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Biochemical Changes in the Kernel of Four Walnut Cultivars under Two Different Storage Temperatures

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A R T I C L E I N F O

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A B S T R A C T

Walnut is one of the most important dry fruits with a high nutritional value; it serves as a natural source of antioxidants. However, the quality of this product is decreased during storage. This study explored the effect of different types of cultivars (common, nok klaghi, stone, paper), temperatures (4 and 25°C), and storage times (6 months) on carbohydrate, protein, peroxide value (PV), total antioxidant capacity (TAC) and phenol (TP) of the walnut kernel. There was a significant difference between the cultivars in the studied traits. The common cultivar showed higher carbohydrate, proteins, antioxidant capacity (TAC) and phenol (TP) content. In terms of storage, the peroxide value was increased in all cultivars, but its intensity for the kernel stored at 25°C was more. At the end of the experiment, the Nok kalaghi cultivar was found to have the highest peroxide value, as compared to other cultivars. The content of phenol, protein and total antioxidant in different genotypes of the kernel was decreased during storage. In all genotypes, samples stored at 4°C showed a better quality than those stored at 25°C. Generally, the results showed that the low temperature could play an important role in maintaining the quality of the walnut kernel, although its effect may vary depending on the genotype

Introduction

Persian walnut (*Juglans regia* L.) is a temperate nut crop; it is one of the most important nuts in the world, with wide distribution and cultivation around the world. China, with 440,321 hectares of the cultivation area and the production of 1602,373 tons, is the largest producer of different walnuts in the world; this country is followed by the United States as the second largest walnut producer (FAOSTAT 2014). Iran, which has the third position, is one of the largest active countries in the global walnut production. According to the latest FAO statistics (2014), the cultivation of walnuts in Iran has been 69,833 hectares; from this area, 445,829 tons of walnuts have been harvested. Some walnut genotypes including paper, Chandlar, stone, needles, damavand, Ziaabadi, cluster and Sabzevari are among the most important ones in Iran (Tajeddin, 2004). They are cultivated in Kerman, Azarbaijan, Fars, Hamedan, Qazvin, Khorasan and Kordestan provinces (Ministry of Jihad-e-Agriculture, 2002).

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Walnut is one of the most important sources of essential fatty acids, fiber, plant proteins, vitamins, minerals, magnesium, potassium, and arginine amino acid (Davis *et al.*, 2007). Due to the high levels of antioxidant compounds, such as vitamin E and polyphenols (Christopoulos *et al*, 2011), eating walnut can play an important role in protecting against oxidative damages and the increased cholesterol (Spaccarotella *et al.*, 2008). According to Miraliakbari and Shahidi (2008), the high antioxidant activity in the walnut kernel can be due to the presence of tocopherol compounds. However, the walnut kernel, as a dry product, is corrupted very quickly because of chemical and microbial agents. Temperature, light, moisture and exposure to oxygen have been found to be the main contributing factors to oxidation (Stark *et al.*, 2000).

Jensen *et al.*, (2003) reported that the increased temperature and the enhanced oxygen can raise the sensitization of products in terms of fat oxidation (rancidity and volatile matter production) and sensory properties. Tsantili and Christopoulos (2011) investigated the effect of temperature and packaging atmosphere on total antioxidants and walnut color during storage. They reported that low temperatures and packaging under N_2 or CO_2 prevented an increase in antioxidant and browning. Research has shown that packaging in the N_2 atmosphere and at low temperatures (1°C instead of 20°C) could prevent the decrease of phenolic and antioxidant compounds in eight pistachio cultivars (Tsantili *et al.*, (2011). Currently, there is not enough information regarding the changes in such nutritional compounds as carbohydrate, proteins, antioxidant properties and phenol of the Iranian walnut kernel during the storage period. Accordingly, the aim of the present study was to investigate the changes in some quality characteristics of four walnut genotypes from Kerman during storage at different temperatures.

Materials and Methods

Four (common, nok klaghi, stone, paper) walnut genotypes with high-marketability were harvested from a commercial garden around Kerman and immediately their green peels were removed and dried for 4 days in shade through natural air circulation. Then, the walnuts were transferred to the laboratory and their wooden shells were removed using a walnut breaker and the walnut kernels were packaged immediately. About 25 grams of walnut kernel were packed in polyethylene. Then stored at 4°C and ambient temperature for 6 months and their various traits were evaluated monthly.

