



Phenolic composition, antioxidant capacity and hexanal content of hazelnuts (*Corylus avellana* L.) as affected by different storage conditions



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ABSTRACT

The effect of five different storage conditions on the total phenolic content (TPC), the antioxidant capacity (AC), the phenolic compound profile, the total amount of quantified phenolics, the hexanal content, and the sensory characteristics of hazelnuts of two cultivars (Tonda Gentile Trilobata or TGT and Delisava—harvest 2010 and 2011) were investigated for two consecutive years. The storage variables were time (0, 4, 8 and 12 months), temperature (ambient temperature, refrigeration at 5 °C, or frozen at −25 °C) and O₂ availability (ambient air, vacuum or modified atmosphere). Comparing the cultivars, Delisava exhibited the highest levels of TPC and AC for both harvests and all storage conditions; however, it was characterized by the highest hexanal content (more than sixfold higher than TGT). At the end of the storage, the TPC and AC decreased with respect to day 0 in both cultivars, with AC losses ranging between 12% and 35% and TPC losses of approximately 15%. However, these parameters were not able to distinguish the storage conditions. The hazelnut phenolic compound profiles did not seem to be affected by storage techniques in either cultivar; additionally, the sensory analysis panellists were not able to discriminate between the storage conditions. Hexanal was confirmed to be a good marker of lipid oxidation, and its content generally increased during storage in both cultivars; nevertheless, changes were well-controlled by storage conditions where low temperature and reduced oxygen worked synergistically. The absence of oxygen seemed to be more relevant with respect to low temperature, and a good preservation of raw hazelnut kernels was achieved by storage under vacuum with or without preliminary nitrogen flushing.

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1. Introduction

Hazelnuts are popular nuts that are particularly appreciated because of their unique flavour and texture. Hazelnuts not only are tasty and nutritious, but their consumption is extensively related to beneficial effects on human health (King et al., 2008; Sabaté and Ang, 2009) because of the content of monounsaturated fatty acids,

phytosterols and other non-nutrient phytochemicals, such as polyphenols, that can help to protect heart health and promote consumers' well-being (Alasalvar and Shahidi, 2009; Torabian et al., 2009). However, the health-promoting capacities of hazelnuts are dependent on the processing and storage history of the nuts. Hazelnuts are seasonal products; therefore, correct storage is fundamental in order to preserve their nutritional components and reduce the production of fat oxidation off-flavours (De Santis et al., 2009). Temperature, humidity and O₂ availability are the most important factors that affect hazelnuts' storage. To extend the shelf-life and protect against rancidification processes, hazelnuts must be dried immediately after harvest to a kernel moisture content of less than 5% (Richardson, 1988), and the relative humidity during storage must never exceed 70% (Tombesi, 1985). Furthermore, controlling the atmospheric composition and storage

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temperature and employing packaging are very important techniques for extending the storage time (Lin et al., 2012).

Because of their very high lipid content (approximately 60%), hazelnuts can be susceptible to rancidity. Lipid deterioration during tree nuts storage is well known and has been well studied (Shahidi and John, 2013). Most of the papers available in literature are focused on the effect of handling (e.g. drying and shelling), processing (e.g. roasting) and storage on the rate of hazelnut lipid oxidation, or study the changes of fat content and fatty acid composition of hazelnuts during storage (Koyuncu et al., 2005; Koyuncu, 2004). Although it was reported that the acidity and peroxide value are powerful in discriminating hazelnut storage stability (Ghirardello et al., 2013), the variations in these two parameters are generally low, and the hazelnut lipid fraction can maintain the characteristics of freshness and stability for a long time, particularly under cold storage conditions. The autoxidation of unsaturated lipids occurs via a self-sustaining free radical mechanism that produces hydroperoxides (primary products), which in turn undergo scission to form various aldehydes, ketones, alcohols, and hydrocarbons (secondary products) (Kim and Min, 2008). The presence of secondary lipid oxidation products influences the overall quality of a lipid. Hexanal is the main volatile aldehyde that is produced during the oxidation of unsaturated fats (Shahidi, 2001), and for this reason, it is a representative marker of the oxidative rancidity as an alternative to traditional oxidation indicators (e.g., acidity or peroxide values) and is used to follow lipid oxidation in lipid-containing foods.

As mentioned above, the effect of storage on the lipid oxidation attributes is well documented; nevertheless, there is a lack of data concerning the effect of different storage conditions on hazelnut antioxidants. In particular, there are limited data on the changes of the phenolic content and phenolic profile. In addition to lipids, phenols are also prone to oxidation during storage. Acting as antioxidants, phenols can preserve the lipid fraction from rancidity (Shahidi and Naczki, 2004); therefore, the study of the effects of particular postharvest conditions on phenolics and their antioxidant capacity is of interest.

The study of the effect of prolonged storage is even more interesting if it is considered that the most wide-spread reason for reduction in hazelnut quality is the production of “admixtures”, with part of the fresh crops admixed with old nuts (Schäfer et al., 2002).

In this work, we investigated the changes of hexanal and polyphenolic content and antioxidant capacity of two hazelnut cultivars, in shell and shelled, since the results could be useful to optimize and/or choose the more efficient storage conditions at industrial scale. A set of five storage conditions (including different temperatures in the presence or absence of O₂) were investigated for up to 12 months. In order to obtain more information in relation to the harvest year, as well as, the relationship between this and the cultivars, the study was performed for two consecutive years.

2. Materials and methods

2.1. Sample preparation

Commercial hazelnuts from two cultivars, the Turkish “Delisava” and the Italian “Tonda Gentile Trilobata” (TGT), that were harvested in 2010 and 2011 were provided by La Gentile s.r.l. (Cortemilia, Cuneo, Italy). The hazelnuts were purchased within one month from the harvest. At the first sampling time (day 0), the hazelnuts (three replicates of 2 kg each cv.) were analysed, and the data were used as references for all treatments. The hazelnuts were then divided into in-shell and shelled (kernels) batches. Delisava hazelnuts were imported in-shell and shelled; for this reason, two corresponding references were analysed. The in-shell and shelled

(calibrated and selected) hazelnuts were packaged in 25-kg bags and stored by Soremartec Italia s.r.l. (Alba, Cuneo, Italy).

Five different storage conditions, chosen among the most common and innovative storage conditions of nuts, typical of industrial storage, were tested: in-shell hazelnuts stored at ambient temperature (ranging between 10 and 25 °C) and 60–80% relative humidity (RH) in woven polypropylene bags (code AT), kernels cold-stored at 5 °C and 55% RH in woven polypropylene bags (code RF), kernels stored at 5 °C in aluminium foil vacuum bags with (code RVN₂) or without (code RV) a preliminary nitrogen flushing, and kernels stored at –25 °C in vacuum bags (code FZ). In the light of the results observed in the first year of the project, storage of kernels at 5 °C in a modified atmosphere (1% oxygen, 99% nitrogen) in woven polypropylene bags was introduced in the second year of work (code RFN₂) in order to shed light on the effect of oxygen. The analyses were conducted at 0, 4, 8 and 12 months of storage in each year. At every sampling time, batches of approximately 2 kg of kernels were taken for the analyses. The in-shell hazelnuts were manually cracked and shelled immediately before sampling.

2.2. Chemicals

Standards of hexanal, phloridzin, (–)-epigallocatechin, (–)-epigallocatechin 3-gallate, (+)-gallocatechin 3-gallate, (–)-epicatechin 3-gallate, procyanidin B1, procyanidin B2, and quercitrin were purchased from Sigma–Aldrich (Milan, Italy); 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium persulfate, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), formic acid, 2,2'-azino-bis-(3-ethylbenzothiazolin-6-sulfonic acid) diammonium salt (ABTS), Folin–Ciocalteu reagent, and gallic acid were purchased from Fluka Chemicals (Milan, Italy). Acetone, methanol, and *n*-hexane were of an analytical or higher grade and were purchased from Fluka Chemicals. Aqueous solutions were prepared using ultra-pure water produced with a Milli-Q System (Millipore, Milan, Italy).

