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# The Effect of Modified Atmosphere Packaging and Packaging Material on Walnut Kernel Shelf-life

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#### Abstract

Walnuts with essential and unsaturated fatty acids from omega-3 group, are considered as one of the most important dried fruits with nutritional value. Genotypes 25 and 29 (superior walnut genotypes) were selected to investigate the effect of modified atmosphere packaging on the beneficial compounds of their kernels oil. Walnut kernels were packaged under modified atmospheric conditions into a 90- $\mu$ m metalized plastic. After the oil cold extraction and purification of walnut kernels, the quantitative and qualitative composition of fatty acids was measured. The percent of fatty acids, aflatoxin content, peroxide index, iodine value, acidity and sensory evaluation of samples were determined in the 0-day and during one year storage. The results showed that the unsaturated fatty acids content are predominant in walnut kernels oil, and the linoleic acid is the dominating fatty acid. The saturated fatty acids content was less than 10%. The aflatoxin content of genotypes 25 and 29 were measured 0 and 5 ppb, respectively. The moisture content and the peroxide value were determined 1.55-4.32% and 0.48-4.65 meqO<sub>2</sub>/kg, respectively. The highest level of acidity was observed in the 25 genotypes. The peroxide index, titratable acidity, and weight loss increased with the increasing of storage time, while the iodine value decreased. Totally, the packaged genotype 25 in the PA/PE/PA/PE/Aluminum foil films under modified atmospheric containing 5-6% O<sub>2</sub>, 15% CO<sub>2</sub> and 79-80% N<sub>2</sub> is recommended due to the high quality of chemical and organoleptic properties and the lack of aflatoxin in the walnuts kernel.

# Introduction

Given its climate, Iran is one of the most significant walnut planting centers in the world. Average annual production of walnuts in the world is 1.14 million tons and with more than 379000 tons Iran is the largest producer of walnuts in the world. The major walnut production centers in Iran are Kerman and then Isfahan, Hamedan, and Khorasan Razavi. According to the Agricultural Census of 2017, the area under walnut cultivation in Kerman amounted to 18993 out of 195280 hectares and has the first position. Kerman walnut is high in quality dating back 5000 years. The average crop yield per hectare is

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#### Keywords

Chemical and organoleptic properties Metalized polypropylene Modified atmosphere packaging (MAP) Packaging Walnut kernel 3.5 tons per hectare using mechanized methods (Ahmadi *et al.*, 2018).

Walnut, with scientific name *Juglans regia* L., is from Juglanaceae family: a multifunctional plant with various uses, such as walnut kernels. The high fat content (over 60%) of good quality protein, minerals, and vitamins in walnut kernels (Table 1) has made it fresh or dried for commercial value (Bayat & Mahjub, 2017). Most plant proteins are incomplete given the lack of essential amino acids, but walnut kernel proteins contain essential amino acids and are therefore classified as valuable animal proteins such as meat or eggs (Golzari, Rahemi, Hassani, Vahdati, & Mohammadi, 2013).

 Table 1. Compounds in 100 g of raw walnut kernels

Kerners		
Composition		Value
Water		3.50 g
Protein		14.80 g
Fat		65.00 g
Starch		13.00 g
	Calcium	100.00 mg
	Phosphorus	380.00 mg
Minerals	Iron	3.00 mg
	Sodium	2.00 mg
	potassium	50.00 mg
	Vitamin A	30.00 units
	Vitamin B <sub>1</sub>	0.35 mg
Vitamins	Vitamin B <sub>2</sub>	0.12 mg
	Vitamin B <sub>3</sub>	1.00 mg
	Vitamin C	2.00 mg

Walnuts kernel has compounds called naturally produced in plant sterols compounds and are chemically similar to cholesterol. Some plant sterols are not absorbed along the digestive tract during the digestive process and block the cholesterol absorption pathway in the bloodstream. 100 g of walnut contains 150 mg of plant sterol. This substance has a significant role in the chemical and vascular protection of the body. Moreover, walnut has a flavonoid called ellagic acid that can block the growth of cancer cells (Stampar, Solar, Hudina, Veberic, & Colaric, 2006).

Walnut kernel is one of the dried fruits that is rapidly decayed by chemical and

microbial agents and the cause of spoilage is the presence of significant values of fat (about 64 to 71%) with unsaturated fatty acids like oleic acid, linoleic acid, linolenic acid and arachidonic acid and the oxidation problem always threatens fat (Tajeddin, 2004). Usually, the moisture content of the walnut harvested varies between 34-40% that decreases to 5% after the peeling and drying process. In long-term storage of kernels, the oxidation of lipids and hydrolysis reactions in monolayer water is at its lowest (Maskan & Karatag, 1997). If the moisture of walnut and its kernel reaches below the layer of monolayer water, the lipase enzyme is activated on its lipid and increases the peroxide value (Hamedi, 2015). Using suitable coatings and packaging in modified atmosphere and appropriate temperature is essential to avoid this process (Yaman, 2004).