The Bradford method (1976) was used to determine the protein concentration. Carbohydrates were measured using the phenol – sulphuric acid method (Dubois *et al.*, 1956).

The peroxide value measurement method is based on the Standard Test of Food Association of Official Analytical Chemists (AOAC) (Horwitz *et al*., 1975). 30mL of solvent (mixture of acetic acid and chloroform) was added to 5g of sample. Then 5 ml of potassium iodine is added and mixed for 1minute. Then, 30ml of distilled water was added and then titration with soluble thiosulfate 0.02%.

Antioxidant content (AC) was determined using DPPH assay (Brand-Williams *et al.*, 1995). Ten gram of kernel was homogenized with 100mL methanol for 1min and centrifuged at 10,000rpm for 15min at 4°C. 100µL of clear supernatant was added to 5mL DPPH solution, and the absorbance of DPPH reagent was determined at 515nm after 30 min of incubation. The inhibition percentage of the absorbance was calculated as follows:

$$\text{Inhibition \%} = \frac{Abs\ t0 - Abs\ t30}{Abs\ t0 \times 100}.$$

Abs t_0 min was the absorbance of DPPH at time 0.
Abs t_{30} min was the absorbance of DPPH after 30min of incubation.

The TP concentration was measured by a modified Follin - Ciocalteu colorimetric method (Tsantili *et al.*, 2010a). 0.2 mL Folin-Ciocalteu reagent was added to 0.2 mL sample, and the tube was stirred after 20 min at room temperature, Two milliliter of Na_2CO_3 (7%, w/v) was added to the mixture and the absorbance was measured at 750 nm. The results were expressed as milligram of gallic acid equivalents (GAE) per gram Fresh weight (mg g^{-1} FW).

Results

Peroxide index

Peroxide index values of different genotypes and temperatures are presented in Table 1. The initial peroxide index values for the walnut kernel were very low at the first storage, equal to 0.023, 0.16, 0.016 and 0.021 meq /g, for the common, nok klaghi, stone and paper cultivars, respectively. As the storage time was increased, the peroxide values were increased gradually in all cultivars until the fifth month, while in the last experiment, this increasing trend was accelerated. However, samples kept at the temperature of 4°C showed the lower peroxide values, as compared to the control sample maintained under 25 °C.

Table1. The mean of peroxide values (meq /g) for samples stored for a period of six months

Cultivar	Temperature (°C)	Time	Peroxide values					
		0	1	2	3	4	5	6
common	4	0.02 $^{q..t}$	0.05 $^{p..t}$	0.06 $^{n..t}$	0.09 $^{m..t}$	0.11 $^{l..t}$	0.15 $^{j..s}$	0.26 $^{g..k}$
	25	0.02 $^{q..t}$	0.16 $^{j..q}$	0.16 $^{j..q}$	0.24 $^{g..l}$	0.26 $^{g..k}$	0.33 $^{e..h}$	0.65 ^{b}
Nok klaghi	4	0.16 $^{j..s}$	0.16 $^{j..s}$	0.17 $^{i..p}$	0.19 $^{i..o}$	0.24 $^{g..l}$	0.29 $^{g..j}$	0.43 ^{def}
	25	0.16 $^{j..s}$	0.18 $^{i..p}$	0.20 $^{h..n}$	0.23 $^{g..l}$	0.31 $^{f..i}$	0.65 ^{b}	0.85 ^{a}
stone	4	0.016 ^{t}	0.019 st	0.02 $^{q..t}$	0.05 $^{o..t}$	0.08 $^{m..t}$	0.16 $^{j..r}$	0.18 $^{i…p}$
	25	0.016^{t}	0.04 $^{p..t}$	0.07 $^{n..t}$	0.14 $^{k..s}$	0.27 $^{g..k}$	0.44 ^{de}	0.47 ^{cd}
paper	4	0.02^{rst}	0.02^{rst}	0.02 ^{rst}	0.14 $^{k..s}$	0.15 $^{j..s}$	0.24 $^{g..k}$	0.29 $^{g..j}$
	25	0.02^{rst}	0.04 $^{p..t}$	0.05 $^{p..t}$	0.22 $^{h..m}$	0.37 $^{d..g}$	0.56 ^{bc}	0.63 ^{b}

Means with the same letter are not significantly different

Protein content

Figs. 1 and 2 show that the common cultivar had the highest (18.39 mg / 100 g fresh weight) and the paper genotype contained the lowest protein content (14.51 mg / 100 g fresh weight). Over time, the amount of protein in each genotype was gradually decreased. However, the decrease at the temperature at 25°C was significantly more severe. At the end of the experiment, the protein reduction at 25°C was approximately 25%. On the other hand, samples at a temperature of 4 °C showed a 12% reduction in their protein content.

