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Effect of antioxidant treatment, packaging materials (laminated vacuum packed), storage temperatures and periods on the nutritive value of almond kernels

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Abstract
The present investigation indicated that antioxidant treated samples packed in laminates under vacuum and stored under ambient conditions proved significantly superior by exhibiting highest crude protein content (15.16%), crude fat content (54.33%), crude fiber content (9.81%), carbohydrate content (9.80%), total phenols (110.20), flavonoid content (16.03) and minimum peroxide value (109.99 meq/100g) and free fatty acid value (0.75 mg/g) compared to untreated kernels packed in LDPE under accelerated conditions throughout the storage period. These samples again proved significantly superior in maintaining colour, taste, texture and mouthfeel compared to untreated, LDPE packed samples stored under accelerated conditions. Storage studied suggested that the product can be stored in cellophane pouches for one month at ambient conditions.

Keywords: Almond, crude protein, fat, packaging

Introduction
Almonds (*Prunus dulcis*) are native to Mediterranean region and are considered as one of the oldest tree nuts in the world. They are closely related to peach and are probably evolved from the same ancestral species in South-Central Asia. From there they spread along the shores of the Mediterranean in Northern Africa and Southern Europe through Egyptians, Greeks and Romans (Anonymous, 2011). The production of almond in India is concentrated in Utter Pradesh, Himachal Pradesh and Jammu and Kashmir. The Kashmir enjoys hub of almond industry in the country and almond production is increasing every year that is the case the share of Jammu and Kashmir in national production is increasing. Annual production of almond in Kashmir valley is 1.5 million tonnes as per the report of horticulture department (Anonymous, 2016).

Almonds being highly nutritious are considered healthiest and well-balanced cholesterol free food (Kendall *et al.*, 2002). On an average 100g of almond contains 575 calorie, fibre 12.2g, vitamin E 26 mg, total fat 949g, monounsaturated fat 31g, total Omega-3 fatty acid 6mg, total Omega-6 fatty acid 12065 mg, protein 21g, potassium 670 mg, magnesium 268 mg, phosphorus 484mg, calcium 265mg and iron 3.5mg. Almonds are low in saturated fats and high in many other protective nutrients which help in preventing cardiovascular, cancer diseases and in reducing heart attack risks. Almonds also contain more alkaline forming minerals than acid forming minerals, which makes them a rare protein-rich, bone protecting and alkalizing food. Emerging research demonstrates that the combination of dietary fiber from cereals along with the protein from almonds contributes to overall satiety and therefore may play a role in controlling caloric intake. Most other protein sources do not provide dietary fiber as is found in almonds (Annoynmous, 2013).

Material and Methods
The present investigation was carried out at Division of Food Science and Technology, Sher-e-Kashmir University of Agricultural Science and Technology of Kashmir (SKUAST-K), Shalimar. This section enlists the raw material used and elaborates the processing techniques, analytical procedures, and statistical methods followed during research.The following parameter were investigated.

Crude protein (%)

Micro-kjeldhal apparatus was used to determine nitrogen content for all the flour samples (AOAC, 2005). Protein estimation was carried out in the Kel Plus (KESO6L, Pelican equipment, Chennai).

1 g of sample was weighed in a dry Kjeldahl flask. To this, 3 g of digestion mixture (potassium sulphate + copper sulphate + iron sulphate in ratio of 0.5:2:0.5) and 10 ml of pure concentrated H_2SO_4 was added. The mixture was digested by heating for about 4-5 hours. Glass beads were added to prevent bumping. After the contents of the flask becomes clear, the digestion was further continued for at least 1 hour till the colour changed to green. The contents of the Kjeldahl flask were cooled, diluted with distilled water and the mixture was made alkaline by adding excess (about 75 ml) of 40% NaOH. The ammonia so liberated was distilled into a receiver containing 25 ml of boric acid and indicator was then titrated with 0.1N HCl till colour changed to pink indicating the end point.