2.3. Extraction of phenolic compounds

The extraction of phenolic compounds was carried out as reported by El Monfalouti et al. (2012) with modifications. Briefly, 2 g of finely ground kernels was placed in a 50-mL centrifuge tube and added to 20 mL of a fresh mixture of acetone/water/formic acid (70:29.5:0.5, v/v/v) and phloridzin as an internal standard (5000 µg L⁻¹ final concentration). The suspension was shaken on a VDRL 711 orbital shaker (Asal S.r.l., Milan, Italy) at a constant oscillation (1.67 oscillations s⁻¹) in the dark at room temperature for 3 h. Afterward, the extract was centrifuged (10 min, 10 °C, 733 rad s⁻¹), and the supernatant was collected in an amber vial and frozen at –18 °C. The residue was re-extracted for an additional 12 h, and extracts were combined in a 50-mL centrifuge tube. The extracts were defatted by washing with *n*-hexane (3 × 10 mL in a 50-mL centrifuge tube), and the acetone was subsequently evaporated under nitrogen flux with stirring (Glas-Col[®], Terre Haute, IN, USA). The extracts were diluted to 10 mL with a methanol/water/formic acid solution (50:49:1, v/v/v); then, the extracts were filtered (0.45 µm) and stored at –18 °C in an amber vial. Every sample was prepared in triplicate and was used for the determination of the total phenolic content, antioxidant capacity, and chromatographic analysis.

2.4. Total phenolic content (TPC) assay

The amount of total phenolics was assayed spectrophotometrically by means of the modified Folin–Ciocalteu method (Singleton et al., 1999; Singleton and Rossi, 1965). Briefly, 2.5 mL of water-

diluted Folin–Ciocalteu reagent 1:10 (v/v), 2 mL of 7.5% aqueous sodium carbonate solution, and 0.5 mL of phenolic extract were mixed well. After 15 min of heating at 45 °C (Pinelo et al., 2004), the absorbance was measured at 765 nm with a UV–vis spectrophotometer (UV-1700 PharmaSpec, Shimadzu, Milan, Italy). A mixture of solvent and reagents was used as a blank. The phenolic content was expressed as g of gallic acid equivalents (GAE) per kg of sample.

2.5. In vitro antioxidant capacity (AC) assays

To assess the antioxidant capacity of the crude hazelnut extracts, two spectrophotometric assays involving chromogen compounds of a radical nature were applied as previously detailed (Ghirardello et al., 2013).

The Trolox equivalent antioxidant capacity (TEAC) was estimated according to the original analytical procedure described by Re et al. (1999) with slight modifications. The scavenging effect of

the ABTS radical cation (ABTS^{•+}) was recorded at 734 nm, and the results were expressed as millimoles of Trolox equivalents (TE) per kilogram of sample, by means of a dose–response curve for Trolox (0–350 μmol).

The radical scavenging activity (RSA) was measured by the discoloration of the purple-colored methanol solution of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Von Gadov et al., 1997) recorded at 515 nm. The results were expressed as millimoles of Trolox equivalent (TE) per kilogram of sample.

2.6. HPLC–DAD analysis

HPLC–DAD analysis was performed by using a Thermo-Finnigan Spectra-System HPLC system (Thermo-Finnigan, Waltham, USA) that was equipped with a P2000 binary gradient pump system, a SCM 1000 degasser, an AS 3000 automatic injector and a Finnigan Surveyor PDA Plus detector (PDA). ChromQuest software (version 5.0) was used for instrument control and UV-data collection and

Table 1

Total phenolic content (TPC), Trolox equivalent antioxidant capacity (TEAC) and radical scavenging activity (RSA) of the hazelnuts during the first storage test. IS: in-shell hazelnuts; S: shelled hazelnuts (kernels); AT: stored at ambient temperature (60–80% RH); RF: refrigerated at 5 °C and 55% RH; RVN₂ and RV: refrigerated under vacuum with or without preliminary nitrogen flushing; RFN₂: refrigerated under nitrogen (5 °C, 55% RH–1% O₂, 99% N₂); FZ: stored at –25 °C under vacuum.

| | | Day 0 | 4th month | 8th month | 12th month | P ^a _{sc} | P _{st} | P _{sc} × P _{st} |
|----------|------------------|-----------------------------------|------------------|-----------------|-----------------|------------------------------|-----------------|-----------------------------------|
| Delisava | | | | | | | | |
| | | TPC (GAE, g kg ⁻¹) | | | | *** | *** | *** |
| IS | AT | 5.43 ± 0.11a | 4.13 ± 1.27ab | 5.21 ± 0.10a | 4.44 ± 0.26b | | | |
| S | RF | 5.73 ± 0.04bB | 3.81 ± 0.49aA | 5.11 ± 0.09aB | 3.40 ± 0.15aA | | | |
| S | FZ | 5.73 ± 0.04bB | 5.03 ± 0.40abB | 5.60 ± 0.04bC | 3.44 ± 0.07aA | | | |
| S | RV | 5.73 ± 0.04bC | 4.96 ± 0.62abBC | 4.87 ± 0.14aAB | 4.09 ± 0.15bA | | | |
| S | RVN ₂ | 5.73 ± 0.04bC | 5.92 ± 0.17bC | 5.09 ± 0.21aB | 4.33 ± 0.12bA | | | |
| | | TEAC (TE, mmol kg ⁻¹) | | | | *** | *** | *** |
| IS | AT | 38.20 ± 0.81 | 29.01 ± 8.69a | 34.59 ± 1.27 | 29.92 ± 1.25c | | | |
| S | RF | 41.26 ± 1.80C | 26.75 ± 3.70aAB | 32.66 ± 3.54B | 21.86 ± 0.98aA | | | |
| S | FZ | 41.26 ± 1.80B | 36.51 ± 3.58abB | 34.31 ± 4.75B | 22.06 ± 0.15aA | | | |
| S | RV | 41.26 ± 1.80C | 33.81 ± 3.43abB | 32.37 ± 1.17B | 26.60 ± 0.77bA | | | |
| S | RVN ₂ | 41.26 ± 1.80C | 43.01 ± 2.06bC | 36.00 ± 1.54B | 29.40 ± 1.36cA | | | |
| | | RSA (TE, mmol kg ⁻¹) | | | | *** | *** | *** |
| IS | AT | 20.65 ± 0.77a | 17.32 ± 4.61 | 23.32 ± 0.66a | 17.13 ± 1.20b | | | |
| S | RF | 22.53 ± 0.65bC | 17.28 ± 1.69B | 24.60 ± 0.74aC | 13.59 ± 0.54aA | | | |
| S | FZ | 22.53 ± 0.65bB | 21.20 ± 0.74B | 28.41 ± 1.14bC | 13.96 ± 0.34aA | | | |
| S | RV | 22.53 ± 0.65bB | 20.72 ± 1.51B | 22.82 ± 1.06aB | 16.25 ± 0.44bA | | | |
| S | RVN ₂ | 22.53 ± 0.65bB | 23.31 ± 0.43B | 24.03 ± 0.93aB | 17.16 ± 1.14bA | | | |
| TGT | | | | | | | | |
| | | TPC (GAE, g kg ⁻¹) | | | | *** | *** | *** |
| IS | AT | 3.94 ± 0.13B | 3.92 ± 0.75cB | 4.28 ± 0.13bB | 1.83 ± 0.04aA | | | |
| S | RF | 3.94 ± 0.13C | 3.90 ± 0.24cC | 3.39 ± 0.23aB | 1.47 ± 0.04aA | | | |
| S | FZ | 3.94 ± 0.13B | 2.44 ± 0.19abA | 3.04 ± 0.49aA | 3.12 ± 0.29bA | | | |
| S | RV | 3.94 ± 0.13B | 3.17 ± 0.28bcA | 3.34 ± 0.38aAB | 2.73 ± 0.13bA | | | |
| S | RVN ₂ | 3.94 ± 0.13C | 1.58 ± 0.36aA | 2.96 ± 0.08aB | 3.07 ± 0.12bB | | | |
| | | TEAC (TE, mmol kg ⁻¹) | | | | *** | *** | *** |
| IS | AT | 27.61 ± 0.73B | 21.42 ± 7.44bB | 28.67 ± 1.28bB | 11.22 ± 0.53aA | | | |
| S | RF | 27.61 ± 0.73C | 22.25 ± 2.49bB | 22.99 ± 2.04abB | 8.13 ± 1.08aA | | | |
| S | FZ | 27.61 ± 0.73B | 14.66 ± 3.51abA | 20.94 ± 3.96aAB | 20.14 ± 2.56bAB | | | |
| S | RV | 27.61 ± 0.73C | 19.12 ± 2.07abAB | 22.34 ± 2.55abB | 17.31 ± 0.74bA | | | |
| S | RVN ₂ | 27.61 ± 0.73C | 8.46 ± 2.06aA | 19.82 ± 0.51aB | 20.08 ± 1.18bB | | | |
| | | RSA (TE, mmol kg ⁻¹) | | | | *** | *** | *** |
| IS | AT | 17.17 ± 0.11B | 17.36 ± 3.11cB | 22.31 ± 0.40bC | 8.40 ± 0.24bA | | | |
| S | RF | 17.17 ± 0.11B | 17.75 ± 1.61cB | 16.83 ± 0.38aB | 5.93 ± 0.31aA | | | |
| S | FZ | 17.17 ± 0.11B | 11.93 ± 0.82abA | 15.33 ± 2.96aAB | 14.36 ± 1.68cAB | | | |
| S | RV | 17.17 ± 0.11B | 13.94 ± 0.92bcA | 16.90 ± 1.70aB | 12.55 ± 0.51cA | | | |
| S | RVN ₂ | 17.17 ± 0.11C | 7.13 ± 2.00aA | 14.91 ± 0.92aBC | 13.97 ± 0.39cB | | | |

