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Influence of Storage on the Aflatoxin and Fatty Acid Composition in Turkish Hazelnut (*Coryllus avellana*) Varieties

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ABSTRACT

In this study, fatty acid compositions of Palaz, Sariyagli, Delisava, Karayagli and Yomra hazelnut varieties stored under room conditions during in the years 2005-2008 were investigated. The hazelnut samples of 2009 were harvested fresh and not stored. Then, the effects of storage on aflatoxin formation and fatty acid composition were investigated: the aflatoxin using HPLC and fatty acid with GC. Results showed that identifiable level of aflatoxin was not determined in stored hazelnut samples. It was showed that average the highest oleic acid, stearic acid, palmitic acid and linoelaidic acid contents in investigated hazelnut varieties changed from 79.930 to 86.812%, 4.202 to 9.134%, 5.550 to 6.192% and 1.504-2.890%, respectively. In addition, it was determined that average values of SFA, MUFA and PUFA changed from 10.530 to 15.594%, 80.598 to 89.104% and 1.504 to 2.890%, respectively. According to their storage periods, values in fatty acids also were found similarly to these values. Although many differences in between fatty acids weren't important as statistical according to these varieties and storage periods, these values were important as statistical in oleic asit and linoelaidic acid. In addition, the differents weren't important with respect to SFA but were important with respect to MUFA and PUFA ($P<0.05$). © 2011 Friends Science Publishers

Key Words: Turkish hazelnut; Fatty acid composition; Aflatoxin; Storage

INTRODUCTION

Turkey is the largest hazelnut (*Coryllus avellana* L.) producing and exporting country in the world, with approximately 70% in the global production, followed by Italy, Spain and USA (Anonymous, 2001). Hazelnuts are consumed all over the world as a fruit in a diversity of manufactured food products, such as snacks chocolates, cereals, bakery, dairy, salad, entree, sauce, ice creams and other dessert formulations (Ozdemir & Akinci, 2004; Amaral *et al*., 2006). Hazelnut plays a major role in human nutrition and health, because of its special composition of fat (around 60%) most of which are highly, rich in unsaturated fatty acids, proteins, carbohydrate, dietary fiber, vitamins, minerals, phytosterols, squalene, antioxidants, phenolics, and phytochemicals (Alasalvar *et al.,* 2003; Beyhan *et al*., 2010). Varieties, location, composition of soils, usage of fertilizer and irrigation affect the fatty acid, mineral and vitamin composition of hazelnuts, and consequently influence the stability and quality of the product (Ackurt e*t al*., 1999) Hazelnuts are rich in polyunsaturated fatty acids, and essential for human health (Garcia *et al*., 1994; Pala *et al*., 1996; Alphan *et al*., 1997). Unsaturated fatty acids, antioxidants and minerals are involved in rancidity. Therefore, high levels of unsaturated/saturated rations are important (Ackurt *at al*., 1999). Many researchers demonstrated that mono-and poly-unsaturated fatty acids (MUFA & PUFA) had lower serum cholesterol levels in humans (Bracco *et al.,* 1994; Kritchevsky *et al.,* 1994; Nydahl *et al.,* 1994; Berry *et al.,* 1995). Some other researchers reported that high levels of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) content were protective effects against ischemic cardiovascular diseases (Fraser *et al.,* 1992; Sabate *et al.,* 1993; Abbey *et al.,* 1994). Because nuts abundantly containing fat is not only an energy source, but also the human diet rich in nutritious elements in certain compounds (MUFAs, PUFAs, vitamin E, natural sterols, etc: Anonymous, 1993).

