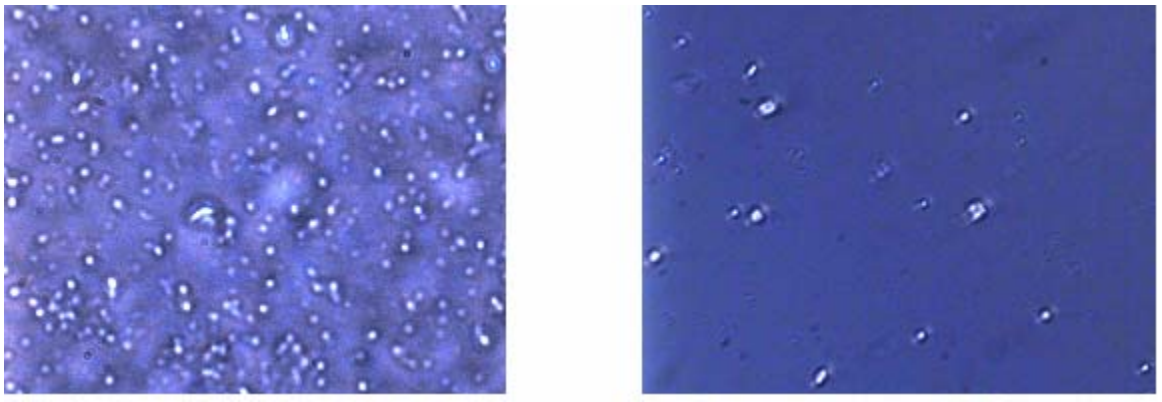
Table II. Molecular weight of storage protein bands in Kalleghoochi pistachio nuts (kDa)

Dry	Water Soaked	2 days	4 days	6 days	8 days	10 days	12 days	14 days	16 days
65.5	65.5	65.5	65.5	65.5	66	65.5	65.5	65.5	64
64	64	64	62.5	64	64	64	64	64	62.5
62.5	62.5	60	60	62.5	62.5	62.5	59.5	59	57.5
60	60	57.5	56	57.5	57.5	59.5	57.5	57.5	54
59	57.5	52.5	54	54	54	57.5	54	54	50
57.5	54	46	46	50	50	54	50	50	42
54	52.5	35	42	47	47	50	46	40.5	38
52.5	50	34	38.5	46	46	46	35	35	35
51	46	33.5	35	42	42	42	33	33	34
50	45	32.5	33	38.5	38.5	38.5	32.5	32.5	32.5
46	40.5	30	32.5	35	35	35	30	30	30
45	35	29.5	30	33.5	33.5	32.5	25	25	25
42	33.5	25	29.5	33	33	32	23.5	23.5	23.5
40.5	33	23.5	25	32.5	32.5	30	23	23	23
35	32.5	22.5	23.5	30	30	25	22.5	22.5	22.5
34.5	30	22	22.5	29.5	29.5	23	20	20	
34	29.5	20	20	28.5	28.5	21.5			
33.5	25	17		25	25	20			
33	24.5			23.5	23.5	19			
32.5	23.5			22.5	22.5				
30	22.522			21.5	21.5				
29.5	20			20	20				
29	19			19	19.5				
25	17			17	19				
24.5					17				
23.5									
23									
22.5									
20									
19									
17									
15									

Table III. Molecular weight of storage protein band in Akbari pistachio nuts (kDa)

Dry	Water Soaked	2 days	4 days	6 days	8 days	10 days	12 days	14 days	16 days
68.5	68	66	66	66.5	66	66	66	66	60
68	66.5	64.5	64.5	64.5	64	64	64	64	59
66.5	64.5	62	62	62	62	62	60	60	57
64.5	63	60	60	57	60	60	57	59	53
62.5	62	59	59	56.5	59	57	56.5	57	52.5
62	60.5	57	57	55	57	56.5	54	54	46
60.5	59	54	54	54	55	53	51	53	44
60	57	53	53	53	54	46	44.5	46.5	42
59	56	46	46	46.5	53	45.5	42	44.5	36
56.5	54.5	39	39	46	46.5	41	37	42	33.5
56	53.5	36	36	45.5	46	36	36	36	31
55	47.5	35	35	45	44.5	35	35	33.5	25.5
54.5	46	33.5	33.5	43	44	33.5	34	31	24
54	43.5	32.5	32.5	42.5	43	32.5	31	25.5	23.5
48	39	31	31	39	39.5	32	25.5	24	21
47.5	38	27	27	36	36	31	24	22.5	
46.5	36.5	26.5	26.5	35	35	30	23.5	21	
43.5	36	25.5	25.5	33.5	33.5	29	22.5		
39	35.5	21		32.5	32.5	25.5	21		
38	35	18		31	31	23.5			
36.5	32.5			30	30	22.5			
36	31			27	29.5	21			
35	26.5			25.5	25.5				
34	25.5			24	24				
32.5	22			23.5	23.5				
32	21			22.5	22.5				
31	17.5			22	21				
27	16.5			21	18				
26.5	16			18					
25.5	15.5								
22.5									
21									
18									
17.5									
16.5									
16									
15.5									

Fig. 1. Protein bodies in Akbari (right) and Kalleghoochi (left) pistachio nuts



Extracted proteins were loaded on SDS-PAGE gel (Fig. 3). Based on Rm and molecular weight of marker protein, standard curve was drawn. By using Rm and standard curve the molecular weight of storage proteins were determined (Table II & III). The amounts of proteins were the highest in dry grains. This amounts decreased in other grains, except in aleurone of pistachio grains 6 and 8 days.