Sedaghat, Pourazarang, Raee, & Hashemi (2007) packed the pistachio and its kernels in crude polypropylene film with nitrogen and vacuum and then stored at 5 and 10 °C and ambient temperature at 65% relative humidity. The results showed that packaging in metallized film and fivelayer film was effective in maintaining the quality of pistachio. At the end of 28 months storage at two mentioned temperatures, the fatty acids were reported as 0.6, 0.8 and 0.4%. At the end of storage, all samples except for vacuum packing contained a large value of insects and larvae and vacuum packing did not increase aflatoxin levels.

Ghanei Zare, Tavakolipour, & Elhamirad (2012) conducted a study on the evaluation of different types of packaging materials including cellophane, nylon, and metal cans combined with vacuum conditions for raw pistachios. The results showed that nylon, especially under vacuum, was more privileged than other treatments.

Sattar, Mohammad, Saleem, Jan, & Ahmad (1990) examined the effect of fluorescent light, gamma rays, and the type of packaging on the oxidation of walnuts, almonds and peanuts. They stored these products at 25-40 °C for 200 days. The results showed that the oxidation rate increased with the use of fluorescent light and gamma rays. Moreover, glass and polyethylene packaging materials protected the product against oxidation.

The purpose of modified atmosphere packaging (MAP) is to increase the shelf life of perishable foods so that they can maintain the quality of freshness or almost freshness. Hence, MAP is a natural way to extend the life of products without using preservatives. On the other hand, hybrid packaging materials like multilayers improve the barrier features of packaging. For instance, with the presence of cardboard and plastic on the top wall of the beverage packaging, the cardboard provides stability and product protection while the plastic material prevents water vapor they provide the optimum for liquid materials (Stiles & Ooraikul, 1991).

According to the above points, for examining the viability of walnut kernels as one of the nutritious and valuable agricultural products of Iran, this product uses PA/PE/PA/PE composite film and aluminum foil, and modified atmosphere method for packaging and some of its important physical and chemical properties were evaluated during storage.

# Materials and methods

Forty kg of walnuts were purchased from two genotypes 25 and 29 from Rabar (Walnt planting area of Kerman) 180 km south of Kerman. After peeling, it was dried in a cabinet dryer at 42 °C to 12% moisture contents. Walnuts were then removed from the dryer and stored at room temperature until 4-6% moisture contents and the kernels were prepared. Two types of 90  $\mu$ m thick packaging films: a) (purchased from Freeman cellophane Company) and b) five metallized layers (polypropylene + polyethylene+ polyamide +polyethylene with aluminum layer) Fireplace) were used. For packaging under modified atmospheric conditions, two gas mixtures (2-3%  $O_2$ , 5-5%  $CO_2$ , and 92%  $N_2$ ; 5-6%  $O_2$ , 15%  $CO_2$ , and 80-79%) were applied.

It should be explained that walnut kernel samples were packed with modified atmosphere in the five-layer film, and control samples in the conventional atmosphere with both types of films using the Hankelman Model A200. Additionally, the approximate weight of walnut kernel in each package was 200±5 g and 12 similar samples were prepared from each treatment.

After counting and coding, the samples were stored in a refrigerator at  $10\pm1$  °C and relative humidity 60% for one year. Once every three months, three replications of each sample were removed from the fridge and chemical tests were performed as follows. Sensory evaluation was done performed every three months, and aflatoxin contamination and fatty acid profiles were measured on walnut kernels samples at harvest.

# Extraction of oil

The walnut kernel was first mixed with manual grinding and then mixed with 1 to 4 volumes of normal hexane. Extraction was done for 48 h in the dark and at ambient temperature with vigorous shaking intensities. The solvent was separated in an oven under vacuum at 40  $^{\circ}$  C.

# Analysis and identification of fatty acids

Fatty acid composition of the oil sample was determined using HP-5890 gas chromatograph (Hewlett-Packard, USA) equipped with CP-FIL silica glass capillary columns, 60 m in diameter 0.22 mm, and ionic flame detector. Nitrogen was used as carrier gas at a flow rate of 0.75 mL/min. The oven was maintained at 198 °C and the injector and indicator at 25 °C.