The most important aflatoxins (AFS, B1, B2, G1 & G2) are produced by *Aspergillus flavus* and *A. parasiticus* fungal species. Aflatoxins have high toxicity and cancerogenic effects; specifically, AFS B1 is the most toxic form. The level of aflatoxins of 2-6 mg taken from foods is said to be lethal. Several countries have restricted the amount of aflatoxin in the products like peanut, hazelnut,

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and almond as they are sensitive to aflatoxin. Some studies have been conducted on nut samples grown in Turkey. Demir *et al.* (2002) found no detectable levels of aflatoxins in 30 stored hazelnut samples. Ozcakmak and Dervisoglu (2007) reported that nut samples stored in Black Sea region, characterized by humid and rainy conditions, showed an increase in mold growth, which by producing aflatoxins caused the quality and economic losses.

In this study, we determined the levels of aflatoxin (AFS; B1,B2,G1 & G2), and its impact on fatty acid composition in the stored Palaz, Sariyagli, Delisava, Karayagli and Yomra shelled hazelnut samples between 2005-2008 and compared them with the fresh samples harvested in 2009.

MATERIALS AND METHODS

This study was carried out in the town of Sakarya province Akyazi in Turkey; on nut varieties stored in ordinary warehouses. Most widely grown varieties of nuts Palaz, Sariyagli, Delisava, Yomra, Karayagli and varieties were used. Warehouses used for storage were built of brick, while the ceiling was made of steel frame with eternity. Ventilation was natural through the grid window on the walls. Also, the ventilation was provided by opening the doors in the summer. Sakarya had the annual average temperature of 14.4°C, mean relative humidity of 73.9%, while average rainfall was 1016 mm (Anonymous, 2009). Stores had 600 tons of maximum capacity of products, storage high was 10 m, storage humidity was 65% and storage volume was 450 m^3. Storage products from the area in 2005, 2006, 2007 and 2008 taken from farmers were stored in bulk.

Extraction of hazelnut oil: The 2009 samples were taken as not stored for the purpose of control in the harvest period. Eight mL hexane was added to 4 g crushed nuts and extraction was performed for 16 h at 55ºC. The obtained hazelnut oil was used for fatty acid analysis (Hofstetter *et al.,* 1965).

Analysis of fatty acids by GC-FID: Fatty acids were determined by gas chromatography with flame ionization detection (GC-FID) capillary column (30 m × 0.25 mm × 25 µm of film). The oven temperature was programmed as follows: 180ºC for 2 min, increased to 200ºC at 2ºC/min, held at 200ºC for a further 10 min, and then increased to 250ºC at 2ºC/min and kept there for 10 min. The injector and detector temperatures were 200 and 250ºC, respectively. Helium was used as the carrier gas at a flow rate of 1.5 mL/min (Hofstetter *et al.,* 1965). Fatty acids were identified by comparison with retention times of external standards.

Determination of aflatoxins: The extraction and determination of aflatoxins by HPLC were based on the method described by VICAM (Anonymous, 1999) and AOAC (Hofstetter *et al.,* 1965; Anonymous, 1995; Anonymous, 1999) with some modifications. A sample of 25 g was extracted with 5 g of sodium chloride and 125 mL methanol-water (70/30; v/v) by warring blender for 2 min. The extract was filtered with Whatman filter paper (Whatman No. 4) and 15 mL of filtrate was diluted with 30 mL of deionized water and mixed well. After filtering with Whatman filter paper, 15 mL filtrate was passed through an immune affinity column (Vicam) at a rate about 1-2 drops and washed with 10 mL water twice at the same flow rate. Final elution was accomplished by passing 1 mL of HPLC grade methanol at the same flow rate, which was collected in a clean vial. Eluent was diluted with 1 mL of deionized water and 20 µL was used for HPLC analysis. HPLC system equipped with a gradient pump, and a fluorescence detector (FLD) with post column was used for quantification of aflatoxins. Separation was performed on a C18 column (2.1×100 mm i.d. 3 µm particle size, inert sill OD-2) using a water-methanol-acetonitryl mobile phase with a flow rate of 0.3 mL/min. Fluorescence detector was operated at an excitation wavelength of 365 nm. As aflatoxins are subject to light degradation, the samples were protected from light with amber colour vials (Hofstetter *et al.,* 1965).

