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Abstract

This study evaluated the effect of different storage conditions currently used by the industry, on the chemical, physical and sensory characteristics of 'Tonda Gentile delle Langhe' hazelnuts, during one year of storage. The traditional method of in-shell preservation in a storage room at ambient temperature was compared with refrigerated storage of shelled nuts at 4 °C and 55% relative humidity, with or without modified atmosphere (1% oxygen, 99% nitrogen). The following parameters were measured: moisture content, lipid content, total phenolic content, and antioxidant capacity of the kernel; acidity and peroxide value of the oil. The kernel resistance to breakage was evaluated by texture analysis using a compression test. The hazelnuts were also evaluated by sensory analysis. The results showed that the acidity and the peroxide value were the most discriminating parameters. After one year of storage, the acidity of hazelnuts stored at ambient temperature (0.47% oleic acid) was higher than the value considered the acceptable limit after storage (0.40% oleic acid), while refrigerated storage maintained a low level of acidity and lipid oxidation, with the best performance in modified atmosphere (0.13% oleic acid; 0.057 O_2 mmol kg^{-1}). Sensory analysis after 12 months also showed differences among the three storage

treatments. In-shell storage of hazelnuts at ambient temperature was able to preserve the kernels below threshold limits of acidity and oxidative degradation for up to 8 months, but refrigeration was necessary to maintain high quality for up to one year. The use of modified atmosphere is recommended for long periods of storage.

Keywords: *Corylus avellana* L., storage conditions, lipid oxidation, polyphenols, antioxidant, mechanical properties.

Highlights

The effect of three storage conditions on quality parameters of hazelnuts was studied in commercial scale conditions.

Time and storage conditions affected significantly acidity and peroxide value.

Low temperature and modified atmosphere (1% oxygen, and 99% nitrogen) decreased additively the lipid oxidation.

Low temperature maintained high level of phenolic content and antioxidant capacity.

1. Introduction

Italy is the world's second largest producer of hazelnuts (*Corylus avellana* L.) after Turkey. Italian hazelnut cultivars are highly valued by the food industry for the quality and sensory characteristics of their nuts, in particular for use in confectionery.

Storage conditions affect hazelnut quality and are thus a concern for both food industry and direct consumption. The resistance to oxidation of lipids is frequently associated with the shelf-life of foods, but there are many other factors that contribute to defining the quality of hazelnuts, such as appearance, texture, flavor, chemical composition, nutritional value, and of course, food safety.

Hazelnuts are one of the most nutritious nuts that contain valuable amounts of nutrients, among which lipids predominate (Venkatachalam and Sathe, 2006). The particular fatty acid composition

of hazelnuts, rich in monounsaturated fatty acids, primarily oleic acid (Amaral et al., 2006; Parcerisa et al., 1998), has a recognized beneficial effect on human health (King et al., 2008; Sabaté and Ang, 2009; Torabian et al., 2009); although as a fatty food, hazelnuts are easily susceptible to rancidity. During storage, the lipid fraction can be subjected to hydrolysis and oxidation, resulting in undesirable odors and flavors, and in the reduction of the nutritional value of the kernels. Very few research articles discuss the effects of postharvest handling and storage on chemical and physical characteristics of hazelnuts (Mencarelli et al., 2008; San Martín et al., 2001), and focused on rate of lipid oxidation, and on fat content and fatty acid composition changes (Koyuncu, 2004; Koyuncu et al., 2005).

Hazelnuts also contain significant quantities of dietary fiber, mineral elements, and vitamins. Hazelnuts are an excellent natural source of the antioxidant vitamin E due to their α-tocopherol content (Kornsteiner et al., 2006), and are also rich in other biologically active compounds such as polyphenols (Alasalvar and Shahidi, 2009). Recently, there is much interest in phenolic compounds because of potential health benefits related to their antioxidant and antiradical activities, anti-inflammatory properties, anticarcinogenic and antimutagenic effects, and antiproliferative potential. Although antioxidant capacity and phenolic composition of hazelnut kernels and hazelnut by-products have been extensively investigated (Alasalvar and Shahidi, 2009), there is a lack of data concerning the stability of the phenolic fraction and the antioxidant capacity of hazelnuts during storage.