Data were expressed as mean ± SD (n = 3). 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.

GAE: gallic acid equivalent; TE: Trolox equivalent.

^a 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 < 0.001.

processing. The separation was achieved at room temperature (maintained at 22 °C) on a C18 RP Lichrospher 250 × 4.6 mm, 5- μ m (Merck Millipore, Darmstadt, Germany) column that was equipped with a C18 RP Lichrospher 5- μ m guard column (Merck Millipore). The mobile phase was composed of trifluoroacetic acid/ultrapure water (0.1:99.9, v/v) (solvent A) and methanol (solvent B); the flow rate was 0.8 mL min⁻¹, and the injection volume was 20 μ L. The elution program was as follows: 95% A kept in isocratic for 2 min, 80% A in 8 min, 25% A in 55 min kept in isocratic for 5 min, 95% A in 3 min kept in isocratic for 5 min. The PDA spectra were recorded in full-scan mode over a wavelength (λ) range of 200–600 nm, and quantification was performed recording the peak area at a maximum λ (λ_{\max}) of each compound. The calibration curves

were constructed by plotting the peak area ratios of each analyte/internal standard vs. analyte concentration. Identification was achieved by comparing the retention times and spectra with those of authentic standards.

2.7. HS-SPME-GC/qMS analysis

Hexanal was extracted from raw kernels using the headspace (HS) solid-phase micro-extraction technique (SPME) and was analysed by GC-qMS according to a previously reported method (Mexis and Kontominas, 2009). The SPME fibre, a carboxen/polydimethylsiloxane (CAR/PDMS), 75- μ m film thickness (Supelco, Bellafonte, PA, USA), was exposed to the headspace of

Table 2
Total phenolic content (TPC), Trolox equivalent antioxidant capacity (TEAC) and radical scavenging activity (RSA) of the hazelnuts during the second storage test. IS: in-shell hazelnuts; S: shelled hazelnuts (kernels); AT: stored at ambient temperature (60–80% RH); RF: refrigerated at 5 °C and 55% RH; RVN₂ and RV: refrigerated under vacuum with or without preliminary nitrogen flushing; RFN₂: refrigerated under nitrogen (5 °C, 55% RH–1% O₂, 99% N₂); FZ: stored at –25 °C under vacuum.