Statistical analysis: Statistical analyses were performed using the SAS software. The obtained data were analyzed using One-way ANOVA. Significant differences were determined by Duncan's Multiple Range Tests (Orhan *et al.,* 2004).

RESULTS AND DISCUSSION

In investigated varieties, the highest fatty acid had oleic acid with 83.546% in Delisava. After oleic acid, stearic acid, palmitic acid and linolaidic acid contents were followed as 8.630%, 6.040% and 1.540%, respectively. The values of other fatty acids changed between 0.006 and 0.314%. The lowest fatty acid ratio of Papaz variety was found as 0.006% in pentadecanoic acid. In this variety, total MUFA was found as 83.738% although total SFA and total PUFA (Polyunsaturated Fatty Acid) were found as 15.054% and 1.540%, respectively (Table I).

The highest fatty acid value in Karayagli variety was found as 86.812 in oleic acid. This value was the highest value in all varieties. In addition it was determined that palmitic asit, stearic asit and linolaidic acid contents in this variety changed as 6.142%, 4.202% and 1.868%, respectively. But, the lowest stearic acid content in all varieties was found in this variety. The lowest fatty acid ratio in this variety as 0.004% in pentadecanoic acid. Other fatty acid contents of this variety changed from 0.018 to 0.116%. In this variety, total MUFA was found as 87.104% although total SFA and total PUFA were found as 10.530% and 1.874 %, respectively (Table I).

Table I: Comparison of fatty acid composition according to hazelnut varieties

Fatty Acid Tipes	Delisava	Karayagli	Palaz	Sariyagli	Yomra
Butyric acid (C4:0)	0.314±0.124 a	0.108±0.124 a	0.508±0.124 a	0.220±0.124 a	0.270±0.124 a
Myristic acid (C14:0)	0.026±0.028 b	0.040±0.028 b	0.136±0.028 a	0.040±0.028 b	0.036±0.028 b
Pentadecanoic acid (C15:0)	0.006±0.010 b	0.004±0.010 b	0.050±0.010 a	0.004±0.010 b	0.004±0.010 b
Cis10-Pentadecaenoic acid (C15:1)	0.140±0.169 a	0.150±0.169 a	0.674±0.169 a	0.120±0.169 a	0.160±0.169 a
Palmitic acid (C16:0)	6.040±0.227 a	6.142±0.227 a	5.550±0.227 a	5.634±0.227 a	6.190±0.227 a
Palmitoleic acid (C16:1c)	0.032±0.069 b	0.054±0.069 b	0.272±0.069 a	0.046±0.069 b	0.048±0.069 b
Heptadecanoic acid (C17:0)	0.010±0.040 b	0.018±0.040 b	0.214±0.040 a	0.026±0.040 b	0.026±0.040 b
Stearic acid (C18:0)	8.630±2.008 a	4.202±2.008 a	9.134±2.008 a	7.152±2.008 a	7.722±2.008 a
Oleic acid (C18:1,c)	83.546±2.029 ab	86.812±2.029 a	79.930±2.029 b	84.528±2.029 ab	83.842±2.029 ab
Linoelaidic acid (C18:2t)	1.540±1.196 b	1.868±1.196 b	2.890±1.196 a	1.880±1.196 b	1.504±1.196 b
Cis11-Eicosaenoic acid (C20:1)	0.024±0.147 b	0.116±0.147 b	0.612±0.147 a	0.076±0.147 b	0.102±0.147 b
SFA (Saturated fatty acid)	15.054±2.021 a	10.530±2.021 a	15.594±2.021 a	13.090±2.021 a	14.192±2.021 a
MUFA (Monounsaturated fatty acid)	83.738±1.932 ab	87.104±1.932 a	80.598±1.932 b	84.750±1.932 ab	84.154±1.932 ab
PUFA (Polyunsaturated fatty acid)	1.540±0.198 b	1.874±0.198 b	2.890±0.198 a	1.880±0.198 b	1.504±0.198 b
SFA/MUFA (%)	17.940±2.930 a	12.140±2.930 a	19.656±2.930 a	16.200±2.930 a	17.080±2.930 a
SFA/UFA (Unsaturated fatty acid(%)	17.560±2.870 a	11.880±2.870 a	18.950±2.870 a	15.860±2.870 a	16.740±2.870 a