The esterified fatty acids were in line with the methyl esters of fatty acids, created by intensive shaking of oil solutions in hexane (0.3 g/7 mL) with 2 mL methanol potassium hydroxide at 50

(2)

°C for 10 min. Methyl esters of fatty acids were identified using the above model gas chromatograph.

#### **Iodine number**

The iodine absorbed by one gram of fat is called iodine number. There is a relationship between the oxidation effect of fat and the iodine number. Oils with more double bonding are more rapidly oxidized under identical conditions and are more susceptible to oxygen degradation. Iodine number was calculated according to the fatty acid analysis (AOAC, 2005b).

#### Peroxide number

Li method was used to measure peroxide index. Hence, at first about 5 g of the extracted oil samples were weighed into a 250 mL Erlenmeyer flask and added to a 30 cm<sup>3</sup> acetic acid-chloroform ratio of three to one. About 0.5 cm<sup>3</sup> of saturated potassium iodide solution was added and stirred after shaking Erlenmeyer flask to dissolve the oil in the solvent. After 2 min, 30 cm<sup>3</sup> of distilled water was added and titrated in the presence of starch reagent with 0.1 N sodium hyposulphite solution. The peroxide index was obtained from the following equation (Iranian National Standardization Organization [ISIRI], 2018).

Peroxide number 
$$= \frac{(a-b) \times N \times 1000}{M}$$
 (1)

a=mL of sodium thiosulfate consumed as a sample

b=mL of sodium thiosulfate used as control

N=normality of thiosulfate used

M=weight of walnut oil in grams

#### Acidity

In an Erlenmeyer flask, first 20 cm<sup>3</sup> of alcohol and 20 cm<sup>3</sup> of chloroform were poured and neutralized in the presence of phenolphthalein reagent to measure the acidity. Then it was added to another Erlenmeyer flask containing 10 g of walnut oil titrated with 0.1 N NaOH, and the

acidity was obtained from the following equation after stirring to dissolve the oil (Hosseini, 1990).

Acidity = 
$$\frac{N \times 0.0282 \times 100}{M}$$

N=mL of one-tenth normal Sodium hydroxide M=walnut oil weight

#### Moisture

A weighing plate and 15 g sample were added to measure moisture percent. Then, it was incubated with the oven for 70 h at 70 °C. The percentage of moisture was calculated from the following equation after fixing the weight by desiccator (AOAC, 2005a).

$$= \frac{\text{dry samle weight} - \text{fresh sample weight}}{\text{fresh weight}} \times 100$$

### Sensory evaluation

The sensory evaluation test was used to evaluate sensory properties. In doing so, 25 people in the age group 15-45 years were selected. Hedonic test was administered after sufficient explanation of the observers' adherence to the test points. The samples were incubated at ambient temperature for 12 h prior to the test to reach temperature equilibrium. The traits examined and their definitions for walnut color. taste or bitterness. firmness. (including pest-infestation appearance rate), and walnut kernel uniformity ranged from very poor (score 1) to very good (score of 100) (Piggott, Simpson, & Williams, 1998).

#### Aflatoxin

Aflatoxin content of walnut kernel packed of the samples was measured by HPLC device in three replications. In doing so, 75 g of each sample was mixed with a mixer (model Hobart VCM 40, USA) for a full three minutes and after several steps of separation with 5% sodium chloride solution, the mixture of acetonitrile and water (84:16), methanol and water ratio (80:20) and trifluoroacetic acid, aflatoxin content using Chromspher C18 column reverse phase with 5  $\mu$ m particle size and 46×100 mm (Chrompack cat. No. 28264) injection rate 20  $\mu$ L was measured. Solvent flow rate of 0.5 mL/min and aflatoxin values were used as controls according to B1, B2, G1 and G2 standards (Cheraghali *et al.*, 2007).

#### Statistical analysis of data

Statistical analysis was done as split plot factorial based on randomized complete block with two factors of packing type in two levels and storage time in three levels with three replications.

#### **Results and discussion**

Walnut characteristics and its changes during storage were analyzed in SPSS17 and data were compared using Duncan's multiple range test (Table 2).

According to the results of variance analysis, the effect of treatment, storage time and their interaction on peroxide, moisture, iodine, and humidity were at 1% significance level, but the effect of replication on these traits was insignificant. Table (2) shows the effect of treatments on the indices. As the shelf life of the peroxide number increased, the acidity of the titer and the flux increased, but the iodine number reduced significantly (Table 3).