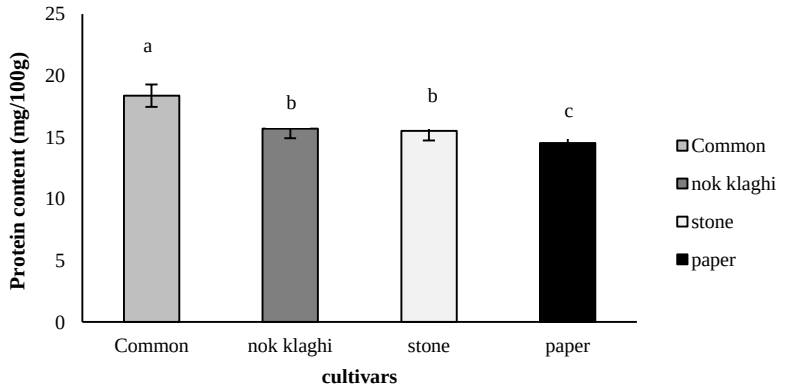

Fig 1. The protein content of four genotypes of walnuts kernel. Columns with the same letter are not significantly different

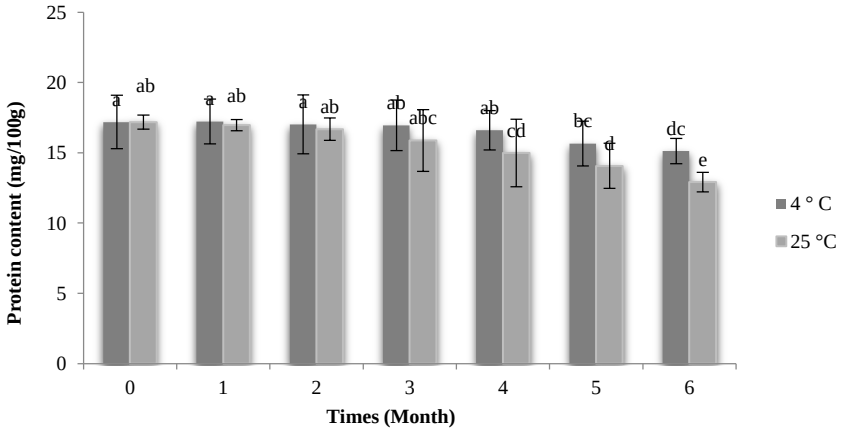

Fig 2. The effect of temperature on the amount of protein in the walnut kernel during storage. Columns with the same letter are not significantly different.

Carbohydrate content

As shown in Figs. 2 and 3, in terms of the protein factor, the common genotype showed the highest carbohydrate content among other varieties. Carbohydrate content of all varieties was also decreased during storage. Temperature showed a significant effect on this factor at the end of storage.

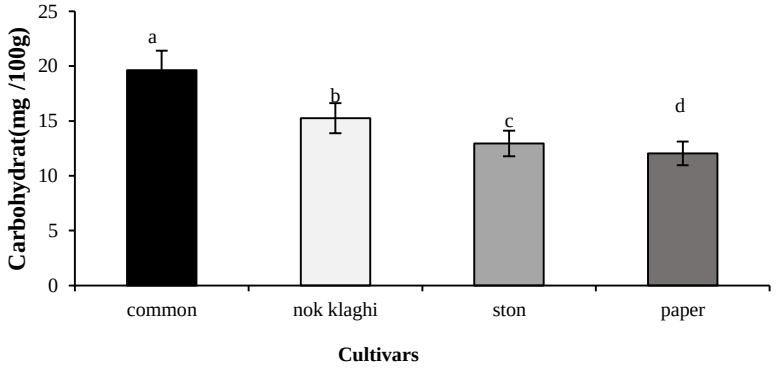

Fig 2. Comparison of carbohydrate content of different genotypes. Means with the same letter are not significantly different