Table II: Comparison of fatty acid composition according to storage times

Fatty Acid Tipes	2005	2006	2007	2008	2009
Butyric acid (C4:0)	0.124±0.123 a	0.428±0.123 a	0.338±0.123 a	0.386±0.123 a	0.144±0.123 a
Myristic acid (C14:0)	0.036±0.028 a	0.028±0.028 a	0.092±0.028 a	0.082±0.028 a	0.040±0.028 a
Pentadecanoic acid (C15:0)	0.006±0.010 a	0.010±0.010 a	0.026±0.010 a	0.026±0.010 ab	0.000±0.010 a
Cis10-Pentadecaenoic acid (C15:1)	0.132±0.169 a	0.100±0.169 a	0.508±0.169 a	0.350±0.169 a	0.154±0.169 a
Palmitic acid (C16:0)	6.306±0.227 a	5.282±0.227 b	6.244±0.227 a	5.904±0.227 ab	5.820±0.227 ab
Palmitoleic acid (C16:1c)	0.044±0.069 a	0.012±0.069 a	0.184±0.069 a	0.170±0.069 a	0.042±0.069 a
Heptadecanoic acid (C17:0)	0.030±0.040 a	0.050±0.040 a	0.092±0.040 a	0.100±0.040 a	0.022±0.040 a
Stearic acid (C18:0)	4.230±2.008 a	6.842±2.008 a	10.080±2.008 a	7.228±2.008 a	8.460±2.008 a
Oleic acid (C18:1,c)	87.262±2.029 a	85.064±2.029 ab	80.082±2.029 b	83.030±2.029 ab	83.220±2.029 ab
Linoelaidic acid (C18:2t)	2.026±0.196 a	2.106±0.196 a	1.918±0.196 a	1.940±0.196 a	1.692±0.196 a
Cis11-Eicosaenoic acid (C20:1)	0.106±0.147 a	0.036±0.147 a	0.358±0.147 a	0.356±0.147 a	0.074±0.147 a
SFA (Saturated fatty acid)	10746±2.020 a	12.694±2.020 a	16.888±2.020 a	13.748±2.020 a	14.384±2.020 a
MUFA (Monounsaturated fatty acid)	87.474±1.932 a	85.204±1.932 ab	80.306±1.932 b	83.908±1.932 ab	83.452±1.932 ab
PUFA (Polyunsaturated fatty acid)	2.026±0.198 a	2.106±0.198 a	1.918±0.198 a	1.904±0.198 a	1.698±0.198 a
SFA/MUFA (%)	12.332±2.930 a	14.920±2.930 a	21.542±2.930 a	16.818±2.930 a	17.404±2.930 a
SFA/UFA (Unsaturated fatty acid(%)	12.040±2.870 a	14.510±2.870 a	21.022±2.870 a	16.368±2.870 a	17.050±2.870 a

Means with different letter in same column are significantly different level (P<0.05)

It was determined that oleic acid, stearic acid, palmitic acid and linolaidic acid contents in Palaz variety was found as 79.930%, 9.134%, 5.550% and 2.890%, respectively. According to these values, Palaz variety had the lowest values with respect to oleic and palmitic acids but the highest values with respect to stearic ve linolaidic acids. As a result of these values, SFA and PUFA ratios were found as highest with 15.594% and 2.890%, respectively but, MUFA ratio was found as lowest with 0.050%. The lowest fatty acid ratio in this variety was found as 0.050% in pentadecanoic acid. Other fatty acid ratios in this variety changed between 0.050 and 0.674% (Table I).