It is well known that many extrinsic factors, such as humidity and temperature, can affect the quality of hazelnuts. One of the most important factors is moisture, since water activity influences quality parameters, including mold if moisture is too high, shrivel if too low, color changes, and rancidity. Consequently, to ensure a long shelf-life and to extend protection from rancidification processes, the nuts have to be dried, immediately after harvest, to 3.5-5% kernel moisture content (Richardson, 1988). In addition, the relative humidity (RH) during storage must never exceed 70% (Tombesi, 1985). Enzymatic and chemical rancidification processes, and vitamin E degradation, are

considerably retarded at low temperature. Mold and insect activity is virtually eliminated near freezing temperatures. In addition, reduced oxygen levels can positively affect long-term kernel storage or prolong the shelf-life of roasted kernels. As reported by Ebrahem et al. (1994), in-shell and un-roasted kernels may be stored for 24 months with minimal loss in quality at temperatures below 10 °C, while roasted kernels, stored at 0 °C, 5 °C or 10 °C, may only be held for 6 months prior to development of detectable rancidity. Although low temperatures are recognized as effective means to prolong hazelnut storage, usually the nuts are stored at ambient temperature because of the high energy cost for refrigeration. However, Johnson et al. (2009) reported that hazelnuts can also be stored at ambient temperature under 99% nitrogen atmosphere, with effects comparable to storage condition at 3-6 °C and 50-60% RH. Optimal storage conditions may be provided by a combination of low temperature and modified atmosphere (saturated with N_2 and/or CO_2) or vacuum. Recently, Mencarelli et al. (2008) demonstrated that a high concentration of nitrogen (98-100%) and low temperature (4 °C) are best for maintaining color, firmness, acidity and peroxide values of the kernels.

Storage stability or shelf-life of foods could be defined as maintenance of the sensory and physical characteristics associated with becoming stale (Baixauli et al., 2008). Texture is one of the most important characteristics of edible fruits and vegetables (Kilcast, 2004), that can be affected by postharvest treatments and processing. The mechanical behavior of hazelnuts has been studied to characterize different varieties (Güner et al., 2003; Valentini et al., 2006), or different cracking and roasting systems (Özdemir and Özilgen, 1997; Saklar et al., 1999; Demir and Cronin, 2004). However, very little data are available on the effect of particular storage conditions on hazelnut textural characteristics (Mencarelli et al., 2008; Ghirardello et al., 2009b).

This work focuses on the evaluation of the effects of three different storage conditions on the quality of ‘Tonda Gentile delle Langhe’ hazelnuts harvested in 2009, during one year of storage. Usually, data reported in literature are from pilot storage systems. In this research, nuts were stored in industrial storage rooms, typical of commercial storage.

2. Materials and Methods

2.1. Sample preparation

The experiments were carried out on ‘Tonda Gentile delle Langhe’ hazelnuts harvested in a single orchard, located in Cravanzana (Langhe district, Piedmont, NW Italy). Immediately after harvest, the nuts were dried to about 5% moisture content in the kernels. The nuts, at 10% starting moisture content, were dried in a food dryer with a slow stream of warm air (50 °C) for 8 h, After an additional cooling step of 6 h, the samples were stored in storage rooms provided by Ascopiemonte – Organizzazione Produttori Frutta a Guscio S.c.a.r.l. (Santo Stefano Belbo, Piedmont, Italy). Just before the samples were placed in storage rooms (Beginning), samples of hazelnuts (four replicates of 2 kg each) were analyzed, and data were used as references for all treatments. Hazelnuts were then divided into in-shell and shelled batches. The kernels were obtained using an industrial shelling-machine (P8O Sheller, Chianchia, Cherasco, Italy). The samples were packaged in 25-kg food polypropylene bags.

Three different storage conditions were tested: in-shell hazelnuts were stored at ambient temperatures (ranging between 10 and 26 °C) and 60-80 % RH, while kernels (shelled hazelnuts) were cold stored (4 °C, 55% RH) with or without modified atmosphere (1% oxygen, 99% nitrogen). Analyses were conducted after 8 and 12 months of storage, except for kernels stored under nitrogen. The modified atmosphere chamber was opened only after 1 year, according to the standard methods in the industry. Therefore, in this case the kernels were analyzed at the end of the experiment. At each sampling time, batches of about 2 kg of kernels and 5 kg of in-shell nuts were taken for analysis. Just before analysis, the in-shell hazelnuts were manually cracked and shelled.

2.2. Chemical analyses

Moisture content was determined by vacuum oven at 70 ± 1º C (method 934.06; AOAC, 1990). Total fat content was determined by using the Soxhlet petroleum-ether extraction method (method 920.39C; AOAC, 1990).

