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Fatty acids, tocopherols and oxidative stability of hazelnuts during storage

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Hazelnuts of three cultivars (*Ata Baba*, *Ran Trapezundski* and *Tonda Gentile*) grown in Bulgaria were stored for 1, 3, 6 and 12 months at different conditions: unshelled (in shell) or shelled (kernels), at 4ºC (fridge) or at 20ºC (in shadow), and then were analyzed about fatty acids composition, tocopherols and oxidative stability (induction period) of their oil. The results revealed that fatty acids were not practically changed up to 12 months in spite of the different storage conditions whereas the tocopherols amount gradually decreased and that trend was slightly stronger at 20ºC than at 4ºC as well as for nuts in shell than the kernels. Concerning oxidative stability, the oil from initial hazelnuts had long induction period (40, 47 and 58 hours for *Ata Baba*, *Ran Trapezundski* and *Tonda Gentile* cultivars, respectively). This oxidative stability gradually decreased during the storage period with the same trend, namely the hazelnuts stored at 20ºC in shell had slightly shorter induction periods than the corresponding kernels stored at 4ºC. All these results indicate that it is possible to store hazelnuts up to 12 months without considerable and harmful changes in their main lipid characteristics. In order to keep their quality at most it is preferable to store them as kernels at 4ºC.

Key words: *hazelnuts, storage, fatty acids, tocopherols, oxidative stability*

INTRODUCTION

Nuts have been known and used as food since ancient times [1] and during the years their health benefits have been clearly recognized [2, 3]. Among all nuts hazelnuts are preferable because of their delicious taste, delicate flavor and beneficial nutritional properties. However, due to high fat content (above 60%) they tend to deteriorate as a result of lipid oxidation. Since the hazelnuts are consumed mainly as kernels the storage conditions are of crucial importance to keep their quality for longer periods. In spite of the significance of that problem, there are not too many publications on the effects of storage conditions on the hazelnut lipid characteristics. Up to now the following have been studied: the storage atmosphere (air or enriched with oxygen vs. inert gases or vacuum [4–8]), temperature (mainly 4-7ºC vs. 20-25ºC [4, 7–10], in single cases –25ºC [8, 11] or even 55ºC [12]), the presence of nut shell [4, 5, 9, 10, 13]. The storage periods are usually up to 12 months [4, 5, 7–12, 14], but also periods of two [13] or four [15] years have been tested. The investigated hazelnut characteristics are: textural [6, 11] or sensory [6, 7, 10] attributes, total phenolics [7, 8, 10], acidity and/or peroxide value [4, 7, 10, 13], hexanal content [7, 8], conjugated dienes and trienes [4], α-tocopherols [12, 13], fatty acids [5, 9, 13–15]. Unfortunately, in these investigations the effects of only single storage condition on particular lipid attribute have been examined. Moreover, some results are contradictory. For that reasons the aim of our work was to study the effects of the two most popular and easy to perform even at home storage conditions, namely the temperature (4ºC in fridge and 20ºC in shadow) and the presence of nut shell, on the fatty acids, tocopherols and oxidative stability (Induction period in hours) of the oil from hazelnuts stored up to 12 months. Thus, some more general recommendations could be given in order to save the quality of nuts not only as industrial/market product but also as preferable dietary stored at home.

EXPERIMENTAL

Samples

Hazelnut cultivars *Ata Baba* (Corylus pontica C. Koch), *Ran Trapezundski* (Corylus maxima Mill.) and *Tonda Gentile* (Corylus avellana L.) were grown in orchards near the town of Kardzhali, Bulgaria. Fruits of three consecutive crops (2009-2011) were collected and corresponding portions of them were stored as kernels (shelled) or in shell, both types in polyethylene bags, at room temperature (20°C, in shadow) or in refrigerator

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(4°C) for 1, 3, 6 and 12 months. Initial portions of hazelnuts were used as respective references (0 months storage).

Reagents

All reagents and solvents were of analytical grade and were used without additional purification. The reference fatty acid methyl esters and tocopherols were from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Extraction of lipids

Hazelnuts (each sample of about 20 g kernels after removing the shell) were ground and the oil was extracted with n-hexane in Soxhlet apparatus for 8 h [16]. The solvent was distilled in rotary evaporator and stock hexane solution of the oil was prepared.