It was determined that oleic acid, stearic acid, palmitic acid and linolaidic acid contents in Sariyagli variety was found as 84.528%, 7.152%, 5.634% and 1.880%, respectively. The lowest fatty acid content of this variety was found as 0.004% in pentadecanoic acid. Other fatty acid ratios in this variety changed between 0.026 and 0.220%. In addition, in this variety, SFA, MUFA and PUFA ratios were found as 13.090%, 84.750% and 1.880%, respectively (Table I).

It was determined that oleic acid, stearic acid, palmitic acid and linolaidic acid contents in Yomra variety was found as 83.842%, 7.722%, 6.190% and 1.504%, respectively. The lowest fatty acid of this variety was found as 0.004% in pentadecanoic acid. Other fatty acid ratios in this variety changed between 0.026 and 0.027%. In addition, in this variety, SFA, MUFA and PUFA ratios were found as 14.192%, 84.154% and 1.504%, respectively (Table I).

As can be seen in both values in above and Table I, it was found significant differences with respect to fatty acid ratios among cultivars. These differences were found significant in oleic and linoelaidic acids, which had higher fatty acid ratio, while they weren't found significant in stearic and palmitic acids. In addition, These differences were found significant in MUFA and PUFA while they weren't found significant in SFA (p<0,05) (Table I).

It was said to be similar situation in comparison between years with respect to storage periods. Although most of fatty acids had some differences according to years, most of these differences weren't found significant as statistically. In this research, differences in oleic acid, palmitic acid and MUFA contents bound to them were found significant in percent level (Table II).

Many workers in Turkey have focused on the hazelnut varieties of the common chemical composition (Alasalvar *et*

al., 2003; Ozdemir & Akinci, 2004) and lipid composition (Koksal *et al.,* 2006). Seyhan *et al.* (2007), while working on chubby Palaz and almond type nut samples in fruit development period (early stage, middle stage, harvest stage) stated that out of 16 fatty acids 18:1 had highest quantity followed by 18:2, 16:0 and 18:0, respectively. They found that MUFA's level changed between 71-81%, increasing levels of MUFA in middle stage and harvest stage levels, on the contrary PUFA levels reduced. Seyhan *et al.* (2007) were determined oleic acid in three periods as 77.92%, 82.29% and 82.29% and linoleic acid as 11.92%, 7.91% and 8.10%, respectively. Total MUFA were 78.21, 82.89 and 82.71% and total PUFA were 12.26, 8.22 and 8.40%. In their research of Seyhan *et al.* (2007) found that oleic acid levels were consistent with the amounts of oleic acid in 2005 and 2009 samples. In our study, total MUFA were found 87.474, 85.204, 80.306, 83.908 and 83.452%, respectively in 2005-2009, while total PUFA levels were 2.026, 2.106, 1.918, 1.904 and 1.698%, respectively. In another study, oleic acid was determined as 77.65% and linoleic acid as 15% in cultivated Palaz species, whereas total MUFA and PUFA were found 92.9 and 15.1% (Koksal *et al.,* 2006).

Ozdemir *et al.* (2001) reported the amount of palmitic acid as 6.6-8.3%, stearic acid as 2.8–3.2%, oleic acid as 75.7-77.7%, and linoleic acid as 11.1–13.8% for some commercial Turkish hazelnut varieties under the ecological conditions of Giresun. Koyuncu *et al.* (1997a) reported palmitic acid of 5.92–6.93%, stearic acid of 0.88–2.36%, oleic acid of 70.38–84.38%, and linoleic acid of 8.25–21.37% for 10 genotypes of Palaz and Tombul Turkish varieties from Samsun provinces. Balta *et al.* (2006) detected fatty acids whose percentages decreased from oleic towards linoleic, palmitic, stearic acids, respectively in the samples chosen from Turkey eastern Anatolia region nuts genotypes. Other researchers worked their studies in ecological conditions, or in the same year during fruit development in different periods, or hazelnut samples grown in culture conditions, etc. Geographical conditions like altitude differences could affect the fatty acid composition (Hadorn *et al.,* 1967; Parcerisa *et al.,* 1993).