| | | | Day 0 | 4th month | 8th month | 12th month | P_{sc}^a | P_{st} | $P_{sc} \times P_{st}$ |
|----------|------------------|--|-----------------------------------|-----------------|------------------|-----------------|------------|----------|------------------------|
| Delisava | | | | | | | | | |
| | | | TPC (GAE, g kg ⁻¹) | | | | *** | *** | *** |
| IS | AT | | 9.50 ± 2.41B | 6.03 ± 1.00AB | 8.90 ± 0.86bAB | 5.35 ± 1.23aA | | | |
| S | RF | | 7.14 ± 0.24B | 4.00 ± 1.47A | 10.76 ± 0.14cC | 8.93 ± 0.42bBC | | | |
| S | RFN ₂ | | 7.14 ± 0.24BC | 5.24 ± 0.36A | 8.05 ± 0.16bC | 7.03 ± 0.53abB | | | |
| S | FZ | | 7.14 ± 0.24 | 5.21 ± 1.81 | 5.49 ± 0.45a | 5.68 ± 0.91a | | | |
| S | RV | | 7.14 ± 0.24B | 4.94 ± 0.34A | 9.06 ± 0.57bC | 6.55 ± 0.86aB | | | |
| S | RVN ₂ | | 7.14 ± 0.24AB | 6.21 ± 1.67A | 9.18 ± 0.58bB | 9.15 ± 0.78bB | | | |
| | | | TEAC (TE, mmol kg ⁻¹) | | | | *** | *** | *** |
| IS | AT | | 52.25 ± 6.89B | 39.53 ± 5.44AB | 51.76 ± 1.95bcB | 30.27 ± 11.21A | | | |
| S | RF | | 49.78 ± 1.48B | 20.01 ± 9.39A | 55.20 ± 1.23cB | 46.27 ± 1.43B | | | |
| S | RFN ₂ | | 49.78 ± 1.48B | 35.48 ± 1.38A | 49.15 ± 0.66bB | 37.04 ± 3.45A | | | |
| S | FZ | | 49.78 ± 1.48B | 34.90 ± 11.93AB | 33.58 ± 2.02aAB | 29.20 ± 5.98A | | | |
| S | RV | | 49.78 ± 1.48B | 33.05 ± 2.50A | 52.45 ± 1.86bcB | 36.11 ± 9.37A | | | |
| S | RVN ₂ | | 49.78 ± 1.48AB | 38.39 ± 9.80A | 53.02 ± 1.54bcB | 47.49 ± 3.10AB | | | |
| | | | RSA (TE, mmol kg ⁻¹) | | | | *** | *** | *** |
| IS | AT | | 30.36 ± 4.80B | 24.78 ± 2.11AB | 27.57 ± 0.80bcB | 21.69 ± 2.56aA | | | |
| S | RF | | 27.26 ± 0.55B | 17.28 ± 5.38A | 29.86 ± 0.79cB | 29.64 ± 0.29cB | | | |
| S | RFN ₂ | | 27.26 ± 0.55B | 22.17 ± 0.76A | 26.67 ± 0.27bB | 27.43 ± 0.70bcB | | | |
| S | FZ | | 27.26 ± 0.55 | 21.66 ± 5.52 | 21.20 ± 1.46a | 24.92 ± 1.78ab | | | |
| S | RV | | 27.26 ± 0.55BC | 21.70 ± 1.12A | 28.19 ± 0.89bcC | 24.87 ± 1.68abB | | | |
| S | RVN ₂ | | 27.26 ± 0.55AB | 24.51 ± 3.47A | 28.63 ± 0.62bcAB | 30.12 ± 0.83cB | | | |
| TGT | | | | | | | | | |
| | | | TPC (GAE, g kg ⁻¹) | | | | *** | *** | *** |
| IS | AT | | 3.33 ± 0.17 | 2.89 ± 0.21b | 2.82 ± 0.38ab | 3.14 ± 0.11b | | | |
| S | RF | | 3.33 ± 0.17A | 3.31 ± 0.28bA | 3.80 ± 0.21bcA | 4.98 ± 0.05eB | | | |
| S | RFN ₂ | | 3.33 ± 0.17B | 1.58 ± 0.55aA | 3.35 ± 0.12abcB | 3.71 ± 0.00cB | | | |
| S | FZ | | 3.33 ± 0.17B | 2.38 ± 0.42abA | 2.64 ± 0.40abAB | 3.28 ± 0.12bB | | | |
| S | RV | | 3.33 ± 0.17 | 3.05 ± 0.33b | 2.41 ± 0.79a | 2.60 ± 0.26a | | | |
| S | RVN ₂ | | 3.33 ± 0.17B | 1.44 ± 0.40aA | 4.05 ± .030cC | 4.52 ± 0.05dC | | | |
| | | | TEAC (TE, mmol kg ⁻¹) | | | | ** | ** | *** |
| IS | AT | | 22.64 ± 0.45C | 19.44 ± 1.35bBC | 17.32 ± 2.85abAB | 14.54 ± 1.10bA | | | |
| S | RF | | 22.64 ± 0.45B | 18.65 ± 1.38bA | 24.93 ± 1.46bB | 24.03 ± 0.52eB | | | |
| S | RFN ₂ | | 22.64 ± 0.45B | 9.86 ± 4.27aA | 19.87 ± 1.84abB | 17.80 ± 0.39cB | | | |
| S | FZ | | 22.64 ± 0.45B | 15.14 ± 3.05abA | 15.98 ± 2.89aA | 14.08 ± 1.11bA | | | |
| S | RV | | 22.64 ± 0.45B | 20.56 ± 2.63bB | 14.66 ± 5.87aAB | 11.51 ± 1.40aB | | | |
| S | RVN ₂ | | 22.64 ± 0.45B | 8.94 ± 2.67bA | 25.38 ± 2.47bB | 21.07 ± 0.59dB | | | |
| | | | RSA (TE, mmol kg ⁻¹) | | | | ** | ** | *** |
| IS | AT | | 14.97 ± 0.47 | 12.86 ± 1.00b | 12.48 ± 1.41a | 14.76 ± 0.86ab | | | |
| S | RF | | 14.97 ± 0.47A | 15.22 ± 1.19bAB | 17.28 ± 1.44cB | 21.68 ± 0.33dC | | | |
| S | RFN ₂ | | 14.97 ± 0.47B | 5.31 ± 3.32aA | 15.31 ± 0.63abcB | 17.22 ± 0.14cB | | | |
| S | FZ | | 14.97 ± 0.47B | 10.23 ± 2.45abA | 12.67 ± 1.70abAB | 16.01 ± 0.02bcB | | | |
| S | RV | | 14.97 ± 0.47 | 13.72 ± 1.89b | 11.01 ± 3.08a | 13.34 ± 1.08a | | | |
| S | RVN ₂ | | 14.97 ± 0.47AB | 4.46 ± 2.00aA | 17.17 ± 0.60bcAB | 20.15 ± 0.60dB | | | |

Data were expressed as mean ± SD ($n = 3$). 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 $P < 0.05$.

GAE: gallic acid equivalent; TE: Trolox equivalent.

^a 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 $P < 0.001$.

the sample using an SPME autosampler (PAL System, Combi PAL, Zwingen, Switzerland). One millilitre of ultrapure water, 0.1 g of finely powdered sample, and 10 μL of 4-methyl-2-pentanone (internal standard, 10.84 mg L^{-1}) were placed in a 10-mL screw-cap glass vial fitted with silicone-PTFE septum (Supelco, Milan, Italy). The sample vials, stirred at 26.18 rad s^{-1} , were thermostated at 60 °C for 10 min; then, the fibre was exposed to the headspace for a sampling period of 10 min (Pastorelli et al., 2006). The fibre was then removed and immediately inserted into the GC-qMS injector in splitless mode at 300 °C for 1 min.

GC/qMS analysis was performed with a Shimadzu GC-2010 gas chromatograph equipped with a Shimadzu QP-2010 Plus quadrupole mass spectrometer (Shimadzu Corporation, Kyoto, Japan) and a DB-WAXETR capillary column (30 m \times 0.25 mm, 0.25 μm film thickness, J&W Scientific Inc., Folsom, CA, USA). The temperature program started at 45 °C and was maintained for 2 min, then increased at a rate of 5 °C min^{-1} –50 °C (held for 1 min), then increased at a rate of 8 °C min^{-1} –170 °C, and finally, increased at a rate of 18 °C min^{-1} –230 °C for 8 min. The carrier gas (He) flow rate was 1 mL min^{-1} . The injection port temperature was 300 °C, the ion source temperature was 240 °C, and the interface temperature was 230 °C. The detection was carried out by electron impact mass spectrometry in total ion current (TIC) mode using an ionization energy of 70 eV. The mass acquisition range was m/z 30–330. Peak identification of hexanal was performed by comparison of the retention time and mass spectra of eluting compound to those of the pure standard. The amount of hexanal was performed as a normalized area by the peak area of a selected quantifier ion of the volatile metabolite ($m/z=56$) with respect to the peak area of the selected quantifier ion ($m/z=85$) of the internal standard (4-methyl-2-pentanone). For each run, the precision of the method was performed, using both a standard solution of hexanal and internal standard both on the performance of the fibres. All analyses were performed in triplicate.

2.8. Sensory analysis

The sensory evaluation of the samples was performed with a pairing test that allows determination of the sensory “proximity” of a set of products (Touraille, 1990). In this case, the aim of the test was to determine whether different storage techniques led to different and significantly recognizable products. A group of 20 trained panellists (15 male, five female, 25–35 years old) was used. Each panellist was simultaneously presented with two sets of samples. Each set was made up of all of the products that were coded with different three-digit numbers, and the panellists had to match them. Hazelnut samples were furnished in white plastic cups containing 6–7 raw kernels. Water was provided for palate cleaning. The testing was carried out in a sensory laboratory that was designed in accordance with ISO 8589: 1988.

2.9. Statistical analyses

Statistical analyses were performed with SPSS software (version 18.0 for Windows, SPSS Inc., Chicago, Illinois). For the chemical data, two-way analysis of variance (ANOVA) was performed using all factors and their interactions. Intra-storage condition and intra-storage time differences ($P < 0.05$) were analysed using one-way ANOVA (single factor was storage time or storage condition), followed by Tukey's HSD post hoc comparison test at a fixed level of $\alpha = 0.05$. For the sensory analysis, the χ^2 test was used ($\alpha = 0.05$).

3. Results

3.1. Total phenolic content and in vitro antioxidant capacity assays

The TPC and AC values of hazelnuts (two years, two cultivars) as a function of storage condition and storage time are reported in Table 1 and Table 2. For each cultivar, the effects of storage condition, storage time and their interaction were all significant. Delisava exhibited higher values than TGT ($P < 0.001$) beginning from day 0.