Analysis of fatty acid (FA) composition

Fatty acids were determined by gas chromatography (GC) of their methyl esters (FAME). For the purpose, about 50 mg oil were transmethylated with 1 % sulfuric acid in methanol [17]. Then the FAME were purified by preparative silica gel thin-layer chromatography (TLC) on 20 x 20 cm glass plates developed with hexane-acetone (100:6, v/v) mobile phase. A drop of methyl oleate solution was used as a reference near the edge of the plate. After development, the zones were visualized under UV light (366 nm) after spraying with 0.1 % ethanolic 2`,7`-dichlorofluorescein. FAME zone (Rf ~ 0.7) was scraped and eluted with diethyl ether in a small glass column. The solvent was evaporated under a gentle stream of nitrogen and the rest was dissolved in hexane to give 1 % solution of FAME. GC was performed on a Trace GC Ultra (Thermo Scientific, Bremen, Germany) gas chromatograph equiped with FID and a DB-225 60 m x 0.25 mm x 0.25 μm column (J&W Scientific, USA). The column temperature was programmed from 150ºC to 240°C with 5°C/min and held at this temperature for 20 min. The injector and detector temperatures were 260ºC and 280ºC, respectively. Helium was the carrier gas at flow rate of 1.0 mL/min. Reference FAME mixture was used for peak identification according to the retention times. The analyses were performed in triplicate. The software package *XcaliburTM 2.0*, Revision 2.0 SR 1 (Thermo Scientific, Bremen, Germany) was used to record and process the data.

Analysis of tocopherols

Tocopherols were analyzed directly by HPLC on 250 mm x 4 mm Nucleosil Si 50-5 column (Macherey-Nagel), eluted by hexane-dioxane (96:4, v/v) and fluorescent detection at 290 nm excitement and 330 nm emission [18].

Determination of oxidative stability

Oxidative stability (Induction period in hours) was measured at 100°C (3 g oil sample, air flow 20 L/h) by Rancimat 679 (Metrohm, Switzerland) equipment.

Data processing

Mean values from three consecutive years, each with three parallel measurements, were compared by Student`s t-test (Microsoft Excel software) [19].

RESULTS AND DISCUSSION

Fatty acid composition

The main fatty acids in the investigated hazelnuts are oleic (18:1, from 73.3 % to 80.4 % depending on the cultivar, Fig. 1), linoleic (18:2, with values of 7.2 %, 9.8 % and 14.5 % for Tonda Gentile, Ran Trapezundski and Ata Baba cultivars respectively, Fig. 2), palmitic (16:0, in the range 6.8 – 7.8 %, Fig. 3) and stearic (18:0, between 2.3 % and 2.8 %, Fig. 4). Other fatty acids as palmitoleic (16:1), linolenic (18:3), arachidic (20:0) etc. are below 0.2 %. As could be seen (Figs. 1 – 4), the cultivars Tonda Gentile and Ata Baba differ almost twice in their linoleic acid proportion. Nevertheless, fatty acids composition of the investigated hazelnuts in general is similar to that of Turkish [5], Italian, Spanish [12, 20], Croatian, French [21] cultivars. Unlike the available information about fresh hazelnuts the findings published about fatty acids composition of stored nuts are not too many, not systematic and indeed discrepant. Moreover, usually the effect of only single storage condition on fatty acids has been examined. Thus, Koyuncu *et al.* [14] have observed in kernels from three Turkish cultivars slight decrease of 18:2 after the 6th month storage at 21 ºC in vacuum bags, without effect of the shell presence or absence on the fatty acids composition [5]. Other authors [9] have noticed increase of 18:0 (from 2.6 % to 3.1 %) after the 8th month and decrease of 18:3 (being 0.1 % in the initial oil) after the 12th month in whole hazelnuts stored at 20 ºC but without such changes in kernels stored at 4 ºC. Unfortunately, no comparative experiments with whole nuts stored at 4ºC and kernels at 20ºC were implemented.

On the other hand, Ebrahem *et al.* [13] have shown that 18:1 and 18:2 have not changed in kernels and in whole hazelnuts stored at 0 ºC up to 12 months. Similarly, Beyhan *et al.* [15] have stored whole hazelnuts in warehouse up to 4 years practically without differences in their fatty acid composition. In addition to these results, our

experiments confirme that fatty acids have not been changed during hazelnut storage up to 12 months irrespective of different conditions such as temperature (4 ºC or 20 ºC) and the presence or absence of shell, as well as irrespective of the differences in the initial fatty acid composition of the three analyzed cultivars (Figs. 1 – 4).