DISCUSSION

Morphological study of aleurone grains showed that there were crystalloid protein bodies in pistachio nuts but we have no information about globoid protein bodies. Similar results were obtained by Tabatabaee-panah *et al.* (2003) on Ohadi cultivar. Tully and Beevers (1975) and Youle and Huang (1976) on Castorbean extracted

Fig. 2. O.D. curve of the first extraction (left) and the second extraction (right)

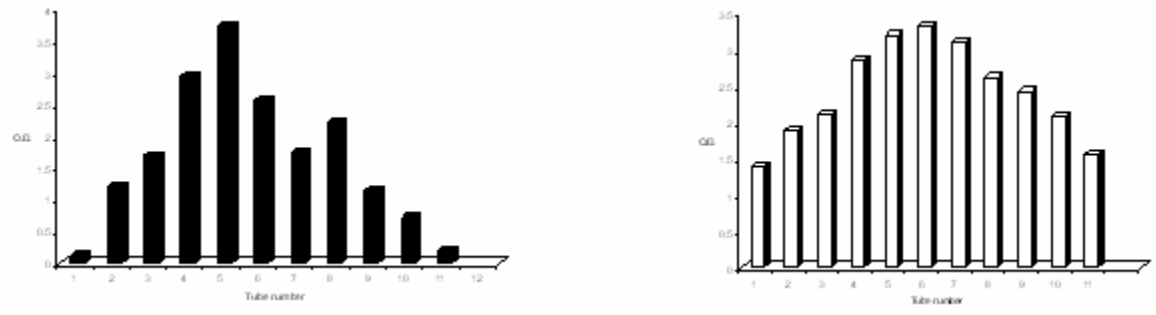
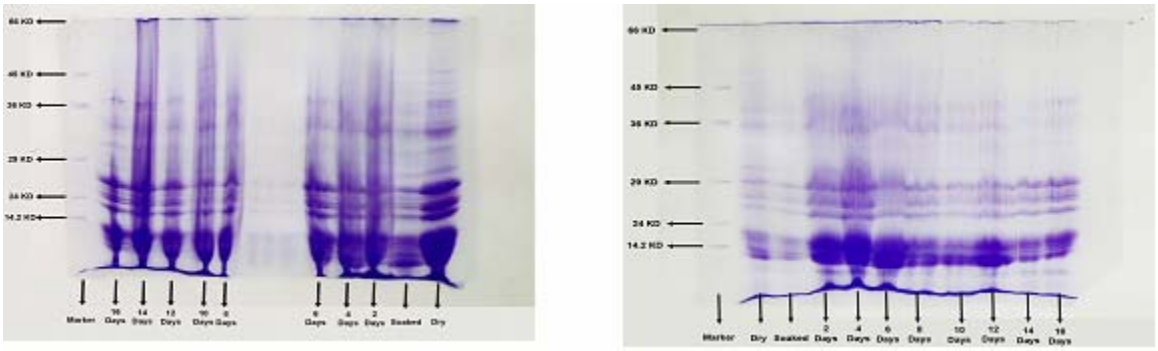


Fig. 3. SDS-PAGE gel of Akbari (left) variety storage proteins and Kalleghoochi (right) variety storage proteins



crystalloid protein and total protein during different stages of germination. Their results showed that there was a rapid disappearance of the crystalloids after one day of germination. The disappearance of the crystalloids paralleled that of the crystalloid proteins as evident from electrophorogram, but preceded the drop of the total protein. This lag in the drop of the total protein was expected since the crystalloid proteins were degraded into lower molecular weight subunits before their final breakdown into amino acids. Shewry and Halford (2002) surveyed variation of storage proteins in many of cereals such as rice, wheat and corn during germination and their results agreement with our's. Tully and Beevers (1978) reported that CPase, Hb-ase and BANAase were relatively low during the early stages of germination in Castorbean. However, whatsoever activity present might be sufficient to account for the observed loss of storage protein.

There are undoubtedly other proteases or peptidases, which have not been assayed yet. We believe that amount of storage protein in dry nuts was highest because dry nuts had not started growing and storage proteins were not used. An increase in storage might be due to their synthesis and gene expression of some proteins may have started again. Of course the kinds of storage proteins and the reason why the genes expression and synthesis stopped and started again on days of 6^{th} and 8^{th} after germination is not clear yet.

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