Overall, during the first year of storage, the TPC and AC levels decreased significantly between 0 and 12 months in both cultivars; however, after an initial decrease at 4 months, the values increased at 8 months to levels often near those from the beginning. The ranking of the storage conditions in order of increasing losses of TPC between the references and the samples at the 12th month of storage was AT < RVN₂ < RV < FZ < RF in Delisava and FZ < RVN₂ < RV < AT < RF in TGT (with 18.2, 24.4, 28.7, 40.1, 40.8 and 20.9, 22.0, 30.8, 53.5, 62.8 as the corresponding loss percentages). During storage, decreases in both TEAC and RSA values followed a pattern similar to that of TPC. Data from the second year of analyses highlighted a significant overall decrease of TPC and AC after 4 months of storage for both cultivars, followed by a significant ($P < 0.001$) increase at the 8th month. At the 12th month of storage, the mean value of each parameter increased or decreased significantly in different ways; in Delisava extracts, TPC and AC levels were lower or near those of the references. TGT had the highest recorded values of TPC and RSA, while TEAC was near the value that was assessed at the 8th month. The changes of TPC between the references and 12-month-stored samples were sometimes positive. In Delisava extracts, the increase was 28.2 and 25.1% in RVN₂ and RF, respectively; the decrease was 1.5, 8.3, 20.4 and 43.7% in RN₂, RV, FZ and AT, respectively. In TGT extracts, increases of 49.5, 35.7 and 11.4% were recorded in RF, RVN₂ and RN₂, respectively; a decrease of 1.5, 5.7 and 21.9% was reported in FZ, AT and RV, respectively. Similarly to the first year, the TEAC and RSA parameters followed a pattern that was analogous with that of TPC. In particular, after 12 months, the highest values of TEAC and RSA were observed for Delisava in RVN₂, RF and RN₂, and for TGT,

Table 3

Probabilities of the effects of storage condition (P_{sc}), storage time (P_{st}) and their interactions on total phenol content (TPC), Trolox equivalent antioxidant capacity (TEAC), radical scavenging activity (RSA), total amount of phenolics, and hexanal content assessed in hazelnut kernels stored under various conditions (two-year data as a whole).

| | TPC | | TEAC | | RSA | | Total phenolics | | Hexanal | |
|------------------------|----------|-----|----------|-----|----------|-----|-----------------|-----|----------|-----|
| | Delisava | TGT | Delisava | TGT | Delisava | TGT | Delisava | TGT | Delisava | TGT |
| P_{sc} | NS | NS | * | NS | NS | NS | NS | NS | *** | NS |
| P_{st} | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| $P_{sc} \times P_{st}$ | NS | *** | NS | *** | NS | *** | NS | ** | *** | *** |

NS not significant

* Significant at $P < 0.05$.

** Significant at $P < 0.01$.

*** Significant at $P < 0.001$.

the highest TEAC values were observed in RF, RVN₂ and RFN₂ storage methods.

Overall, the values determined for all storage conditions in the first year of analyses were not significantly different in either Delisava or TGT extracts. In the second year, significant differences ($P < 0.05$) were highlighted in TGT extracts only for TPC and ranged between 2.85 and 3.85 GAE g kg⁻¹ in RV and RF, respectively.

Two-way ANOVA of the two-year data as a whole (Table 3) showed a significant effect ($P < 0.001$) of storage time for TPC and AC in both Delisava and TGT cultivars; the storage condition effect was not significant. A significant interaction effect was observed ($P < 0.001$) only for TGT samples.

3.2. HPLC–DAD analysis

The HPLC analysis of the extracts highlighted the presence of 11 compounds; eight compounds were identified by comparison with analytical standards (Table 4). The identified compounds can be classified into four groups: benzoic acids (gallic acid), flavanols ((–)-epigallocatechin, (–)-epigallocatechin 3-gallate, (+)-gallo catechin 3-gallate, (–)-epicatechin 3-gallate), procyanidins (procyanidin B1 and procyanidin B2), and flavonols (quercitrin). The compound at Rt 16.62 min and λ_{\max} 277 nm was tentatively identified as a B-type procyanidin dimer. In addition, two unidentified compounds were also detected at Rt 9.8 and 13.1 min, and λ_{\max} 264 and 297, respectively. Epigallocatechin 3-gallate was detected only in TGT samples. The phenolic compound profiles showed differences between cultivars and years (Supplemental Table 1 and 2). In the first year of analysis, the most abundant phenolic compound in Delisava extracts was procyanidin B1, followed by epigallocatechin, which showed mean values of 19.62 and 17.08 mg kg⁻¹, respectively. In the TGT extracts, the order of the same compounds was inverted, with epigallocatechin as the most abundant compound (mean value 13.39 mg kg⁻¹) followed by procyanidin B1 (mean value 9.52 mg kg⁻¹). In the second year of analysis, epigallocatechin was found to be the most abundant of both Delisava and TGT extracts (mean value 11.43 and 11.57 mg kg⁻¹, respectively). For both cultivars and years, the most stable compound was gallic acid, which ranged between 7.70 and 8.58 mg kg⁻¹ overall. Two-way ANOVAs revealed the significant effects of storage condition, storage time and their interaction for every quantified compound (Table 5).

The total amount of quantified phenolic compounds is reported in Table 6. In the first year of analyses, Delisava extracts were characterized by a higher amount of phenolics than TGT. A significant effect of storage time was detected in both cultivars, with an increase in the average amount of phenolics after four months of storage. In Delisava, the increase continued until the eighth month, and then the amount decreased; at the 12th month (mean value 112.52 mg kg⁻¹), the amount of phenolics was higher than that at the beginning (mean value 96.78 mg kg⁻¹). In TGT, the

phenolic content decreased at the 8th month and then remained almost unchanged (mean value at the 12th month 70.49 mg kg⁻¹) and comparable the initial content (mean value 71.15 mg kg⁻¹). The effect of storage conditions and the interaction effect of storage conditions and storage time were also found to be significant. In the second year of analysis, the amount of phenolics in Delisava and TGT extracts were quite similar. Two-way ANOVA showed a significant effect of both storage conditions and storage time; their interaction was also significant. The amount of phenolics in Delisava increased between 0 and 4 months and then decreased. In TGT, the amount of phenolics was higher at the beginning and then decreased progressively during the next sampling times.

The two-year overall data two-way ANOVA (Table 3) showed that the effect of storage conditions was not significant, while the storage time effect was significant for both cultivars, with $P < 0.05$ and 0.01 in Delisava and TGT, respectively. The interaction effect was significant only for TGT ($P < 0.01$).

3.3. HS–SPME–GC/MS analysis

The changes in hexanal content (normalized area) are shown in Fig. 1A–D. For each cultivar, the effects of storage conditions, storage time and their interaction were all significant, with $P < 0.001$ (the only exception was the effect of storage time for TGT that was analysed in the second year, with $P < 0.01$). The differences between cultivars were significant; Delisava was characterized by the highest hexanal values. The formation of secondary oxidation products that were most likely a result of the availability of previously shelled Delisava hazelnuts was evident. Indeed, the hexanal mean values assessed at day 0 for in-shell and shelled references were 0.077 and 0.284 normalized area, respectively.