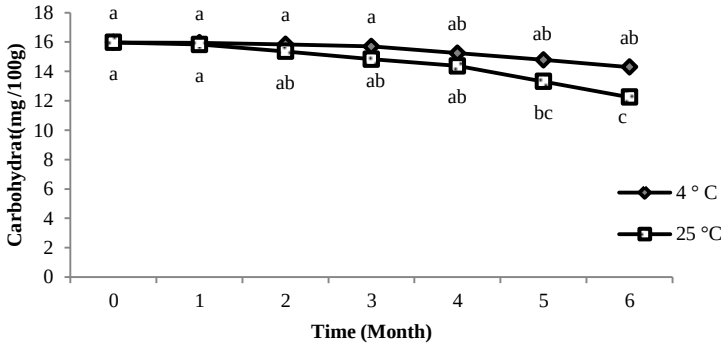

Fig 3. Effect of temperature on carbohydrate content of walnut kernel for 6 months. Means with the same letter are not significantly different.

Antioxidant capacity (TAC)

The antioxidant levels of the samples varied according to the genotypes. The results showed that the antioxidant capacities were decreased during storage, but their intensity for the samples stored at 25°C was greater. Accordingly, at the end of the 6-month period, in all genotypes, the samples stored at 25°C showed approximately 30% reduction in the antioxidant capacity, but the antioxidant capacity of the samples stored at 4 °C was decreased only by 4% (Fig. 4)

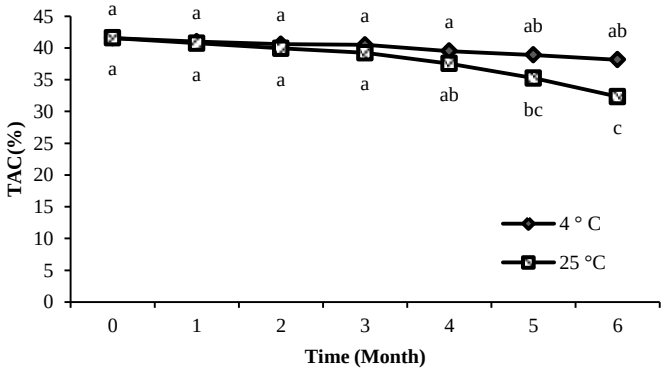

Fig 4. Effect of temperature on the antioxidants activity of walnut kernel in six months of storage.
Means with the same letter are not significantly different

Phenolic compounds

According to Figs 5 and 6, the total phenol content was decreased gradually over time, but the temperature treatment at 4°C significantly maintained phenolic compounds (P> 0.05), so that the maximum phenol content was found in the samples stored at 4°C and the lowest one was found in the samples under the temperature of 25 °C.

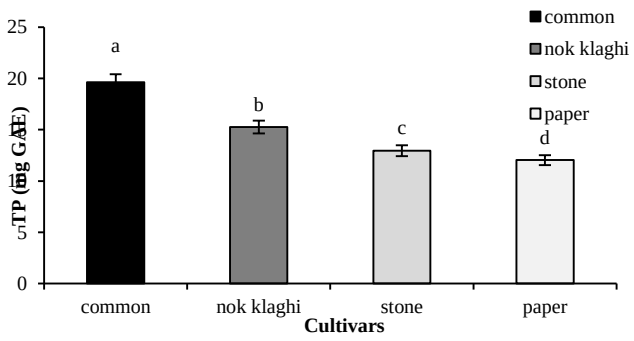

Fig 5. Comparison of total phenol content of different genotypes. Means with the same letter are not significantly different

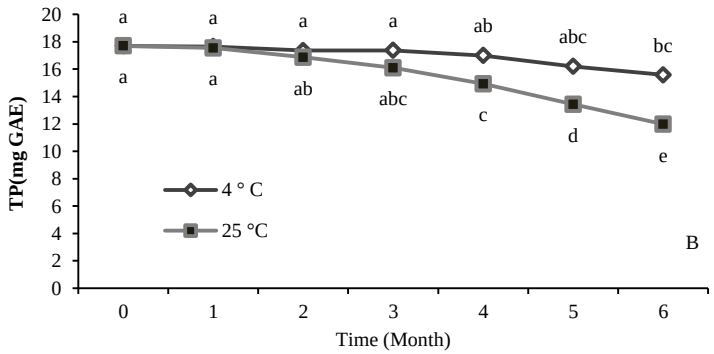

Fig 6. Effect of temperature on total phenol content of walnut kernel in six months of storage. Means with the same letter are not significantly different