As Table (3) shows, the rate of changes in the peroxide value of walnut kernel samples varied from 1.55 to 4.32 during one year of storage. Of the two genotypes used in the project, genotype 29 had less peroxide content with 92% CO<sub>2</sub> and 5-6%  $O_2$  packaging, and the referees rated the genotype more favorable. The moisture content of the samples varied from 0.48 to 4.65%. The highest acidity was observed in walnut cultivar 25. Walnut kernel peroxide index increased during storage. This is in line with the results of Sattar et al. (1990). However, there were no changes in the peroxide index in the first six months. There was also a slight change in the second six months.

**Table 2.** Comparison of the average effect of using modified atmosphere on the walnut kernel indices of the two genotypes

Row	Applied treatments	Peroxide Index (mEq oxygen per kg)	Moisture (%)	Titrable acidity (%)	Iodine number (g/100 g oil)	Weight loss (%)
1	Genotype 29 with 5-6% $O_2$ and 15% $CO_2$	1.55±1.24 <sup>ab</sup>	4.18±20 <sup>bc</sup>	0.69±0.83 <sup>a</sup>	54.00±2.70 <sup>abc</sup>	$0.00 \pm 0.05^{a}$
2	Genotype 29 with 2-2% $O_2$ and 6-5% $CO_2$	$2.44{\pm}1.49^{ab}$	3.29±1.97 <sup>bc</sup>	0.86±0.92 <sup>a</sup>	56.9±2.75 <sup>bc</sup>	$0.00{\pm}0.05^{a}$
3	Genotype 25 with 5-6% $O_2$ and 15% $CO_2$	$2.82 \pm 1.68^{ab}$	4.60±2.15 <sup>c</sup>	0.11±0.33 <sup>a</sup>	$119.70 \pm 3.30^{d}$	$0.00{\pm}0.05^{a}$
4	Genotype 25 with 2-3% $O_2$ and 6-5% $CO_2$	2.56±1.60 <sup>ab</sup>	4.10±2.00 <sup>bc</sup>	0.11±0.33 <sup>a</sup>	$116.90 \pm 3.20^{d}$	$0.01 \pm 0.09^{a}$
5	Control 29 in the five- layer film	$4.32 \pm 2.00^{b}$	4.10±2.00 <sup>bc</sup>	4.32±0.35 <sup>a</sup>	99.60±3.16 <sup>c</sup>	$0.69 \pm 0.38^{b}$
6	Control 29 in ordinary film	$4.02 \pm 2.00^{b}$	4.60±2.01 <sup>bc</sup>	0.12±0.34 <sup>a</sup>	35.70±2.44 <sup>a</sup>	1.69±1.30 <sup>bc</sup>
7	Control 25 in the five- layer film	4.20±2.05 <sup>b</sup>	3.00±1.75 <sup>ab</sup>	$0.22 \pm 0.47^{b}$	73.80±2.90 <sup>c</sup>	1.47±1.21 <sup>bc</sup>
8	Control 25 in ordinary film	4.26±2.06 <sup>b</sup>	$2.50 \pm 1.57^{a}$	$0.28 \pm 0.35^{b}$	47.90±2.63 <sup>ab</sup>	$2.82{\pm}1.68^{c}$

The non-similar letters in each column show the difference in the significance level for that trait.

Row	Storage time	Peroxide Index (mEq oxygen per kg)	Moisture (%)	Titrable acidity (%)	Iodine number (g/100 g oil)	Weight loss (%)
1	Three months storage	1.47±1.21 <sup>a</sup>	4.00±2.00 <sup>b</sup>	0.08±.28 <sup>a</sup>	82.28±3 <sup>b</sup>	0.20±0.45 <sup>a</sup>
2	Six months storage	$1.65 \pm 1.28^{a}$	$4.10\pm2.01^{b}$	$0.12 \pm 0.35^{b}$	84.30±3.01°	$0.25{\pm}0.5^{a}$
3	Nine months storage	$4.79 \pm 2.19^{b}$	$4.10\pm2.01^{b}$	$0.11 \pm 0.33^{b}$	$68.00\pm0.33^{a}$	$1.50 \pm 1.22^{b}$
4	12 months storage	$5.10\pm2.26^{b}$	$3.04 \pm 1.74^{a}$	$0.25 \pm 215^{\circ}$	$67.70\pm0.50^{a}$	$1.40 \pm 1.18^{b}$

Table 3. Comparison of the average effect of storage time on the indices of two genotypes of walnut

The non-similar letters in each column show the difference in the significance level for that trait.