During the first year of storage, the level of hexanal increased significantly between 0 and 4 months in both cultivars. The hexanal content generally decreased at the 8th month to values often near the initial values and then remained almost unchanged. A different behaviour was reported for Delisava stored using AT and RF methods and TGT stored using the RF method, with an increase in the hexanal contents until 12 months of monitored storage. After one year of storage, hazelnuts packaged under vacuum conditions (FZ, RV and RN₂) had the lowest hexanal contents (mean value 0.246 and 0.061 normalized area in Delisava and TGT, respectively), while RF samples showed a 15- and fourfold increase in Delisava and TGT, respectively. Data from the second year confirmed the differences between cultivars and the highest hexanal content of Delisava. Changes in hexanal content in Delisava samples were similar to those assessed in the first year, but in this case, the hexanal values of hazelnuts stored with FR, RV and RVN₂ methods were almost unchanged in the first sampling time and then increased at the 8th month. The introduction of the new storage modality (RN₂) allowed an efficient control of the lipid oxidation

Table 4
Retention time (R_t), detection wavelength (λ_{\max}), calibration curve, investigated linear range, determination coefficient (R²), linear range, LOD and LOQ of the phenolic compound standards.

| | R _t (min) | λ_{\max} (nm) | Calibration curve | R ² | Linear range (mg L ⁻¹) | LOD (mg L ⁻¹) | LOQ (mg L ⁻¹) |
|--------------------------------|----------------------|-----------------------|----------------------|----------------|------------------------------------|---------------------------|---------------------------|
| Gallic acid | 10.7 | 270 | y = 0.7528x - 0.4147 | 1.0000 | 1–10 | 0.007 | 0.050 |
| Procyanidin B1 | 17.4 | 277 | y = 0.1379x - 0.0997 | 0.9889 | 2–30 | 0.515 | 1.694 |
| (–)-Epigallocatechin | 20.0 | 280 | y = 0.8515x - 0.7002 | 0.9983 | 10–100 | 0.075 | 0.250 |
| Procyanidin B2 | 21.6 | 277 | y = 0.2330x - 0.1352 | 0.9998 | 2–10 | 0.100 | 0.200 |
| (–)-Epigallocatechin 3-gallate | 24.9 | 280 | y = 0.0849x - 0.0499 | 0.9995 | 0.5–2 | 0.144 | 0.500 |
| (+)-Gallocatechin 3-gallate | 28.1 | 280 | y = 1.2287x - 0.6064 | 0.9952 | 5–10 | 0.008 | 0.026 |
| (–)-Epicatechin 3-gallate | 30.7 | 280 | y = 0.1303x - 0.0396 | 0.9984 | 0.5–2 | 0.150 | 0.500 |
| Phloridzin (IS) | 40.4 | 283 | y = 0.6625x - 0.3226 | 1.0000 | 2–10 | 0.006 | 0.020 |
| Quercitrin | 44.7 | 350 | y = 0.2861x - 0.1677 | 0.9995 | 2–10 | 0.093 | 0.356 |

IS: internal standard.

Table 5Probabilities of the effects of storage condition (P_{sc}), storage time (P_{st}) and their interactions on phenolic compounds detected in two-year stored hazelnuts.

| 1th year | Phenolic compound | P_{sc}^1 | P_{st} | $P_{sc} \times P_{st}$ | 2nd year | Phenolic compound | P_{sc} | P_{st} | $P_{sc} \times P_{st}$ |
|------------|--------------------------------|-----------------------|----------|------------------------|----------|--------------------------------|-----------------------|----------|------------------------|
| Delisava | Non identified molecule n. 1 | ** | *** | *** | Delisava | Non identified molecule n. 1 | *** | *** | *** |
| | Gallic acid | *** | *** | *** | | Gallic acid | *** | *** | *** |
| | Non identified molecule n. 2 | NS | *** | *** | | Non identified molecule n. 2 | *** | *** | *** |
| | B-type procyanidin dimer | *** | *** | *** | | B-type procyanidin dimers | *** | *** | *** |
| | Procyanidin B1 | *** | *** | *** | | Procyanidin B1 | *** | *** | *** |
| | (-)-Epigallocatechin | NS | ** | NS | | (-)-Epigallocatechin | NS | *** | *** |
| | Procyanidin B2 | ** | *** | *** | | Procyanidin B2 | ** | *** | *** |
| | (-)-Epigallocatechin 3-gallate | ** | *** | *** | | (-)-Epigallocatechin 3-gallate | Occasionally detected | *** | *** |
| | (+)-Gallocatechin 3-gallate | NS | *** | *** | | (+)-Gallocatechin 3-gallate | *** | *** | *** |
| | Quercitrin | * | *** | *** | | Quercitrin | *** | *** | *** |
| TGT | Non identified molecule n. 1 | *** | *** | ** | TGT | Non identified molecule n. 1 | NS | *** | *** |
| | Gallic acid | *** | *** | *** | | Gallic acid | ** | *** | *** |
| | Non identified molecule n. 2 | ** | NS | *** | | Non identified molecule n. 2 | *** | *** | *** |
| | B-type procyanidin dimer | *** | *** | *** | | B-type procyanidin dimers | *** | *** | *** |
| | Procyanidin B1 | *** | *** | *** | | Procyanidin B1 | * | *** | *** |
| | (-)-Epigallocatechin | * | *** | *** | | (-)-Epigallocatechin | ** | *** | *** |
| | Procyanidin B2 | NS | *** | NS | | Procyanidin B2 | ** | *** | *** |
| | (-)-Epigallocatechin 3-gallate | Occasionally detected | *** | *** | | (-)-Epigallocatechin 3-gallate | Not detected | *** | *** |
| | (+)-Gallocatechin 3-gallate | NS | *** | *** | | (+)-Gallocatechin 3-gallate | ** | *** | *** |
| | (-)-Epicatechin 3-gallate | *** | *** | *** | | (-)-Epicatechin 3-gallate | *** | *** | *** |
| Quercitrin | *** | *** | *** | Quercitrin | *** | *** | *** | | |

NS: not significant.

* Significant at $P < 0.05$.** Significant at $P < 0.01$.*** Significant at $P < 0.001$.

that resulted in small changes in hexanal content during storage (0.261 and 0.258 normalized area in reference and 12 months stored samples, respectively). Instead, the behaviour of the TGT samples was changed. With the exception of the AT samples, the highest values of hexanal were detected at the 12th month for every storage condition. Among low temperature storage conditions, the best performance was that of RN₂.

Two-way ANOVA of the two-year data as a whole (Table 3) showed a significant effect ($P < 0.001$) of storage time in both Delisava and TGT cultivars, while a significant effect of storage condition was observed only in Delisava; the interaction effect was significant ($P < 0.001$) in both cultivars. Overall, higher values of hexanal were detected at the 12th month and in RF samples.

3.4. Sensory analysis

The results of sensory evaluations are reported in Table 7. For both cultivars and years of analysis, the number of correct matches was low. For Delisava, the correct matches were detected at the 8th month only for the FZ samples in both years. This response was confirmed at the 12th month of the second year. For TGT, the correct matches were detected for the AT and FZ samples in both years with some differences with respect to storage time. In the second year, the AT and FZ samples were correctly matched already at the 4th month. The correct responses were also detected at the 8th month (for AT samples only) and at the 12th month. The RV and RVN₂ samples were correctly recognized, but only in the first year of observations.

4. Discussion

4.1. Total phenolic and antioxidant capacity

The results showed that changes in TEAC and RSA values followed a pattern similar to that of TPC. As reported by Christopoulos and Tsantili (2011), cultivar, storage time and storage condition affected AC similarly to TPC; however, some differences between either TEAC or RSA and TPC were observed. Delisava exhibited higher AC and TPC values than TGT during the

entire storage period and for both examined years. Storage condition trends did not follow similar developments over time. In addition, at each sampling time, the differences among storage conditions were often significant but did not define a stable pattern. The observed two-year data highlighted the decrease in TPC and AC after 12 months of storage and a significant increase in the same parameters at the 8th month with respect to the beginning. In a previous study (Ghirardello et al., 2013) with hazelnuts that were stored for 12 months, an increase in TPC and RSA was recorded between the 8th and 12th months of storage; however, in this case, at the end of the storage period, both parameters decreased with respect to the beginning. With regards to this phenomenon, Bolling et al. (2010) suggested that a dynamic process affected the changes in flavonoid and phenolic acid contents by an increase in polyphenol extractability, degradation of polymeric polyphenols and, consequently, an increase of soluble phenolics or polyphenol synthesis after harvest. In contrast to the hypothesis that low temperature and modified atmosphere could effectively prevent the decrease in the phenolic content and antioxidant capacity in the long-term storage of nuts, the mean values of all assessed parameters were not able to significantly discriminate among the storage conditions; however, the effect of storage time was significant.