Titratable acidity (expressed as the percentage of oleic acid), peroxide value (PV, determined by iodometric titration and expressed as millimoles of active O_2 per kg of oil), and fatty acid composition of the oil were determined on each sample according to the European Official Methods of Analysis (Council Regulation, EEC-N.2568/91). Fatty acids were converted into methyl esters (FAME) and analyzed using a Shimadzu GC-2010 plus gas chromatograph (Shimadzu, Milan, Italy) equipped with a flame ionization detector (FID). Separation was achieved on a Supelco SP^{TM} 2560 capillary column (Supelco, Bellefonte, USA), 100 m long, 0.25 mm i.d, 0.2 μm film thickness. The split-splitless injector was used at a split ratio of 1:50. The injector volume of the sample was 1 μL. The injector and detector temperatures were both set at 250 °C. The column temperature was 165 °C for 1 min hold and programmed to increase to 200 °C at a rate of 0.05 °C s^{-1} and then held for 45 min. Helium was used as the carrier gas with a flow rate of 16.7 μL s^{-1}. Fatty acid peaks were identified by comparing retention times with FAME stock solution. The quantification was performed by internal normalization.

The extraction of antioxidant compounds was conducted by mixing finely ground kernels with 50% ethanol (v/v) in ultrapure water acidified with formic acid (pH 4). Each sample (1 g) was extracted with 10 mL of extraction solvent in a capped glass tube on a VDRL 711 orbital shaker (Asal s.r.l., Milan, Italy) at a constant oscillation (1.67 oscillations s^{-1}), at ambient temperature (20-22 °C), for 77 min (Ghirardello et al., 2009a). Afterward, the extracts were centrifuged (15 min at 2700 × g) and the supernatants were filtered through a 0.45-μm pore size syringe filter. The extractions were done in quadruplicate for each sample, and the extracts were stored at -18 °C until analysis.

The total phenolic content (TPC) of kernel extracts was assayed spectrophotometrically by means of the Folin-Ciocalteu method, as modified by Singleton and Rossi (1965). Briefly, 0.5 mL of kernel extract was added into a tube containing 2.5 mL of 10-fold dilute Folin-Ciocalteu reagent. The tube was vortexed and allowed to stand at room temperature for 3 min. Then, 2 mL of Na_2CO_3 (7.5%, w/v) was added to the mixture. The absorbance was measured at 756 nm with a UV-1700

PharmaSpek UV-Vis spectrophotometer (Shimadzu, Milan, Italy), after 15 min heating at 45 °C (Pinelo et al., 2003). Phenolic content was expressed as grams of gallic acid equivalent (GAE) per kilogram of sample.

To determine the antioxidant capacity of the extracts, the DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay (RSA) was performed by the method described by von Gadov et al. (1997) slightly modified. Seventy-five microliters of sample extract were added to 3 mL of DPPH$^{\bullet}$ methanol solution (6.1×10^{-5} mol L^{-1}) and incubated for 1 h at room temperature in the dark. The absorbance was measured at 515 nm against methanol solution of DPPH$^{\bullet}$ as a blank. The results were expressed as inhibition percentage (IP) of the DPPH radical calculated according to the following Eq.:

$IP = [(A_{0min} - A_{60min})/ A_{0min}] \times 100$

where A_{0min} is the absorbance of the blank at t = 0 min; and A_{60min} is the absorbance of the samples at 60 min. Results were expressed as millimoles of Trolox equivalent (TE) per kilogram of sample, by means of a dose-response curve for Trolox (0-350 µmol).

2.3. Physical analyses

Instrumental texture properties of the kernels were monitored with a Universal Testing Machine (UTM) TA.XT2i® Texture Analyser (Stable Micro System, Godalming, Surrey, UK). The device was equipped with a 50 kg load cell and a HDP/90 platform. The compression test was performed at 1 mm s^{-1} constant bar speed with a P/75 circular aluminum flat probe (75mm of diameter) (Valentini et al., 2006). The force-deformation curve was acquired as a graph (Fig. 1) and processed by the Texture Export Exceed software rel. 2.54 (Stable Micro Systems, Godalming, UK). Three replicates of 10 nuts for each storage condition were compressed along the longitudinal axis through the hilum containing the major dimension (length) of the kernel (Güner et al., 2003). The breakage characteristics of hazelnut were expressed according to Saklar et al. (1999) by the following parameters: the rupture force (N), represented by the first fracture point (F_1), the slope

(S_{F1}) of the line between the starting point and the first fracture point (N m^{-1}), and the rupture energy (mJ), represented by the area under the curve (W_1) for the region between the starting point and the first fracture point.

[Fig. 1 about here]

2.4. Sensory analyses

The sensory evaluation of the samples was performed with a duo-trio overall difference test (ISO 10399, 2004), by a group of 24 trained panelists. Since 'Tonda Gentile delle Langhe' hazelnuts are generally used by the food industry as roasted kernels, the tastings were conducted on roasted samples. To ensure the characteristic texture and aroma, the kernels were roasted (160 °C, 20 min in a ventilated oven) just before the tasting sessions. All samples were furnished in white plastic cups, containing 6-7 whole roasted kernels. Water was provided for palate cleansing.