As shown in Fig. (1), walnut kernel of five-layer genotype 29 in film PA/PE/PA/PE and aluminum foil was superior over control sample in five-layer film. Genotype 25 had a higher peroxide value than genotype 29. Peroxide values were lower in the gas filled samples than in the control samples. This is in line with the results of (Mexis, Riganakos, & Kontominas, 2011). Control samples kept in PA/PE/PA/PE film and aluminum foil had the same number as those preserved in cellophane film.



Fig. 1. Peroxide value of two walnut kernel genotypes with treatments applied during one year of storage

(P1=genotype 29 in five-layer film with 2-3%  $O_2$ , 6-5% CO<sub>2</sub> gas and 92% N<sub>2</sub> gas, P2=genotype 29 in fivelayer film with 5-6%  $O_2$ , 15% CO<sub>2</sub> gas and 79-80% N<sub>2</sub> gas, P3=genotype 25 in five-layer film with 2-3% O<sub>2</sub>, 5-6% CO<sub>2</sub> gas and 92% N<sub>2</sub> gas, P4=genotype 25 in five-layer film with 5-6% O<sub>2</sub>, 15% CO<sub>2</sub> gas and 79-80% N<sub>2</sub> gas, P5=control, genotype 29 with five-layer film, P6=control, genotype 29 with cellophane film, P7=control, genotype 25 with five-layer film, P8=genotype 25 with cellophane film, P8=genotype 25 with cellophane film,

Fig. (2) shows the iodine number changes. The lowest number of iodine

number was observed in the cellophane (conventional) film, and the highest in the walnut kernel genotype 25. In other words, this genotype has more unsaturated fatty acids. This is in line with the results of (Raee *et al.*, 2007) on cover under modified atmospheric conditions on pistachio kernels. The iodine number decreased during the shelf life, which is in line with the results of (Escobar, Estevez, & Guiñez, 2000).



Fig. 2. Iodine number in walnut kernel genotype with treatments applied during one year of storage

(P1=genotype 29 in five-layer film with 2-3% O<sub>2</sub>, 6-5% CO<sub>2</sub> gas and 92% N<sub>2</sub> gas, P2=genotype 29 in fivelayer film with 5-6% O<sub>2</sub>, 15% CO<sub>2</sub> gas and 79-80% N<sub>2</sub> gas, P3=genotype 25 in five-layer film with 2-3% O<sub>2</sub>, 5-6% CO<sub>2</sub> gas and 92% N<sub>2</sub> gas, P4=genotype 25 in five-layer film with 5-6% O<sub>2</sub>, 15% CO<sub>2</sub> gas and 79-80% N<sub>2</sub> gas, P5=control, genotype 29 with five-layer film, P6=control, genotype 29 with cellophane film, P7=control, genotype 25 with five-layer film, P8=control, genotype 25 with cellophane film, P8=genotype 25 with cellophane film,

Edible fats, both animal and vegetable, have certain and minor values of free fatty acid, but may exceed the permissible limit due to spoilage and hydrolysis factors. Hence, measuring the acidity of oil is a way to show its spoilage. As Fig. (3) shows, the lowest fat content was related to genotype 25 and the highest to genotype-29 spoilage in conventional film. As the storage life increased, the rate of spoilage increased as well.



**Fig. 3.** Acidity percent of two walnut kernel genotypes with treatments applied during one year of storage

(P1=genotype 29 in five-layer film with 2-3% O<sub>2</sub>, 6-5% CO<sub>2</sub> gas and 92% N<sub>2</sub> gas, P2=genotype 29 in fivelayer film with 5-6% O<sub>2</sub>, 15% CO<sub>2</sub> gas and 79-80% N<sub>2</sub> gas, P3=genotype 25 in five-layer film with 2-3% O<sub>2</sub>, 5-6% CO<sub>2</sub> gas and 92% N<sub>2</sub> gas, P4=genotype 25 in five-layer film with 5-6% O<sub>2</sub>, 15% CO<sub>2</sub> gas and 79-80% N<sub>2</sub> gas, P5=control, genotype 29 with five-layer film, P6=control, genotype 29 with cellophane film, P7=control, genotype 25 with five-layer film, P8=control, genotype 25 with cellophane film, P8=genotype 25 with cellophane film,

During drying the fruits using hot and dry air, their moisture reduces and reaches about 4-6%. In this process, temperature and humidity must be controlled to prevent thermal damage to the fruit. The moisture percentage of walnut kernels should not exceed 6 and less than 2. If it is more than 6, conditions are provided for the growth of fungi and bacteria, and if it is lower than 2, oxidation of fats in walnuts will take place.