4.2. Phenolic compounds profile

Numerous data are available on the phenolic composition of hazelnuts; however, investigations are mainly focused on differences resulting from cultivar, origin, and variety of products (hazelnut skin, hard shell, tree leaf, green leafy cover and kernel) including fresh, raw and roasted kernels. No data are available about the effect of different storage conditions on changes in the phenolic compound profiles in long-term stored hazelnuts. According to the assessed TPC, Delisava was characterized by a higher total amount of phenolics than TGT; however, the evolution of these two parameters over time did not follow the same pattern. Storage affected the concentration of each phenolic compound to different degree, and it was very difficult to compare the storage methods. Differences could be explained by the different rates of

Table 6

Total amount of phenolics of the hazelnuts during the two years of testing. IS: in-shell hazelnuts; S: shelled hazelnuts (kernels); AT: stored at ambient temperature (60–80% RH); RF: refrigerated at 5 °C and 55% RH; RVN₂ and RV: refrigerated under vacuum with or without preliminary nitrogen flushing; RFN₂: refrigerated under nitrogen (5 °C, 55% RH–1% O₂, 99% N₂); FZ: stored at –25 °C under vacuum.

| | Day 0 | 4th month | 8th month | 12th month | <i>P</i> _{sc} ^a | <i>P</i> _{st} | <i>P</i> _{sc} × <i>P</i> _{st} |
|-------------------|--|----------------|-------------------|------------------|-------------------------------------|------------------------|---|
| Delisava—1th year | Total amount of phenolics (mg kg ⁻¹) | | | | *** | *** | *** |
| IS | AT | 107.03 ± 0.93A | 115.41 ± 12.36aAB | 130.92 ± 0.06dC | 124.22 ± 3.85cBC | | |
| S | RF | 94.22 ± 7.85A | 104.70 ± 8.65aAB | 124.37 ± 1.25cC | 106.09 ± 0.29aB | | |
| S | FZ | 94.22 ± 7.85A | 120.68 ± 5.48aB | 141.33 ± 3.98eC | 103.14 ± 2.18aA | | |
| S | RV | 94.22 ± 7.85A | 120.94 ± 9.33aB | 102.84 ± 0.81aA | 115.71 ± 5.17bB | | |
| S | RVN ₂ | 94.22 ± 7.85A | 137.27 ± 3.25bC | 114.40 ± 4.22bB | 113.46 ± 4.38bB | | |
| TGT—1th year | Total amount of phenolics (mg kg ⁻¹) | | | | ** | *** | *** |
| IS | AT | 71.15 ± 2.45B | 80.74 ± 5.40abC | 75.23 ± 4.96bBC | 61.47 ± 1.22aA | | |
| S | RF | 71.15 ± 2.45B | 81.42 ± 3.01abC | 75.24 ± 3.25bAB | 66.79 ± 2.04bA | | |
| S | FZ | 71.15 ± 2.45A | 85.53 ± 3.35bB | 67.65 ± 2.17aA | 71.07 ± 2.25cA | | |
| S | RV | 71.15 ± 2.45A | 81.61 ± 3.37abB | 71.52 ± 1.32abA | 80.19 ± 1.88dB | | |
| S | RVN ₂ | 71.15 ± 2.45AB | 75.04 ± 4.76aB | 66.34 ± 3.74aA | 72.95 ± 1.86cAB | | |
| Delisava—2nd year | Total amount of phenolics (mg kg ⁻¹) | | | | *** | *** | *** |
| IS | AT | 73.02 ± 1.51C | 76.96 ± 2.81aC | 58.57 ± 4.12aA | 68.08 ± 0.32B | | |
| S | RF | 72.07 ± 1.51C | 80.79 ± 2.63aD | 62.38 ± 1.4abA | 67.06 ± 2.15B | | |
| S | RFN ₂ | 72.07 ± 1.51C | 78.19 ± 0.20aD | 60.30 ± 0.82aA | 64.34 ± 2.60B | | |
| S | FZ | 72.07 ± 1.51A | 82.28 ± 4.47aB | 67.33 ± 0.87bA | 66.55 ± 4.30A | | |
| S | RV | 72.07 ± 1.51B | 81.14 ± 3.84aC | 62.41 ± 2.57abA | 64.57 ± 2.78A | | |
| S | RVN ₂ | 72.07 ± 1.51A | 91.98 ± 6.09bB | 66.92 ± 3.79bA | 64.89 ± 4.36A | | |
| TGT—2nd year | Total amount of phenolics (mg kg ⁻¹) | | | | ** | *** | *** |
| IS | AT | 89.82 ± 1.78C | 83.45 ± 2.87bcBC | 73.19 ± 9.79abAB | 64.75 ± 3.90aA | | |
| S | RF | 89.82 ± 1.78B | 79.76 ± 0.95bA | 74.66 ± 2.62abA | 75.54 ± 4.87bA | | |
| S | RFN ₂ | 89.82 ± 1.78B | 86.15 ± 0.68cB | 62.48 ± 1.72aA | 60.73 ± 3.04aA | | |
| S | FZ | 89.82 ± 1.78C | 80.69 ± 1.89bcB | 69.62 ± 6.58abA | 74.47 ± 2.33bAB | | |
| S | RV | 89.82 ± 1.78C | 86.84 ± 2.80cC | 65.98 ± 2.97aA | 78.56 ± 2.40bB | | |
| S | RVN ₂ | 89.82 ± 1.78C | 66.23 ± 6.18aA | 77.93 ± 3.26bB | 74.04 ± 2.80bB | | |

Data were expressed as mean ± SD (*n* = 3). 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.

^a 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.

degradation and/or synthesis of each phenolic compound. Despite different changes in absolute total phenolic amount, the overall relative profiles (individual content/sum) were similar for the majority of compounds in all storage conditions and for both harvest years. Therefore, the hazelnuts that were subjected to different storage techniques for up to 12 months had similar phenolic profiles. The total amount and profile of phenolics, affected by dynamic metabolic processes, were ineffective to identify the oxidative state of the hazelnuts during storage. Nevertheless, the contribution of phenolics as antioxidants in long-term stored hazelnuts was confirmed.

4.3. Hexanal content

Hexanal, one of the major secondary products that is formed during the oxidation of linoleic or other ω-6 fatty acids in lipid-containing foods and its concentration, is directly related to the development of oxidative off-flavour (tallowy and green leafy flavour). This compound has a low retronasal odour threshold in oil (75 μg kg⁻¹) (Aparicio and Luna, 2002; Belitz et al., 2009) and, together with propanal, is considered an indicator of the stability of food lipids (Shahidi and Wanasundara, 2008). In hazelnuts, hexanal was found to be useful for detecting the first stage in the oxidative process; nevertheless, data on the hexanal content of hazelnuts during storage are still scarce.

The initial hexanal content of fresh raw hazelnuts are generally rather low and increases as a result of ageing and processing. The obtained results show that barriers against oxygen and low temperature additively prevented the lipid oxidation in long-term stored hazelnuts. The control of the external activators of the lipid

autoxidation, namely high temperatures and O₂, played a key role in preventing lipid degradation. Low temperatures can act by retarding the metabolic reaction involved (the initiation of the free radical chain reaction), while reduced availability of O₂ by delaying the formation of the peroxy-fatty acid radicals. The partial effectiveness of shells as an O₂ barrier is well documented, particularly for TGT. For Delisava, this effect was also evident at the beginning, when in shelled hazelnuts a small but measurable degree of oxidation was already in progress. On the contrary, the comparison of the data in Fig. 1 leads to the conclusion that as the O₂ barrier decreased (RF method), the efficacy of low temperature also decreased. In agreement with the results of De Santis et al. (2009), in most cases, the vacuum-stored samples showed the best protection from lipid oxidation. In the second year of analysis, the introduction of a new storage modality (RFN₂ method) confirmed the tendency, with a better performance of refrigeration in the presence of a very low O₂ concentration.