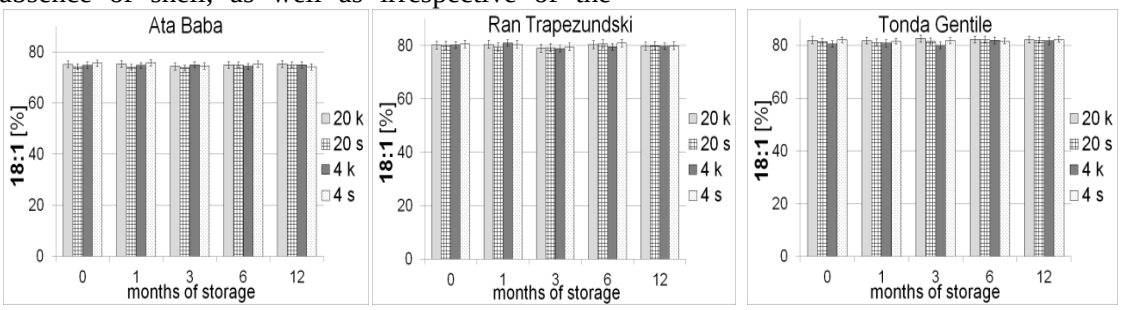

Fig. 1. Oleic acid (9-18:1) content of the oil from three cultivars hazelnuts stored up to 12 months at different conditions: at **20**ºC or **4**ºC, as kernels (**k**) or in shell (**s**).

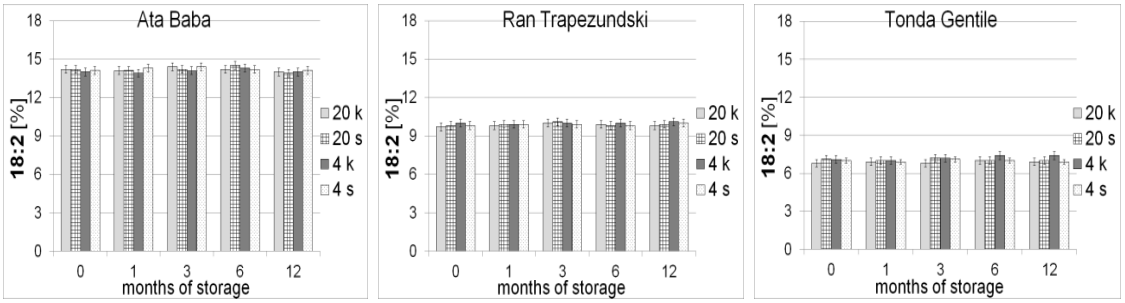

Fig. 2. Linoleic acid (9,12-18:2) content of the oil from three cultivars hazelnuts stored up to 12 months at different conditions: at **20**ºC or **4**ºC, as kernels (**k**) or in shell (**s**).

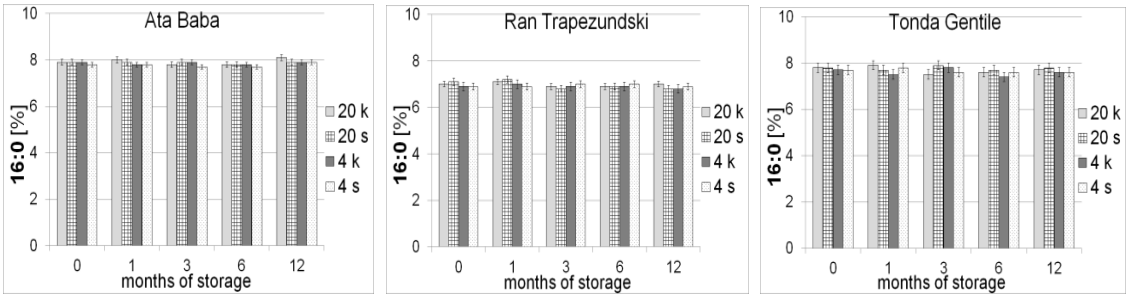

Fig. 3. Palmitic acid (16:0) content of the oil from three cultivars hazelnuts stored up to 12 months at different conditions: at **20**ºC or **4**ºC, as kernels (**k**) or in shell (**s**).