Based on the results, the composition of the inlet gas, the type of packaging and the storage time had a significant effect on moisture content, but the effect of repetition was not significant (Table 2). The moisture content of the samples over time can be due to the low range of relative humidity changes in the storage location and the effectiveness of the impermeable plastic bags in controlling the moisture content during the storage period. On the other hand, walnut kernels contain a high amount of fat, proteins and peptides can react with lipid and affect membrane resistance and ultimately prevent water from leaving the product (Cline & Press, 1990). Fig. (4) shows the moisture changes.



**Fig. 4.** Changes in moisture percentage of two walnut kernel genotypes with treatments applied during one year of storage

(P1=genotype 29 in five-layer film with 2-3%  $O_2$ , 6-5%  $CO_2$  gas and 92%  $N_2$  gas, P2=genotype 29 in fivelayer film with 5-6%  $O_2$ , 15%  $CO_2$  gas and 79-80% N2 gas, P3=genotype 25 in five-layer film with 2-3%  $O_2$ , 5-6%  $CO_2$  gas and 92%  $N_2$  gas, P4=genotype 25 in five-layer film with 5-6%  $O_2$ , 15%  $CO_2$  gas and 79-80% N2 gas, P5=control, genotype 29 with five-layer film, P6=control, genotype 29 with cellophane film, P7=control, genotype 25 with five-layer film, P8=control, genotype 25 with cellophane film, P8=genotype 25 with cellophane film,

According to the results, walnut kernel weight loss was insignificant (at zero level) until six months after storage. Within six months, it reached 1.4 and 1.6% in 12 months. Among the treatments applied, the highest weight loss was in the control sample kept in cellophane film (Fig. 5). Five-layer films with packaging under modified atmospheric conditions could reach zero weight loss. Weight loss was observed in the control samples kept in the five-layer film but it was lower than in the cellophane films.



**Fig. 5.** Weight loss of two walnut kernel genotypes and the effect of treatments applied on it

(P1=genotype 29 in five-layer film with 2-3%  $O_2$ , 6-5% CO<sub>2</sub> gas and 92% N<sub>2</sub> gas, P2=genotype 29 in fivelayer film with 5-6%  $O_2$ , 15% CO<sub>2</sub> gas and 79-80% N2 gas, P3=genotype 25 in five-layer film with 2-3%  $O_2$ , 5-6% CO<sub>2</sub> gas and 92% N<sub>2</sub> gas, P4=genotype 25 in five-layer film with 5-6%  $O_2$ , 15% CO<sub>2</sub> gas and 79-80% N<sub>2</sub> gas, P5=control, genotype 29 with five-layer film, P6=control, genotype 29 with cellophane film, P7=control, genotype 25 with five-layer film, P8=control, genotype 25 with cellophane film, P8=genotype 25 with cellophane film,

According to Table (4), it was clarified that linoleic acid is the dominant fatty acid in walnut oil. Oleic acid, linolenic acid, palmitic acid, and stearic acid were in the next ranks. Amaral, Casal, Pereira, Seabra, & Oliveira (2003) in Portugal found six genotypes of Mayette, Marbot, Franqutte, Lara, Mellanaise, Parisenne, dominant fatty acid, linoleic acid. Genotype 25 had significantly higher linoleic acid content compared to sample 29. The unsaturated fatty acids in genotype 25 were lower. The average unsaturated fatty acids in the two mentioned genotypes were 90.85% and saturated fatty acids less than 10%. The average of unsaturated fatty acids with a double bond was 29.37% and the average of unsaturated fatty acids with multiple double bonds 30.73%. This was in line with the results of Ozkan & Koyuncu (2005).

Table 4. The results of fatty acids composing two fatty acid genotypes

		1 0		· · / ·		
Walnuts	Linoleic acid (%)	Linolenic acid (%)	Oleic acid (%)	Palmitic Acid (%)	Stearic acid (%)	Extraction of total oil (%)
	C2: 18 ω6	C3: 18 ω3	C1: 18 ω9	C0: 16	C0: 18	
Genotype 29	47.55	11.82	30.91	5.72	2.48	65.40
Genotype 25	50.24	13.30	27.82	6.27	2.36	71.00

The sensory test results of walnut kernels are shown in Tables (5) and (6). According to the results of analysis of the effect variance. of repetition, treatment and holding time on color, taste, at 1% level, and the interaction effect of treatment and storage time at 5% level were significant. The effect of repetition on tissue firmness, appearance and uniformity of kernel was significant at 5% level and the interaction between treatment and storage time was insignificant. The best color score for the

control sample was obtained in a fivelayer film and walnut kernel with modified atmosphere (5-6% O<sub>2</sub>, 15%  $CO_2$  and 79-80%  $N_2$ ). The quality of treatments was positive these in measuring the chemical composition. In terms of taste, firmness, which control genotype 25 in conventional film, gained the lowest score. In terms of kernel uniformity, the genotype had 25 points lower because of the difficulty in getting the kernel out of the bony skin.