2.5. Statistical analyses

Results were expressed as mean ± SD. Analyses of variance were done using SPSS software (version 18.0 for Windows, SPSS Inc., Chicago, Illinois). ANOVA was performed on the chemical and physical data, considering all factors and their interaction (two-way ANOVA). Intra-storage condition and intra-storage time differences were analyzed using one-way ANOVA (factor being storage time and storage condition). Significant differences ($P < 0.05$) among means were determined using the Tukey's test at a fixed level of $\alpha = 0.05$. For the sensory analysis, the total amount of correct responses was compared to the critical number of correct responses in a 'duo-trio' difference test for significance with an $\alpha = 0.05$.

3. Results and Discussion

3.1. Chemical analyses

Data on moisture and lipid content of kernels, acidity and peroxide value of oils, are reported in Table 1. The kernel moisture content was nearly stable during storage, except for in-shell hazelnuts stored at room temperature at the twelfth month, probably due to a partial rehydration of the kernels. The moisture content of the kernels never reached 5%, the threshold value for the good preservation of hazelnuts.

The lipid content of the kernels was very stable under all storage conditions. Contrary to data reported by Koyuncu (2004), no significant differences of lipid content were observed between hazelnut stored shelled and in-shell. Table 2 shows the fatty acid compositions of hazelnuts during the storage period. The predominant saturated fatty acid in hazelnuts was palmitic (C16:0), followed by stearic (C18:0). The two most abundant unsaturated fatty acids were oleic (C18:1) and linoleic (C18:2). Their relative amounts were comparable to data reported in literature for the same cultivar (Cristofori et al., 2008). The linoleic acid content varied inversely to the oleic acid content (Koyuncu et al., 2005), and was always less than 9% of total fatty acids, considered a critical threshold value by the food industry (Arcoleo, 1991).

[Table 1 and Table 2 about here]

During storage, the ratio of unsaturated/saturated fatty acids decreased from 0.1203 to 0.1094. Indeed, the total saturated fatty acid content increased from 7.69% to 8.42% at the end of the storage time, while the total unsaturated fatty acid content decreased from 92.30% to 91.69% (Table 2). These changes were significant and related to the decrease of linoleic acid content, probably as the result of its peroxidation and subsequent loss. A similar trend was reported by Koyuncu et al. (2005) for the fatty acid composition of hazelnut kernels stored for one year in vacuum packages.

A significant effect of time and condition of storage on the indices of stability of the lipid fraction was observed. As expected, the acidity and peroxide values increased with storage in all samples (Table 1). Interaction effect of storage condition and storage time was also found significant for the acidity and peroxide value parameters ($P < 0.001$). At the beginning of the experiment, the kernels exhibited very low acidity and peroxide values, close to zero (0.06% oleic

acid; O_2 0.045 mmol kg^{-1}), indicating the absence of initial trygliceride hydrolysis and fatty acid oxidation. After 8 months of storage, in-shell hazelnuts stored at ambient temperature showed higher acidity and PV in comparison with cold stored kernels (0.25 and 0.10% of oleic acid, O_2 of 0.082 and 0.050 mmol kg^{-1}, respectively). The variation of these two parameters was very small, and the lipid fraction of the hazelnuts maintained its characteristics of freshness and stability. This trend is in agreement with Gattuso et al. (1995), where the PV at eight months of storage of in-shell nuts stored in perforated containers in a dry and airy storage room, was O_2 of 0.10 mmol kg^{-1}. After twelve months of storage, the acidity of in-shell hazelnuts stored at ambient temperature was 0.47% oleic acid. This value was higher than the acidity reported for the superior extra-virgin olive oils (0.40% oleic acid) that, in the absence of indications about a critical acidity value for the nut industry, we can choose as a limit of acceptability after storage.

Storage at low temperature permitted to maintain a low level of acidity and lipid oxidation, with the best performance in modified atmosphere (0.13% of oleic acid and O_2 of 0.057 mmol kg^{-1}).

The PV is one of the parameters adopted by the nut industry to evaluate the storage aptitude of hazelnut; higher scores are assigned to lots with O_2 values lower than 0.25 mmol kg^{-1} (Arcoleo, 1991). In this study the PV was lower than O_2 of 0.25 mmol kg^{-1} at all treatments and times, except for in-shell hazelnuts after 12 months of storage. The analysis of variance documented an increasing PV trend for storage time (0 < 8 < 12 months), and for storage conditions (refrigerated under nitrogen < refrigerated < ambient temperature). San Martin et al. (2001) reported, for hazelnuts stored in modified atmosphere conditions, a positive correlation, over time, between oxidative rancidity and oxygen content. Differences in PV with time were noted by the third month of storage, but were almost stable at 6, 9 and 12 months with values of 0.45-0.50. In another study on storage (Mencarelli et al., 2008), prevention of oxidative process in hazelnuts was achieved by modified atmosphere (100% CO_2 or 100% N_2) at 4 °C. A little increase of PV (from O_2 of 0.075 to 0.15 mmol kg^{-1}) with the acidity was reported. Hazelnuts stored for three months at 4 °C under nitrogen (98% N_2) maintained the initial PV, in accordance with our data.