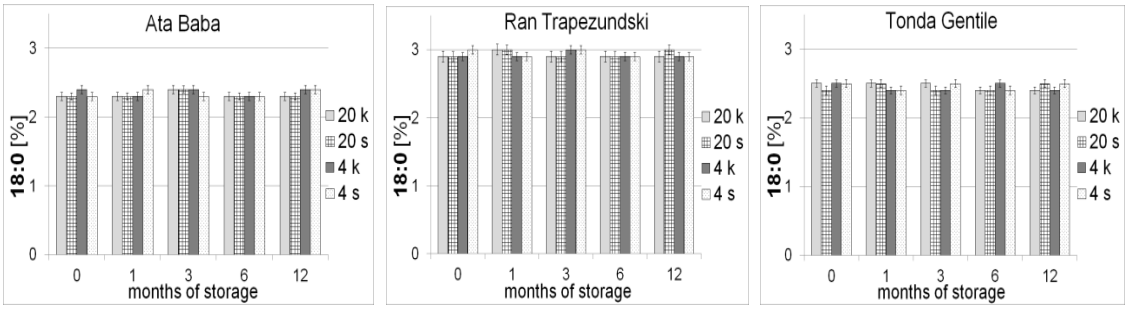

Fig. 4. Stearic acid (18:0) content of the oil from three cultivars hazelnuts stored up to 12 months at different conditions: at **20**ºC or **4**ºC, as kernels (**k**) or in shell (**s**).

Tocopherols

Tocopherols are important biologically active substances with significant role of strong natural antioxidants. Since their availability prevents the oils from lipid oxidation higher amount of tocopherols is valuable and desirable virtue. Hazelnut oil from different varieties contains tocopherols in quite wide range: from 115 to

600 mg/kg [12, 20 – 24]. Oils from the three cultivars presented here have quite high initial amounts: 560 mg/kg in *Ran Trapezundski*, 520 mg/kg in *Ata Baba* and 500 mg/kg in *Tonda Gentile* (Fig. 5).

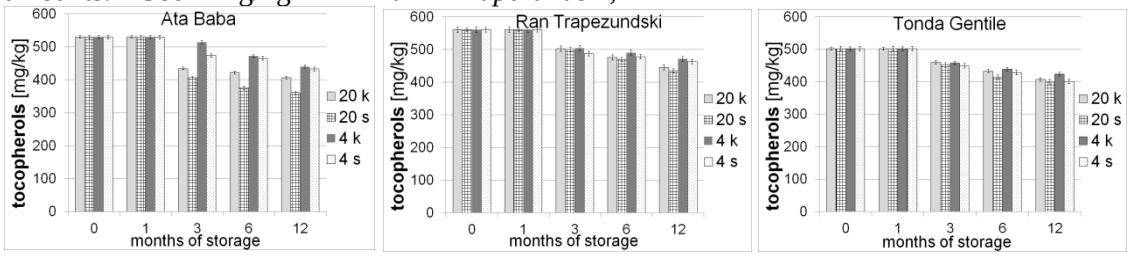

Fig. 5. Total tocopherols in the oil from three cultivars hazelnuts stored up to 12 months at different conditions: at **20**°C or **4**°C, as kernels (**k**) or in shell (**s**).

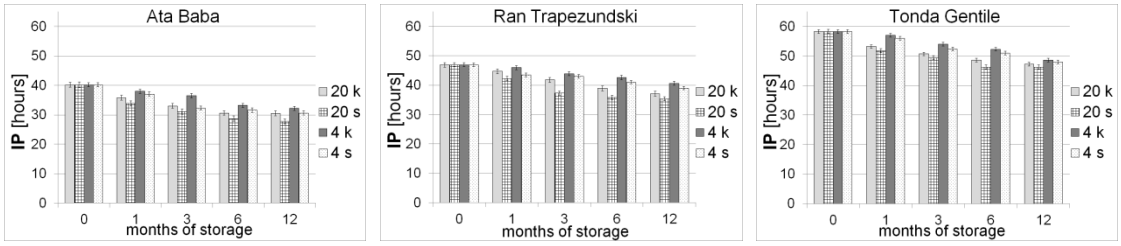

Fig. 6. Oxidative stability (induction period) of the oil from three cultivars hazelnuts stored up to 12 months at different conditions: at **20**°C or **4**°C, as kernels (**k**) or in shell (**s**).

After the first storage month these amounts gradually decrease and that trend is slightly stronger at 20°C than at 4°C as well as for nuts in shell than the kernels. Likewise, respective reducing of the individual α-, β- and γ-isomers is observed (not shown here). As can be seen (Fig. 5) hazelnuts stored at 4°C as kernels contain slightly more tocopherols than that stored at 20°C in shell, irrespective of the differences between the three cultivars. Only two publications were found in the literature concerning determination of tocopherols during hazelnuts storage. They revealed that (i) the remaining amount of α-tocopherol after 9 months storage of kernels was higher at lower temperature (three investigated cultivars) [12], and (ii) hazelnuts stored up to 12 months at 0°C and at 10°C contained slightly higher amount of α-tocopherol as kernels than in shell (one investigated cultivar) [13]. Despite the scarce information, these findings confirm our results that storage of hazelnuts at 4°C as kernels is preferable for keeping their quality for longer period.