<b>Table 5.</b> Comparison of the average effect of modified atmosphere applied on kernel sensory properties of two           walnut genotypes							
Row	Applied treatments	Color	Taste	Tissue firmness	Appearance	Kernel uniformity	
1	Genotype 29 with 5-6% $O_2$ and 15% $CO_2$	78.83±2.98 <sup>bc</sup>	79.44±2.00 <sup>bc</sup>	81.10±3.10 <sup>b</sup>	78.21±2.97 <sup>bc</sup>	79.6±2.97 <sup>b</sup>	
2	Genotype 29 with 2-2% $O_2$ and 6-5% $CO_2$	$78.34{\pm}2.97^{abc}$	84.44±3.10 <sup>bc</sup>	82.10±3.15 <sup>b</sup>	$79.70 \pm 2.98^{b}$	$78.45 \pm 2.97^{b}$	
3	Genotype 25 with 5-6% $O_2$ and 15% $CO_2$	79.60±2.98 <sup>bc</sup>	83.17±3.20 <sup>c</sup>	$82.65 \pm 3.10^{b}$	81.90±3.10 <sup>b</sup>	81.75±3.00 <sup>c</sup>	
4	Genotype 25 with 2-3% $O_2$ and 6-5% $CO_2$	81.41±3.00 <sup>c</sup>	81.33±3.00 <sup>c</sup>	$79.60 \pm 2.98^{b}$	79.00±2.97 <sup>bc</sup>	$80.60 \pm 2.99^{b}$	
5	Witness 29 in the five-layer film	82.14±3.10 <sup>c</sup>	80.42±3.10 <sup>bc</sup>	81.10±1.30 <sup>b</sup>	82.35±3.20 <sup>c</sup>	79.54±2.98 <sup>b</sup>	
6	Witness 29 in ordinary film	$81.47 \pm 3.20^{\circ}$	$80.00 \pm 2.99^{bc}$	$81.40 \pm 3.10^{b}$	$81.16 \pm 3.10^{\circ}$	$77.50 \pm 2.96^{ab}$	
7	Witness 25 in the five-layer film	74.98±2.98 <sup>ab</sup>	$74.79{\pm}2.90^{ab}$	$76.98{\pm}2.96^{a}$	74.10±2.39 <sup>a</sup>	$75.85{\pm}2.95^{a}$	
8	Witness 25 in ordinary film	$72.12\pm2.90^{a}$	$71.90{\pm}2.90^{a}$	$73.37 \pm 2.92^{a}$	$70.80 \pm 2.90^{a}$	$72.25 \pm 2.90^{a}$	

The non-similar letters in each column show the difference in the significance level for that trait.

Table 6. Comparison of the average effect of modified atmosphere use on kernel sensory properties of two walnut genotypes

Row	Storage time	Color	Taste	Tissue firmness	Appearance	Kernel uniformity
1	Three months after storage	84.15 <sup>c</sup>	85.35 <sup>b</sup>	85.52 <sup>b</sup>	84.89 <sup>b</sup>	84.83 <sup>a</sup>
2	Six months after storage	83.84 <sup>c</sup>	85.33 <sup>b</sup>	74.2 <sup>b</sup>	84.85 <sup>b</sup>	$84.86^{a}$
3	Nine months after storage	$74.86^{b}$	73.15 <sup>a</sup>	85.45 <sup>a</sup>	73.53 <sup>a</sup>	83.9 <sup>a</sup>
4	Twelve months after storage	69.97 <sup>a</sup>	$70.82^{a}$	85.52 <sup>a</sup>	69.51 <sup>a</sup>	84.22 <sup>a</sup>

The non-similar letters in each column show the difference in the significance level for that trait.

As the storage life of walnut kernels increased, color and taste decreased, but its tissue firmness increased. The reason is the decrease in walnut kernel moisture with increase in storage time. Appearance decreased and no changes were made to the kernel uniformity. Walnut kernels in this study had a significant difference with the taste of walnut kernels in the market and gained a higher score. Thus, it is desirable to use a five-layer film and a gas composition of 5% O<sub>2</sub> and 15% CO<sub>2</sub>. Anyway, the storage time reduces the taste (Table 6).