The total phenolic content decreased significantly at the 8th month (GAE of 1.05 and 1.09 g kg^{-1} for shelled and in-shell hazelnuts, respectively), then remained almost unchanged, with a slight increase in refrigerated kernels (Table 1). At the twelfth month of storage the higher values of TPC was recorded for refrigerated kernels (GAE of 0.19 g kg^{-1}) with significant differences compared to the other storage conditions. Previous studies reported that low temperature and modified atmosphere can effectively prevent the decrease of phenolic content and antioxidant capacity in long-term stored nuts. For example, during 12 months of storage, low temperature (1 °C instead of 20 °C) and packaging atmosphere with 100% N_2, prevented additively the loss in antioxidants in stored pistachios (Tsantili et al., 2011). Peanuts stored at 20 and 35 °C for up to 4 months had 35% less total phenolics than initially (Talcott et al., 2005), but at 20 °C total phenolics losses were less than those at 35 °C. During one year of storage, Christopoulos and Tsantili (2001) reported a progressive decrease of total phenolics in walnuts. The losses were additively reduced by lower temperature and packaging under elevated N_2 or CO_2.

The present results showed that the decrease in DPPH$^{\bullet}$ values followed a pattern similar with that of TPC. When data were analyzed by two-way ANOVA (Table 1), only the storage time effect was significant ($P < 0.001$) for both parameters. This result was more evident for antioxidant capacity indices, with an increase of the DDPH$^{\bullet}$ scavenging activity between the 8th and the 12th month of storage, and the best performance at the 12th month for the refrigerated kernels (TE of 6.29 mmol kg^{-1}). This was partially in contrast with the hypothesis that storage would decrease the antioxidant potential of hazelnuts due to oxidation of phenolic compounds. A similar behavior was highlighted by Bolling et al. (2010) studying the influence of storage on polyphenol content and antioxidant capacity of California almond skins. They observed an increase of polyphenols and antioxidant capacity in skin extracts of almonds stored for 15 months, without storage temperature effects (4 or 23 °C, 30% RH). They suggested that a dynamic process affected the changes in flavonoid and phenolic acid contents, by an increase of polyphenol extractability, a degradation of polymeric polyphenols and consequently an increase of soluble phenolics, or a polyphenols

synthesis after harvest, just observed in a few foods. Therefore, as reported by Manzocco et al. (2001), the loss of antioxidant capacity of polyphenols is due to their enzymatic or chemical oxidation; however, some authors suggested that partially oxidized polyphenols can exhibit higher antioxidant activity than that of non-oxidized phenols.

3.2. Physical and sensory analyses

As reported in a previous study on hazelnuts of the cultivar 'Tonda Romana' (Ghirardello et al., 2009b), rupture force (N) and slope at the first fracture point ($slope_{F1}$, N m^{-1}) are the most discriminating parameters during storage. These indices can be considered important quality markers because they are correlated with sensory characteristics of crispiness and crunchiness (Saklar et al., 2009). The textural parameters measured in this experiment, despite the high variability of the values, showed significant differences from the starting values at both eight and twelve months of storage (Table 3). The two-way ANOVA analysis showed a significant effect ($P < 0.001$) of storage time for the rupture force and $slope_{F1}$, and of storage conditions for $slope_{F1}$ ($P < 0.05$); an interaction effect ($P < 0.01$) was observed for the rupture energy. All parameters increased with time in in-shell hazelnuts; for those shelled the highest values of rupture force and rupture energy were registered at 8^{th} month. These results are in agreement with those reported by Ghirardello et al. (2009b) showing that the rupture force and the rupture energy of raw hazelnuts refrigerated and cold stored under nitrogen were higher after four and eight months of storage, in comparison to the fresh samples. After 12 months of storage, the in-shell hazelnuts were characterized by the highest values of rupture force and rupture energy, and the lowest value of $slop_{F1}$, therefore they had the highest firmness and resistance to deformation. However, only the rupture energy (mJ) was able to discriminate cold stored kernels, with or without modified atmosphere, from those stored at ambient temperature, characterized by a greater resistance to fracture. Mencarelli et al. (2008) utilized an Instron Universal Testing Machine to study the effect of different temperatures and modified atmospheres on the deformation of hazelnut kernels. They

observed an increase with time of the kernels fracturability for all storage conditions. Though, these data cannot be compared with our results because of the use of different operative conditions during the compression test and of the measurement of different parameters.