Oxidative stability

The oxidative stability of oils is important indicator for their admissible shelf-life. It depends on the fatty acid composition, the availability of natural antioxidants (tocopherols, etc.) as well as on the presence of synergists (e.g. phytosterols). The oxidative stability of hazelnut oil is among the highest for vegetable oils: 16 h of refined [24, 25] or 20 – 40 h of cold-pressed [26] samples (at the same experimental conditions as presented here). The fresh oils from investigated here three cultivars have even better indexes, i.e. 40 h, 48 h and 59 h for *Ata Baba*, *Ran Trapezundsli* and *Tonda Gentile*, respectively (Fig. 6).

During storage of nuts, these values have gradually decreased and some effect of the temperature and shell can be seen, namely the decrease of oxidative stability is stronger at 20°C than at 4°C as well as the kernels have slightly higher stability than nuts in shell. Some reasonable interpretation of these results might be the moisture held by the shell which induces/favors undesirable processes as lipid oxidation and degradation. Comparing the three analyzed cultivars, the highest oxidative stability of *Tonda Gentile* can be explained by the lowest percentage of linoleic acid (Fig. 2) regardless of the lowest amount of tocopherols (as main antioxidant in that oils, Fig. 5). Unfortunately, no data in the literature have been found to compare with these results excepting only partial characteristics of lipid oxidation such as acidity, peroxide value [4, 7, 10, 13], antioxidant capacity (expressed as Trolox equivalent [8]) or conjugated dienes and trienes [4]. Thus, the authors have observed gradual increase during storage of acidity and peroxide values at that in greater extent at higher temperature (25°C vs. 7°C for kernels and whole nuts [4]; or 20°C in shell vs. kernels at 4°C [10]). On the other hand, no significant effects of the storage conditions (temperature and shell) on the antioxidant capacity (as Trolox equivalent, [8])

as well as on the conjugated dienes and trienes [4] have been observed.

CONCLUSION

Fatty acids have not been changed significantly during hazelnuts storage up to 12 months irrespective of different conditions such as temperature (4ºC or 20ºC) and the presence or absence of shell, as well as irrespective of the differences in the initial fatty acid composition of the three analyzed cultivars. The fresh oils have quite high initial amounts of tocopherols which gradually decrease during storage and that trend is slightly stronger at 20ºC than at 4ºC as well as for nuts in shell than as kernels. The oxidative stability of the fresh oils decrease similarly during the hazelnuts storage. Thus, it is possible to store hazelnuts up to 12 months without considerable and harmful changes in their main lipid characteristics. In order to keep their quality at most it is preferable to store the hazelnuts as kernels at 4ºC.

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МАСТНИ КИСЕЛИНИ, ТОКОФЕРОЛИ И ОКИСЛИТЕЛНА СТАБИЛНОСТ НА ЛЕШНИЦИ ПО ВРЕМЕ НА СЪХРАНЕНИЕТО ИМ

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(Резюме)

Лешници от три сорта (*Ата Баба*, *Ран Трапезундски* и *Тонда Джентиле*), отглеждани в България, са съхранявани за 1, 3, 6 и 12 месеца при различни условия: с черупки или като ядки, при 4ºC (в хладилник) или при 20ºC (на сянка), след което са анализирани мастно-киселинния състав, токоферолите и окислителната стабилност (индукционен период в часове) на маслата им. Резултатите показват, че мастните киселини практически не се променят до 12 месеца независимо от различните условия на съхранение, докато количеството на токоферолите постепенно намалява, и тази тенденция е малко по-силно изразена при 20ºC, отколкото при 4ºC, както и при лешниците с черупки, в сравнение с ядките. Подобно съответно намаляване се наблюдава и при индивидуалните α-, β- и γ-изомери. Относно окислителната стабилност, маслата от изходните лешници имат дълъг индукционен период (40, 47 и 58 часа, съответно при сортовете *Ата Баба*, *Ран Трапезундски* и *Тонда Джентиле*). Тази окислителна стабилност постепенно намалява по време на съхранението по същия начин, както при токоферолите, т.е. лешниците, съхранявани при 20ºC с черупки имат малко по-къс индукционен период. Всички тези резултати показват, че е възможно лешниците да бъдат съхранявани до 12 месеца, без да настъпят значителни и вредни за здравето промени в основните им липидни характеристики. За да се запази качеството им в най-голяма степен, е препоръчително те да бъдат съхранявани като ядки при 4ºC.

Ключови думи: *лешници, съхранение, мастни киселини, токофероли, оксидантна стабилност*