In our study, hazelnut samples stored for four years were used and fatty acids were found in all types of nuts, compared with other studies in Turkey. Linoleic acid content was detected as relatively low level compared with the others. PUFA amounts of certain types of nuts can be considered to be affected by storage conditions, because in studies of Turkey, no researcher noticed existence of linoelaidic, which is a transform of linoleic acid. In our study, these fatty acids were found in almost all types of nuts. Other studies were taken into account identified oleic acid contents of our samples were found to be higher than the others. Parcerisa *et al.* (1993) found that fatty acids in hazelnut species harvested in different geographical origins in Oregon, USA, were oleic, linoleic, palmitic, stearic, linolenic, and eicosanoic acid. Lauric and Myristic acids were detected as the lowest level. They informed that MUFA and PUFA were found to be dominant. However, Hadorn and Zurcher found that oleic and linoleic acid content change a lot in hazelnut samples grown in different origins, while Contini *et al.* (1991) found that it didn't change. Oliveira *et al.* (2008) stated in their study in 2008 Portugal, Daviana, Fertillede Coutard and Bollwiller nut species reported that, oleic acid was determined predominant in all samples followed by linoleic acid, palmitic acid, and stearic acid, respectively. They stated PUFA and SFA levels had lower percentage than MUFA. However, PUFA levels were determined lower than F Coutard's in Daviana culture samples.

Aflatoxin determination: Although nut products are less affected by mold contamination in comparison with the others, mold and aflatoxin contamination can be seen. Aflatoxin forming mold developed in dead cells after harvest and aflatoxin is formed at the proper temperature and humidity. During the aflatoxin formation, physical, biological, climatic, especially temperature and humidity are effective (Pıtt, 1981). Mycotoxin risky products such as hazelnuts, pistachios should be stored in warehouses, not under nylon. Used as a storage place must be sealed and insulated and a cool, dry, not in direct sunlight, moisture, high from the ground floor, ceiling and roof. The storage temperature for conditions of nuts should be at 5-7°C, relative humidity of 65% or lower, and grain moisture of 4-5% are needed. If relative humidity is 78-80% and the temperature is 20-30ºC, the development of aflatoxin in stored nuts are most likely. Hazelnut samples used in our study was stored at temperature of 10ºC and relative humidity of 65%. Although storage under modified atmosphere is costly, it is an economical application in stored products, including mitotoxins due to providing all of assurance.

WHO and FAO determined the amount of aflatoxin in foods as 30 μg/kg that can be tolerated (Anonymous, 1995). Leong *et al.* (2010) studied on formation of aflatoxin in nut and nut products in Malaysia, detected high levels of aflatoxin in 2009. Cheraghali *et al.* (2007) found high amounts (2, 0.4, 2, 0.4 ng/g, respectively) of aflatoxin (B1, B2, G1, G2) in Iranian pistachio nuts in 2007. Demir *et al.* (2002) did not find level of aflatoxin that can be determined in 30 nut samples in the region of Giresun (Turkey). Gürsoy and Bicici (2006) were investigated three different periods of aflatoxin. According to post-harvest drying and storage during the post-harvest drying period, a significant amount of aflatoxin levels was reported. Aycicegi *et al.* (2005) determined high levels of aflatoxin in the food products consumed in Ankara. On dehulled 47 hazelnut samples in Cacao Hazelnut cream 97.5% of the aflatoxin was found.

CONCLUSION

In the present study, there were diffecerences for fatty acid compositions among hazelnut cultivars and years,

while no aflatoxins were found. Although many differences in between fatty acids weren't important as statistically according to both these varieties and storage periods, these values were important as statistical in oleic acid and linoelaidic acid. This results showed that climate in the region and storage facilities used are suitable for long term storage of hazelnut.

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