[Table 3 about here]

The duo-trio difference test results are reported in Table 4. The obtained results showed that, after 8 months of storage, no sensory differences were found by panelists between hazelnuts stored under refrigerated and ambient conditions. Instead, after 12 months there were sensory differences among all storage conditions, and particularly between the samples refrigerated and refrigerated under nitrogen.

[Table 4 about here]

4. Conclusions

Acidity and peroxide values in this study indicated the efficacy of low temperature for minimizing lipid oxidation during 8 months of storage. Assays of other important quality parameters did not document significant differences among the samples. Sensory panelists were not able to discriminate the refrigerated hazelnuts from those stored at ambient temperature for 8 months. However, after 12 months, the sensory analysis was able to distinguish the different storage techniques. Again, acidity and peroxide value were the more powerful and discriminating indices. Low temperature and elevated nitrogen atmosphere prevented additively lipid oxidation giving the lowest values of acidity and peroxide value. Furthermore, the use of low temperature was more effective for maintaining high level of phenolic content and antioxidant capacity than low temperature combined with elevated nitrogen atmosphere. The effectiveness of low temperature in delaying the quality decay of hazelnuts is confirmed; refrigeration was effective for maintaining kernel quality for up to one year of storage. On the other hand, when stored as in-shell nuts at ambient temperature, quality was only maintained for a period of about 8 months after harvest.

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Table 1. Moisture content, lipid content, acidity, peroxide value (PV), total phenolic content (TPC), and antioxidant capacity ($DPPH^{\bullet}$) of the hazelnuts during storage. IS: in-shell hazelnuts; S: shelled hazelnuts (kernel); Refrigerated: stored at 4 °C and 55% RH; Under N_2: refrigerated under nitrogen (4 °C, 55% RH - 1% O_2, 99% N_2); Ambient temp.: stored at ambient temperature (70% RH).

		Beginning	8th month	12th month	$P^{\dagger}_{sc}$	P_{st}	$P_{sc} \times P_{st}$
Moisture content (% dry basis)					***	***	***
S	Refrigerated	3.94 ± 0.04^{AB}	3.97 ± 0.05^{B}	3.89 ± 0.01^{aA}			
S	Under N_2	3.94 ± 0.04		3.97 ± 0.05^{b}			
IS	Ambient temp.	3.94 ± 0.04^{A}	3.97 ± 0.02^{A}	4.95 ± 0.04^{cB}			
Lipid content (% dry basis)					NS	NS	NS
S	Refrigerated	61.28 ± 7.53	62.38 ± 1.27	65.36 ± 3.61			
S	Under N_2	61.28 ± 7.53		63.74 ± 4.26			
IS	Ambient temp.	61.28 ± 7.53	63.78 ± 2.33	62.69 ± 3.27			
Acidity (% oleic acid)					***	***	***
S	Refrigerated	0.06 ± 0.01^{A}	0.10 ± 0.01^{aB}	0.27 ± 0.01^{bC}			
S	Under N_2	0.06 ± 0.01^{A}		0.13 ± 0.01^{aB}			
IS	Ambient temp.	0.06 ± 0.01^{A}	0.25 ± 0.02^{bB}	0.47 ± 0.02^{cC}			
PV (O_2, mmol kg^{-1})					***	***	***
S	Refrigerated	0.045 ± 0.006^{A}	0.050 ± 0.004^{aA}	0.093 ± 0.005^{bB}			
S	Under N_2	0.045 ± 0.006^{A}		0.057 ± 0.005^{aB}			
IS	Ambient temp.	0.045 ± 0.006^{A}	0.082 ± 0.006^{bB}	0.263 ± 0.012^{cC}			
TPC (GAE, g kg^{-1})					NS	***	NS
S	Refrigerated	1.40 ± 0.29^{B}	1.05 ± 0.05^{A}	1.19 ± 0.04^{bAB}			
S	Under N_2	1.40 ± 0.29^{B}		1.03 ± 0.04^{aA}			
U	Ambient temp.	1.40 ± 0.29	1.09 ± 0.13	1.07 ± 0.06^{a}			
$DPPH^{\bullet}$ (TE, mmol kg^{-1})					NS	***	NS
S	Refrigerated	8.40 ± 1.79^{B}	4.35 ± 0.36^{A}	6.29 ± 0.32^{bAB}			
S	Under N_2	8.40 ± 1.79^{B}		5.20 ± 0.23^{aA}			
IS	Ambient temp.	8.40 ± 1.79^{B}	3.81 ± 0.94^{A}	5.50 ± 0.28^{aA}			

Data were expressed as mean ± SD (n = 4). Values in the column with different lowercase letters were significantly different at $P < 0.05$. Values in the row with different capital letters were significantly different at $P < 0.05$.