Discussion

The peroxide value and therefore, the primary oxidation product can be a good descriptor of lipid oxidation only in the initial process. Walnut kernels have the fat content of about 65%, of which approximately 70% belongs to unsaturated fatty acids. Lipid oxidation is the most important quality limit in walnuts due to the high content of unsaturated lipids. Naturally antioxidant compounds present in the kernel keep the walnut kernels against oxidation. However, the removal of the shell may damage the kernels, exposing the kernels to oxygen, in addition to storage conditions such as temperature, oxygen, light and relative humidity, all affecting the degree of lipid oxidation (Jensen *et al*, 2001). The walnut kernels used in this experiment had the high initial quality. This can be seen from the low content of peroxides. Osterberg *et al.* (2001) reported that fresh walnut kernels had peroxide values ranging from 0.15 to 0.29 meq O_2/kg oil. Sheen (1999) reported 0.04–0.35 meq O_2/kg oil, depending on the cultivar analyzed. Data from this experiment clearly showed that decreasing the temperature reduced the oxidation changes in the walnut kernel. The results obtained in this study were consistent with those got in other studies. Raisi *et al.* (2015), for instance, showed that temperature (4°C and 25°C) could influence the intensity of almond kernel oxidation during storage period and samples kept at 4°C showed higher stability in the peroxide value, as compared with other treatments. Osterberg *et al.* (2001) also observed that the peroxide in the fresh walnut kernel and the stored walnut were 0.058-29.0 and 0.06-1.66, respectively. Jensen *et al.* (2003) stated that the increased temperature and the enhanced oxygenation led to an increase in the product sensitivity to fatty oxidation (rancidity and

volatile matter production) and sensory properties. Lo´pez *et al*. (1995) did not, however, observe the effect of storage temperature (range of 3-10°C) on the unshelled walnut quality. Senesi *et al*. (1991) found that almonds could be stored for up to 9 months at 4 °C or 20 °C without any significant quality loss. Beyond this storage period, quality could be maintained only by using a packaging material with low oxygen permeability and by storage at 4°C.

The highest amount of protein was observed in the common, Nokkalaghi, Stone and Paper varieties, respectively. The data obtained in this study was in the range of data reported by other researchers. In the study of several cultivars of walnut, Nicola (2003) reported the protein content of the walnut in the range 13 to 14.63 mg, while Gugong *et al.* (2014) reported a higher protein content (17.66 mg). According to the results of this study, the amount of protein was reduced during storage, but this decrease in the samples at 25° C was more intense.

Carbohydrate content was varied from 12.04 to 19.63 mg /100 g in the cultivars under study, and the common and paper cultivars contained the highest and lowest amounts of carbohydrates, respectively. Data obtained in this study were in the range of other data related to Iranian walnut genotypes. Hamidi *et al*, (2014), in the study of several Iranian local walnut genotypes, showed that carbohydrates were between 23 and 11.23%. Also, the results of this study were consistent with the data related to five genotypes reported by Nicola (2003). He reported that the amount of carbohydrate in five genotypes in Turkey was between 23.25 and 33.11 mg /100 g.

Phenol contents showed a decreasing trend through the 6-month storage. This process was mostly apparent in the latter two-time periods. In addition, the temperature of 4°C led to the effective preservation of phenol. This was in the range of data reported by other researchers (Christopoulos, and Tsantili, 2011). Bakkalbaşi *et al.* (2013) showed that the total phenol content was decreased significantly during the walnut kernel storage. Also, studies on peanuts have shown that in the samples kept at a temperature of 20°C, quality could be preserved more than those stored at 35 °C (Talcott *et al*, 2005). The prevention of the reduction of phenolic compounds could be attributed to the limitation of oxidation reactions by low temperatures and less access to oxygen, since phenolic compounds could be susceptible to chemical or enzymatic oxidation (Manzocco *et al.*, 2000). However, there is not enoughinformation regarding the phenol of walnut kernel and the changes in these compounds.

The antioxidant level found in this study was less than that reported by other researchers. Akbari *et al*. (2014), in a study of six different genotypes of walnut, reported the antioxidant capacity between 53.83% and 90.38%,. Our results showed that antioxidant capacity was decreased during the storage; however, samples stored at the temperature of 25 °C showed the higher reduction in terms of antioxidant potential and nutritional value. Samaranayaka *et al*. (2008) stated that the phenol content present in each walnut could have a deep impact on their antioxidant activity. To conclude, it is recommended that walnuts be stored at 4 °C for keeping the oxidation of lipid at the lowest level.

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