Protein percentage was calculated according to the formula

$$\text{Crude protein (\%)} = \frac{(\text{Sample titre-blank titre}) \times 14 \times 6.25 \times 100}{\text{Weight of sample (g)}}$$

Where, 14 is the molecular weight of nitrogen and 6.25 is the nitrogen factor for proteins.

Crude fat (%)

Crude fat was determined by using petroleum ether as a solvent in the Soxhlet apparatus (make PELICAN, model SOCC-PLUS-SCS-2).according to AOAC (2005) method. Extraction was carried out by using a fat-free thimble containing 5 g of flour samples and fitted in apparatus for about 12 hours. The results were expressed as per cent fat.

$$\text{Fat (\%)} = \frac{\text{Weight of fat (g)}}{\text{Weight of sample (g)}} \times 100$$

Crude fibre (%)

Crude fibre was estimated by following the standard procedures given by AOAC (2005). Crude fibre was determined by the Fibre tech apparatus (make PELICAN, model fibraplus-FES06E).

1 g of each sample were subjected to acid hydrolysis with 2.5N HCl followed by alkali digestion with 0.1N NaOH. The residue so obtained was washed with double distilled water and ignited in a muffle furnace at 600ºC for 4-6 hours. At high temperature, the inorganic matter in the residue gets oxidized and inorganic residue or ash is left behind. The difference in the weight before and after process was then determined to calculate the per cent crude fibre in sample.

$$\text{Crude fibre (\%)} = \frac{W_1 - W_2}{W} \times 100$$

Where,

W gm = weight of sample

W_1 gm = Residue (crude fiber + minerals)

W_2gm= ash remained

Result and Discussion

Data presented in Table-1 depicts the effect of antioxidant treatment, packaging materials, storage temperatures and periods on the crude protein content of almond kernels.The antioxidant (T_2) treated almond kernels had significantly higher mean crude protein content (14.81%) compared to the value of 14.72 per cent recorded in untreated (T_1) samples. With respect to packaging materials a significantly higher mean crude protein content (14.88%) was recorded in laminates under vacuum packaging (P_2) compared to the value of 14.59 observed in LDPE (P_1) packed samples. Significantly higher mean crude protein of 15.01per cent was recorded in samples stored under ambient temperatures (C_1) compared to value 14.33 recorded in samples stored under accelerated temperatures (C_2).

Table 1: Effect of antioxidant treatment, packaging materials, storage temperatures and periods on the crude protein content (%) of Almond kernels

Treatment (T)	Storage temperature (C)	Packaging										Storage period mean (Days) (D)				Factor mean	
		LDPE(P_1)					Laminates vacuum packed (P_2)										
		Storage periods (Days)(D)					Storage periods (Days)(D)										
		0	90	180	270	Mean	0	90	180	270	Mean	0	90	180	270	T	C
Control (T_1)	Ambient (C_1)	16.50	15.32	14.57	13.15	14.88	16.50	15.62	14.80	13.32	15.06	16.50	15.47	14.68	13.23	T_1 = 14.72	C_1 =15.01
	Accelerated (C_2)	16.50	15.19	13.53	12.21	14.63	16.50	14.61	13.59	12.67	14.34	16.50	14.90	13.56	12.44		
Sub mean		16.50	15.25	14.05	12.68	14.75	16.50	15.11	14.19	12.99	14.70	16.50	15.18	14.12	12.83		
BHT+BHA (0.015+0.015%) (T_2)	Ambient (C_1)	16.50	15.45	14.40	13.41	14.94	16.50	15.76	14.93	13.46	15.16	16.50	15.60	14.66	13.43		
	Accelerated (C_2)	16.50	14.44	13.40	12.39	14.18	16.50	15.44	14.86	13.09	14.97	16.50	14.94	14.13	12.74		
Sub Mean		16.50	14.94	13.90	12.90	14.56	16.50	15.60	14.89	13.27	15.06	16.50	15.27	14.39	13.08		
Storage Temperature Mean	Ambient (C_1)	16.50	15.38	14.48	13.28	14.91	16.50	15.69	14.86	13.39	15.11	16.50	15.53	14.67	13.33	T_2 =14.81	C_2 =14.33
	Accelerated (C_2)	16.50	14.81	13.46	12.30	14.40	16.50	15.02	14.22	12.88	14.65	16.50	14.92	13.84	12.59		
Overall mean		16.50	15.09	13.97	12.79	14.65	16.50	15.35	14.54	13.13	14.88	16.50	15.23	14.26	12.96		
Grand mean		14.59					14.88										