†Probabilities of the effects: P-level calculated for samples from different storage condition (P_{sc}). P-level calculated for samples from different storage time (P_{st}). P-level calculated from storage condition (P_{sc}) × storage time (P_{st}).

** Significant at $P < 0.01$.

*** Significant at $P < 0.001$.

NS, not significant.

Table 2. Percentage of fatty acids, total saturated fatty acids, total unsaturated fatty acids and unsaturated/saturated fatty acids ratio of lipid fractions extracted from hazelnuts during storage. IS: in-shell hazelnuts; S: shelled hazelnuts (kernel); Refrigerated: stored at 4 °C and 55% RH; Under N_2: refrigerated under nitrogen (4 °C, 55% RH - 1% O_2, 99% N_2); Ambient temp.: stored at ambient temperature (70% RH).

		Beginning	8th month	12th month	$P^{\dagger}_{sc}$	P_{st}	$P_{sc} \times P_{st}$
C16:0 (palmitic)							
S	Refrigerated	5.74 ± 0.07	5.85 ± 0.17	5.76 ± 0.24			
S	Under N_2	5.74 ± 0.07^{B}		4.96 ± 0.24^{A}			
IS	Ambient temp.	5.74 ± 0.07	5.78 ± 0.11^{A}	5.35 ± 0.71			
C18:0 (stearic)							
S	Refrigerated	1.85 ± 0.46	2.40 ± 0.96	2.61 ± 0.69			
S	Under N_2	1.85 ± 0.46^{A}		3.25 ± 0.28^{B}			
IS	Ambient temp.	1.85 ± 0.46^{A}	2.60 ± 0.33^{B}	3.12 ± 0.29^{B}			
C 18:1 (oleic)							
S	Refrigerated	85.70 ± 2.53	85.63 ± 1.34	87.41 ± 0.96			
S	Under N_2	85.70 ± 2.53		85.82 ± 0.96			
IS	Ambient temp.	85.70 ± 2.53	85.01 ± 0.79	84.60 ± 1.90			
C 18:2 (linoleic)							
S	Refrigerated	6.16 ± 2.03	5.78 ± 0.51	3.76 ± 1.54			
S	Under N_2	6.16 ± 2.03		4.95 ± 1.15			
IS	Ambient temp.	6.16 ± 2.03	6.26 ± 0.49	6.87 ± 0.91			
C 18:3 (linolenic)							
S	Refrigerated	0.13 ± 0.01	0.11 ± 0.00	0.16 ± 0.05			
S	Under N_2	0.13 ± 0.01		0.11 ± 0.02			
IS	Ambient temp.	0.13 ± 0.01^{B}	0.11 ± 0.01^{B}	0.00 ± 0.00^{A}			
Total saturated					NS	**	NS
S	Refrigerated	7.69 ± 0.44	8.30 ± 0.78	8.39 ± 0.44			
S	Under N_2	7.69 ± 0.44^{A}		8.37 ± 0.24^{B}			
IS	Ambient temp.	7.69 ± 0.44	8.42 ± 0.22	8.51 ± 1.00			
Total unsaturated					NS	*	NS
S	Refrigerated	92.30 ± 0.44	91.69 ± 0.78	91.64 ± 0.47			
S	Under N_2	92.30 ± 0.44^{B}		91.61 ± 0.23^{A}			
IS	Ambient temp.	92.30 ± 0.44	91.59 ± 0.24	91.81 ± 0.95			
Unsat./Sat.					NS	**	NS
S	Refrigerated	12.03 ± 0.73	11.12 ± 1.07	10.95 ± 0.65			
S	Under N_2	12.03 ± 0.73^{B}		10.95 ± 0.34^{A}			
IS	Ambient temp.	12.03 ± 0.73	10.88 ± 0.31	10.91 ± 1.36			

Data were expressed as mean ± SD (n = 4). Values in the column with different lowercase letters were significantly different at $P < 0.05$. Values in the row with different capital letters were significantly different at $P < 0.05$.

†Probabilities of the effects: *P*-level calculated for samples from different storage condition (P_{sc}). *P*-level calculated for samples from different storage time (P_{st}). *P*-level calculated from storage condition (P_{sc}) × storage time (P_{st}).

** Significant at $P < 0.01$.

*** Significant at $P < 0.001$.

NS, not significant.

Table 3. Rupture force, slope at the first fracture point ($Slope_{F1}$), and rupture energy of the hazelnuts during storage. IS: in-shell hazelnuts; S: shelled hazelnuts (kernel); Refrigerated: stored at 4 °C and 55% RH; Under N_2: refrigerated under nitrogen (4 °C, 55% RH - 1% O_2, 99% N_2), Amb. temp.: stored at ambient temperature (70% RH).