According Table (5), to control genotypes 25 and 29 had the lowest score in conventional film. The highest score is for samples stored in the five-layer film with or without modified atmosphere. The best color for the control sample was in five-layer walnut film with modified atmosphere (5-6% O<sub>2</sub>, 15% CO<sub>2</sub> and 79-80% N<sub>2</sub>). At genotype 25, the samples had better color relative to genotype 29, with the main reason as the later harvest date of genotype 29. These results show that care must be taken in the harvest date. When 85% of the walnut green skin has a cleft, it had to be harvested. Packed samples with 5-6% O<sub>2</sub>, 15% CO<sub>2</sub>, and 79-80% N<sub>2</sub> showed better appearance than other samples. Genotype 25 gained more scores. As the shelf life increased, the appearance of the properties decreased. Regarding the firmness of the tissue and the uniformity of the kernel, no changes were observed in No samples. significant the stored seen differences were between the treatments and the control samples for uniformity (Table 6).

Genotypes 29 were contaminated with aflatoxin and aflatoxin levels were determined at 5 parts per billion. No aflatoxin contamination was seen in genotype 25. This shows that besides controlling cracking of hard walnut skin, improper harvesting date also results in the growth of aflatoxin-producing fungi in walnut kernel samples.

#### Conclusions

Walnut is considered as one of the most important dried fruits given its nutritional value due to its essential and unsaturated fatty acids. Maintaining this nutritional value during maintenance was one of the goals of the project. In doing so, we examined the important physicochemical properties of two walnut kernel genotypes through its MAP, and by using five-layer PA/PE/PA/PE composite packaging materials and aluminum foil. The results showed that unsaturated fatty acids in walnut oil was higher than saturated fatty acids and was the dominant fatty acid in linoleic acid. Overall, genotype 25 packaged in five-layer film containing 5-6%  $O_2$ , 15%  $CO_2$  and 79-80%  $N_2$  is recommended due to lack of aflatoxin and desirable chemical and sensory properties.

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# تأثیر بستهبندی با اتمسفر اصلاحشده و نوع مادهٔ بستهبندی بر ماندگاری مغز گردو

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#### چکیدہ

گردو با داشتن اسیدهای چرب ضروری و غیراشباع گروه امگا-۲۳، بعنوان یکی از مهمترین خشکبار ازنظر ارزش غذایی مطرح است. ژنوتیپهای ۲۵ و ۲۹ (ژنوتیپهای برتر گردو) با هدف بررسی تأثیر بستهبندی به روش اتمسفر اصلاحشده بر ترکیبات سودمند موجود در روغن گردوی آنها، انتخاب شدند. مغز گردوها تحت شرایط اتمسفر اصلاحشده داخل پلاستیک پنچلایه متالیزه با ضخامت ۹۰ میکرون بستهبندی گردیدند. بعد از استخراج روغن به روش سرد و خالصسازی آن، ترکیب کمی و کیفی اسیدهای چرب تشکیل دهنده اندازه گیری شد. میزان اسیدهای چرب، مقدار آفلاتوکسین، عدد پراکسید، عدد یدی، اسیدیته و ارزیابی حسی نیز در روز صفر و طی یکسال نگهداری تعیین گردید. نتایج نشان داد میزان اسیدهای چرب غیراشباع نسبت به اسیدهای چرب اشباع بیشتر بوده و اسید لینولئیک، اسید چرب غالب است. مقدار اسیدهای چرب شران اسیدهای چرب غیراشباع نسبت به اسیدهای چرب اشباع بیشتر بوده و اسید لینولئیک، اسید چرب فالب است. مقدار اسیدهای چرب شیان تعییرات رطوبت و اندیس پراکسید نمونههای مغز گردو طی یکسال نگهداری، بهترتیب صفر و مسمت در بیلیون برآورد گردید. میزان تغییرات رطوبت و اندیس پراکسید نمونههای مغز گردو طی یکسال نگهداری، بهترتیب حدود شد. با افزایش زمان نگهداری، عدد پراکسید، اسیدیتهٔ و از ایش، و عدد یدی کامش م خرب مشاور نیزیبی حدود رفر و م ۲۰۹۸ شد. با افزایش زمان نگهداری، عدد پراکسید، اسیدیتهٔ قابل تیتر و افز ایش، و عدد یدی کاهش یافت. بهطورکلی، ژنوتیپ ۲۵ م گردوی بستهبندی شده در فیلم پنچلایه با اتمسفر اصلاحشده حاوی ۶–۵ درصد اکسیژن، ۱۵ درصد دی اکسیدکرین و ۲۰–۷۹ درصد ازت،

**واژههای کلیدی:** اتمسفر اصلاحشده (MAP)، بستهبندی، پلی پروپیلن متالیزه شده، خواص حسی و شیمیایی، مغز گردو