BHA= butylated hydroxyl anisole, BHT= butylated hydroxyl toluene (CD p≤0.05)

Treatments (T)	=	0.102	T×C	=	NS	T×P ×D	=	0. 289
Packaging (P)	=	0.102	P×C	=	NS	C×D	=	0.204
Storage temperature (C)	=	0.144	T×P×C	=	0.204	T×C×D	=	NS
Storage period (D)	=	0.008	T×D	=	NS	P×C×D	=	NS
T×P	=	0.144	P×D	=	0.204	T×P×C×D	=	0.409

With the increase in storage periods there was a significant decrease in overall mean crude protein content of almond kernels. Highest crude protein content of 16.50 per cent recorded at 0 days of storage decreased to 15.23 per cent at 90 days, which further decreased to 14.26 and 12.96 per cent at 180 and 270 days respectively.

Data revealed that the effect of antioxidant treatment, packaging materials, storage temperatures and periods significantly affected crude protein content of almond kernel. There was a significant decrease in protein content. It was because of the exposure of protein at the high temperatures which caused denaturation of protein cells leading to loss of protein due to weakening of 3D conformation of protein cells. However, decrease in protein content can also be attributed to their involvement in milliard reactions. Similar results were reported by Senesi *et al.* (1991) while the effect of different packaging conditions on the stability of shelled almonds. They used packaging materials with different diffusivities to gases, light and internal atmosphere of the packages and the physical (colour, texture and water activity), sensory (colour, taste and total acceptance) and chemical characteristics (protein and fat content) of almonds were determined after 18 month of storage.

Table 2: Effect of antioxidant treatment, packaging materials, storage temperatures and periods on the crude fat (%) content of Almond kernel

Treatment (T)	Storage temperature (C)	Packaging										Storage period mean (Days) (D)				Factor mean	
		LDPE(P_1)					Laminates vacuum packed (P_2)										
		Storage periods (Days) (D)					Storage periods (Days) (D)										
		0	90	180	270	Mean	0	90	180	270	Mean	0	90	180	270	T	C
Control (T_1)	Ambient (C_1)	58.90	53.59	50.23	47.98	52.67	58.90	55.16	49.15	47.37	52.64	58.90	55.07	55.27	47.80	T_1 = 51.51	C_1=53.79
	Accelerated (C_2)	58.90	49.05	46.81	44.93	49.92	58.90	50.65	47.98	45.76	50.82	58.90	49.85	52.39	45.35		
Sub mean		58.90	51.32	48.52	46.45	51.29	58.90	52.90	48.56	46.56	51.73	58.90	52.46	53.83	46.57		
BHT+BHA (0.015+0.015%) (T_2)	Ambient (C_1)	58.90	54.98	51.39	48.23	53.37	58.90	57.37	55.32	54.33	56.48	58.90	55.48	52.78	51.16		
	Accelerated (C_2)	58.90	50.65	47.36	45.54	50.61	58.90	50.98	49.05	46.78	51.43	58.90	50.82	48.21	46.16		
Sub Mean		58.90	52.81	49.37	46.88	51.99	58.90	54.17	52.18	50.55	54.56	58.90	53.15	50.49	48.66	T_2 = 52.97	C_2=50.69
Storage Temperature Mean	Ambient (C_1)	58.90	54.29	50.81	48.11	51.64	58.90	56.26	57.23	50.85	51.12	58.90	55.28	54.03	49.48		
	Accelerated (C_2)	58.90	49.85	52.08	45.24	50.26	58.90	50.82	48.52	46.27	52.84	58.90	50.34	50.30	45.76		
Overall mean			58.90	52.07	51.44	46.67	50.95	58.90	53.54	52.87	48.56	51.98	58.90	52.81	52.16		
Grand mean		52.27					53.46										