		Beginning	8th month	12th month	$P^{\dagger}_{sc}$	P_{st}	$P_{sc} \times P_{st}$
Rupture force (N)					NS	***	NS
S	Refrigerated	91.83 ± 20.91^{A}	105.39 ± 22.19^{B}	99.17 ± 14.83^{AB}			
S	Under N_2	91.83 ± 20.91		95.88 ± 17.64			
IS	Amb. temp.	91.83 ± 20.91^{A}	97.16 ± 20.29^{AB}	106.39 ± 21.53^{B}			
$Slope_{F1}$ (N m^{-1})					*	***	NS
S	Refrigerated	26063.4 ± 4033.9^{A}	30125.2 ± 6229.1^{B}	33637.3 ± 7082.3^{abC}			
S	Under N_2	26063.4 ± 4033.9^{A}		36170.7 ± 9248.3^{bB}			
IS	Amb. temp.	26063.4 ± 4033.9^{A}	29420.9 ± 6117.2^{B}	31236.3 ± 7232.^{aB}			
Rupture energy (mJ)					NS	NS	**
S	Refrigerated	153.95 ± 68.74^{AB}	181.33 ± 72.06^{B}	137.26 ± 41.70^{aA}			
S	Under N_2	153.95 ± 68.74^{A}		121.69 ± 51.64^{aB}			
IS	Amb. temp.	153.95 ± 68.74	149.60 ± 59.22	177.14 ± 64.41^{b}			

Data were expressed as mean ± SD (n = 10). Values in the column with different lowercase letters were significantly different at $P < 0.05$. Values in the row with different capital letters were significantly different at $P < 0.05$.

†Probabilities of the effects: *P*-level calculated for samples from different storage condition (P_{sc}). *P*-level calculated for samples from different storage time (P_{st}). *P*-level calculated from storage condition (P_{sc}) × storage time (P_{st}).

* Significant at $P < 0.05$.

** Significant at $P < 0.01$.

*** Significant at $P < 0.001$.

NS, not significant.

Figure captions

Figure 1

Typical force-deformation curve for compressed hazelnut. F_1: first fracture point (N); S_{F1}: slope of the line between starting point and the first fracture point (N m^{-1}); W_1: area under the curve between starting point and the first fracture point (mJ).

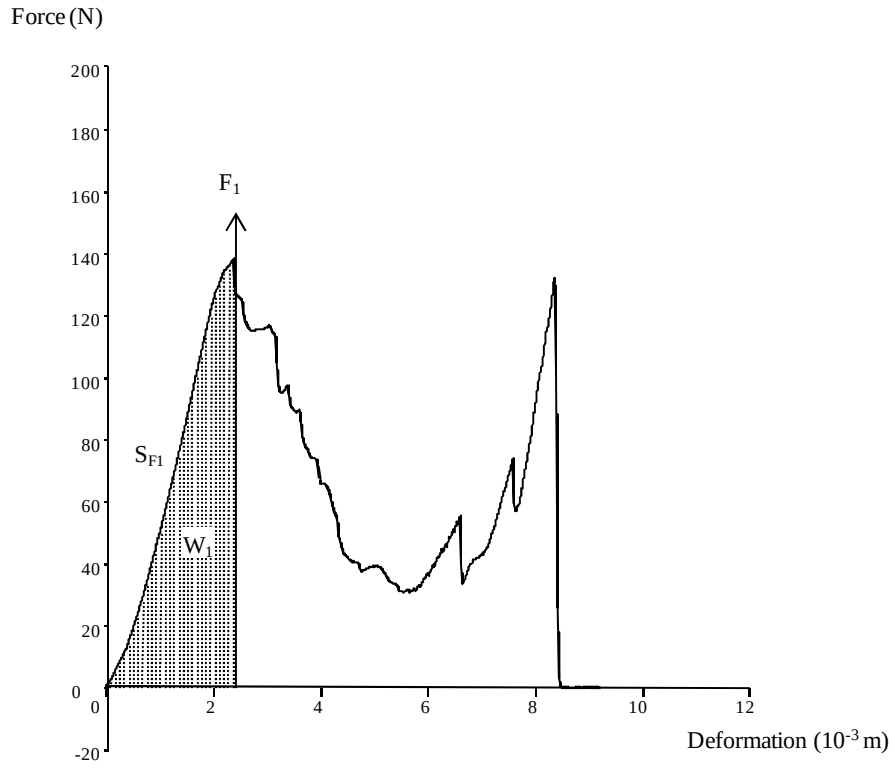
Force (N)
200
180
160
140
120
100
80
60
40
20
0
-20
0
2
4
6
8
10
12
F1
SF1
W1
Deformation (10-3 m)

Figure 1.