The behaviour of the TGT cultivar during two years of observations was less uniform than that of Delisava, but it is interesting to note that TGT was characterized by a much lower hexanal content; therefore, the ranges of variability for the two cultivars were very different. This was not surprising; indeed, it is reported that the concentration of hexanal in nuts is affected by numerous factors including kernel maturity, fat content, and variety (Lee et al., 2014).

4.4. Sensory evaluation

Based on the results reported in Table 7, there was no significant evidence that the storage conditions, even if prolonged, produced

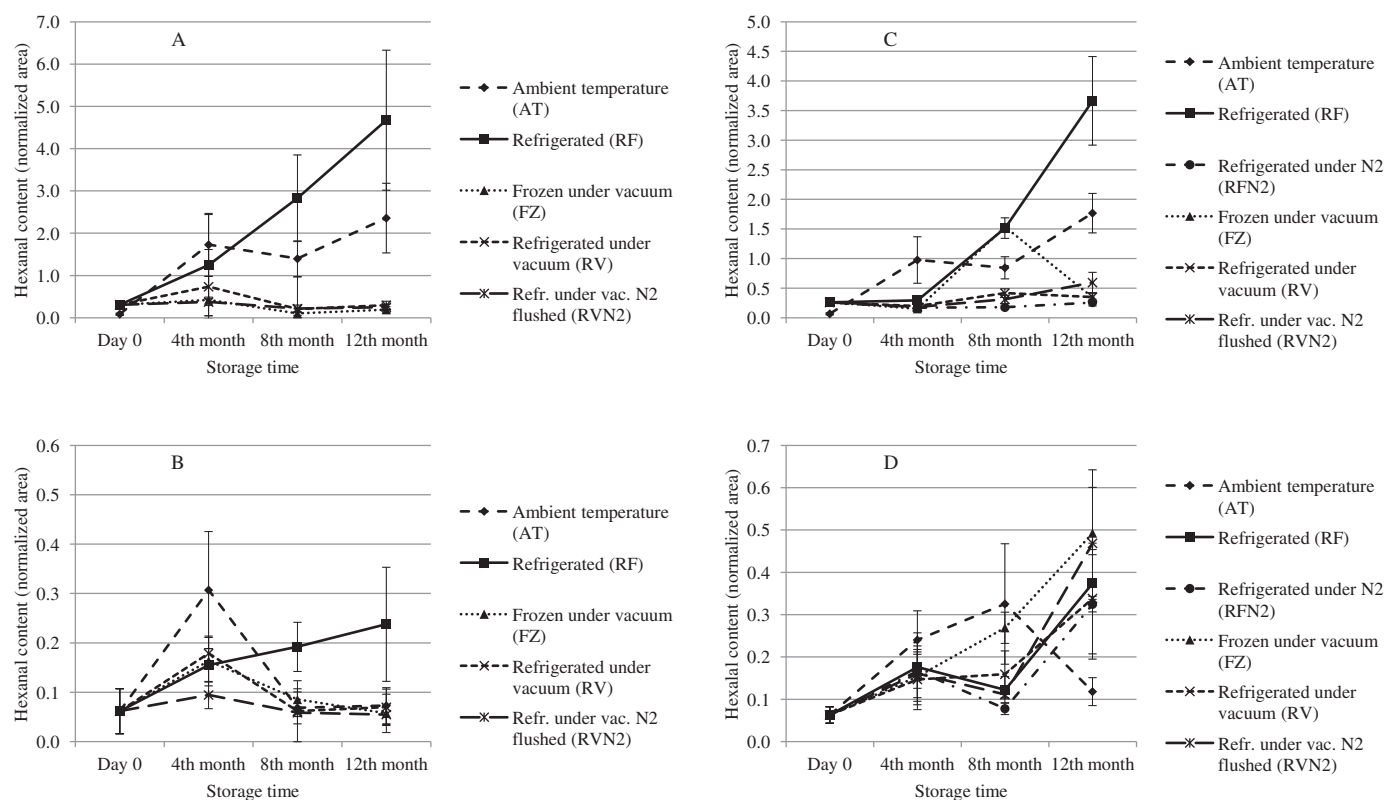


Fig. 1. Change in the hexanal content (mean value; $n=3$) of hazelnuts during the first (A–Delisava; B–TGT) and second (C–Delisava; D–TGT) years of storage.

Table 7

Results of χ^2 test performed on sensory analysis results obtained at four, eight and 12 months of storage for the two years of testing. IS: in-shell hazelnuts; S: shelled hazelnuts (kernels); AT: stored at ambient temperature (60–80% RH); RF: refrigerated at 5 °C and 55% RH; RVN₂ and RV: refrigerated under vacuum with or without preliminary nitrogen flushing; RFN₂: refrigerated under nitrogen (5 °C, 55% RH–1% O₂, 99% N₂); FZ: stored at –25 °C under vacuum.

| | 4th month | | 8th month | | 12th month | |
|--------------------|-----------|----------|-----------|----------|------------|----------|
| | 1st year | 2nd year | 1st year | 2nd year | 1st year | 2nd year |
| Delisava | | | | | | |
| IS AT | – | – | – | – | – | + |
| S RF | – | – | – | – | – | – |
| S RFN ₂ | – | – | – | – | – | – |
| S FZ | – | – | + | + | – | + |
| S RV | – | – | + | – | – | – |
| S RVN ₂ | – | – | – | – | – | – |
| TGT | | | | | | |
| IS AT | – | + | + | + | – | + |
| S RF | – | – | + | – | – | – |
| S RFN ₂ | – | – | – | – | – | – |
| S FZ | – | + | + | – | + | + |
| S RV | – | – | + | – | + | – |
| S RVN ₂ | – | – | + | – | + | – |

+: indicates correct match at $P < 0.05$.

–: indicates no correct match at $P < 0.05$.

sensory changes. Only for the FZ and AT methods were correct matches possible, especially with long-term storage. Overall, contrary to what is observed about hexanal content, TGT seemed to be more affected by storage conditions. Although attempts to relate sensory data to volatile compounds have been frequently reported in literature, often those associations have not been shown to be conclusive. Multiple volatiles are responsible for a flavour sensation and although it is possible to pair some volatile

compounds with some aroma or flavour sensations, this does not always happen (Chambers and Koppel, 2013).

5. Conclusions

All assessed parameters highlighted differences between the cultivars; furthermore, these seemed to be more relevant than that resulting from harvest year. Delisava was characterized by the highest levels of TPC and AC; nevertheless, it seemed to be less stable in terms of lipid oxidation and it was distinguished by the highest hexanal content. The hexanal content was confirmed to be a good indicator to monitor the oxidative state of hazelnut lipids.

The results clearly showed good preservation of raw hazelnut kernels under vacuum with or without nitrogen. The absence of oxygen seemed to be more relevant than low temperature to reduce lipid oxidation, and its positive effect was confirmed.

The hazelnut phenolic profiles did not seem to be affected by storage techniques, and all assessed parameters were generally more affected by storage time than by storage conditions. It was not possible to discriminate the storage conditions by sensory analysis, and there were no correlations between sensory results and fat oxidation.

Cultivar and harvest year seemed to be involved in changing the compositional characteristics of hazelnuts during storage. Initial levels of antioxidants and markers of lipid oxidation along with their changes during storage should be considered for the choice of the cultivar and its ideal storage condition.

Other studies on storage conditions with an emphasis on packaging as a barrier to O₂ are necessary because these materials can be used to prevent lipid oxidation of hazelnuts and as an alternative to more expensive storage at low temperature. The use of eco-friendly food packaging as a barrier against oxygen, starting from the nut shells, could be taken into account for a sustainable food production system.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at <http://dx.doi.org/10.1016/j.postharvbio.2015.09.039>.

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