BHA= butylated hydroxyl anisole, BHT= butylated hydroxyl toluene (CD p≤0.05)

Treatments (T)	=	0.660	T×C	=	0.871	T×P ×D	=	1.231
Packaging (P)	=	0.660	P×C	=	NS	C×D	=	1.321
Storage temperature (C)	=	0.660	T×P×C	=	1.321	T×C×D	=	1.081
Storage period (D)	=	0.934	T×D	=	NS	P×C×D	=	NS
T×P	=	0.934	P×D	=	NS	T×P×C×D	=	NS

Analysis of the data as presented in Table 2 indicated that the effect of antioxidant treatment, packaging materials and storage temperatures significantly affected crude fat content of almond kernels during 270 days of storage. Overall mean crude fat content of 52.97 per cent recorded in antioxidant (T_2) treated samples was significantly higher than the value of 51.51 per cent observed in untreated (T_1) samples.

Significantly higher crude fat content (53.46%) was recorded in almond kernels samples packed in laminates under vacuum (P_2) compared to the value of 52.27 per cent observed in samples packed in LDPE (P_1). Again overall mean crude protein content of 53.79 per cent recorded in samples stored under ambient temperatures (C_1) was significantly higher than the value of 50.69 per cent observed in samples stored at accelerated temperatures (C_2).

With the increase in storage periods there was a significant decrease in overall mean crude fat content. Highest crude fat content of 58.90 per cent recorded at o days of storage decreased to 52.81per cent at 90 days, which further decreased to 52.16 and 47.62 per cent at 180 and 270 days of storage respectively

Data revealed significantly high fat content in antioxidant treated samples in comparison to untreated samples. Use of antioxidants is mainly aimed at maintaining stability of fats in the substrate. This is achieved by preventing oxidative degradation of free fatty acids which may then get converted into other products thus depleting overall fat content. Results indicate that antioxidant treatments work fairly well in controlling fat depletion. However, untreated samples were less effective to prevent fat depletion which is reflected in significantly lower fat content. Fat content in vacuum packed kernels was significantly higher than those packed in LDPE. This could be attributed to higher oxidative changes that occur in thin LDPE packaging. The immediate derivatives of oxidative changes are further reduced to products like esters, aldehydes, ketones, hexanols, which contribute to the decline in fat content during extended storage particularly at high temperatures. Jensen *et al.* (2003) has reported an increased concentration of hexanals in walnuts under high oxygen concentration. Hexanals have been reported as one of the main products of oxidation by Vercellotti *et al.* (1992) which studied the storage stability of roasted peanuts. The results were also in conformity to those reported by Shin and Lee (2002) and Mexis *et al.* (2009) in walnuts kernels.

Perusal of the data in table 3 indicated that the only storage periods had significant effect on crude fiber content of almond kernels. A significant decrease in crude fiber content was observed in almond kernels from 0 to 270 days of storage amongst all the treatment combinations. The maximum value of 10.60 per cent was recorded at 0 days of storage, which decreased to 10.23, 10.14and 10.10 per cent at 90, 180 and 270 days of storage respectively.

However minimum and maximum drop was recorded in antioxidant treated samples (T_2) from laminate vacuum packaging (P_2) stored under ambient temperatures (C_1) and from untreated (T_1) samples under accelerated temperatures (C_2) in LDPE (P_1) packaging at 270 days of evaluation as indicated by mean crude fiber value of 9.06 and 7.95 per cent respectively from an initial value of 10.60 per cent recorded at 0 days of storage.

Crude fiber provides a distinction between the most digestible and least digestable carbohydrate and it reduces the risk of intestinal disorder. The decrease in crude fiber content may be attributed to the change in moisture content. Opeymi and Emmanuel (2014) observed the decrease in crude fiber content in cashews nuts during twenty weeks of storage. The similar findings were recorded by Fagbohun and Faleye (2012) that investigated the nutritional changes and mycoflora of groundnut during storage and reported the decrease in crude fiber from 9.82 in freshly processed groundnuts to 8.92 in samples at twentieth week of storage.

Table 3. Effect of antioxidant treatment, packaging materials, storage temperature and storage period on the crude fiber (%) of Almond kernel

Treatment (T)	Storage temperature (C)	Packaging										Storage period mean (Days) (D)				Factor mean	
		LDPE(P_1)					Laminates vacuum packed (P_2)										
		Storage periods (Days)(D)					Storage periods (Days)(D)										
		0	90	180	270	Mean	0	90	180	270	Mean	0	90	180	270	T	C
Control (T_1)	Ambient (C_1)	10.60	9.79	8.87	8.19	9.36	10.60	10.01	9.43	8.92	9.74	10.60	10.27	10.18	10.14	T_1 = 9.47	C_1 =9.60
	Accelerated (C_2)	10.60	9.20	8.82	8.03	9.16	10.60	9.81	9.39	8.73	9.63	10.60	10.24	10.14	10.10		
Sub mean		10.60	9.49	8.84	8.11	9.26	10.60	9.91	9.41	8.82	9.68	10.60	10.25	10.16	10.12		
BHT+BHA (0.015+0.015%) (T_2)	Ambient (C_1)	10.60	9.86	9.07	8.54	9.51	10.60	10.07	9.52	9.06	9.81	10.60	10.24	10.15	10.10		
	Accelerated (C_2)	10.60	9.13	8.75	7.95	9.10	10.60	10.16	9.86	8.65	9.80	10.60	10.17	10.11	10.06		
Sub Mean		10.60	9.49	8.91	8.24	9.30	10.60	10.11	9.69	8.85	9.80	10.60	10.20	10.13	10.08		
Storage Temperature Mean	Ambient (C_1)	10.60	9.82	8.97	8.36	9.43	10.60	10.04	9.47	8.99	9.77	10.60	10.25	10.16	10.12	T_2 = 9.55	C_2 =9.42
	Accelerated (C_2)	10.60	9.16	8.78	7.99	9.13	10.60	9.98	9.62	8.69	9.71	10.60	10.20	10.12	10.08		
Overall mean		10.60	9.49	8.87	8.17	9.28	10.60	10.01	9.54	8.84	9.74	10.60	10.23	10.14	10.10		
Grand mean		9.28					9.75										

BHA= butylated hydroxyl anisole, BHT= butylated hydroxyl toluene (CD $p \leq 0.05$)

Treatments (T)	=	NS	T×C	=	NS	T×P ×D	=	NS
Packaging (P)	=	NS	P×C	=	NS	C×D	=	0.117
Storage temperature (C)	=	NS	T×P×C	=	0.117	T×C×D	=	NS
Storage period (D)	=	0.012	T×D	=	NS	P×C×D	=	0.166
T×P	=	NS	P×D	=	0.117	T×P×C×D	=	NS

Conclusion

Statistically significant effect of packaging materials and storage periods was observed on crude protein, fat and crude fibre of almond kernel. No deterioration was observed in the samples up to 90 days of storage.laminates under vacuum packaging proved to be superior with respect to maintaining the quality parameters during 270 days of storage of compared to LDPE packaging. Futhermost storage under ambient temperature conditions were found to be better as that of accelerated temperature conditions.

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