ISSN 1330-9862
(FTB-2878)

original scientific paper

The Influence of Atmosphere on the Oxidation of Ground Walnut During Storage at 20 °C

Rajko Vidrih[1], Janez Hribar[1], Anita Solar[2] and Emil Zlatić[1]*

[1]Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia

[2]Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia

Received: July 5, 2011
Accepted: December 23, 2011

Summary

The aim of this study is to determine the impact of atmosphere on the oxidation of ground walnut during storage at 20 °C. Seven varieties of walnut (*Juglans regia* L.) were ground and stored under O_2 or N_2 atmospheres in hermetically sealed vials for 10 months at room temperature. Antioxidative potential, total phenolic content, fatty acid composition, and oxidative degradation products were determined after 10 months of storage. Cultivar, atmosphere and cultivar×atmosphere interactions significantly influenced the antioxidative potential. Cultivar and atmosphere significantly influenced the content of total polyphenols, with more polyphenols found in walnut stored in the N_2 atmosphere. The mass fraction of unsaturated linolenic acid tended to decrease during storage under the O_2 atmosphere; statistically significant differences were only found between individual varieties. The O_2 atmosphere also resulted in an increase in the synthesis of oxidative degradation products. Among the degradation products, hexanal was the most abundant volatile compound, followed by 1-octen-3-ol, octanal, as well as the mixture of 2-octenal and 1-octen-3-ol. In general, higher concentrations of these degradation products were found in walnut stored under the O_2 atmosphere, although these differences were statistically significant only between individual varieties for some compounds.

Key words: walnut, fatty acids, antioxidative potential, oxidation degradation products

Introduction

Since ancient times, the English or Persian walnut (*Juglans regia* L.) has been considered to be a food with health-promoting attributes. Walnuts have often been thought of as 'brain food', not only because of the wrinkled brain-like appearance of their shells, but also because of their high concentrations of ω-3 fatty acids. Of all of the tree nuts, the lipid fraction of walnuts contains the highest levels of polyunsaturated fatty acids (PUFAs). Walnuts are also a rich source of bioactive compounds: they contain polyphenols, dietary compounds, tocopherols, folic acid, minerals, and manganese and copper (*1,2*). Fatty acids in walnuts can provide benefits for patients with coronary artery disease (*3–5*). Indeed, the walnut is unique among the nuts due to the presence of antioxidants (*6*), and it is classified as second among foodstuffs with high amounts of antioxidants (*7,8*). Different compounds with antioxidative potential were isolated from walnut kernels (*9*) or leaves (*10*).

Several studies have reported values of the lipid content of walnut kernels that range from 62 to 70 % of the total fatty acids (*2,11*). Walnut is predominantly a rich source of PUFAs; linoleic acid accounts for 49 to 72 %, and linolenic acid for 8 to 25 % of all of the fatty acids (*12*).

*Corresponding author; E-mail: rajko.vidrih@bf.uni-lj.si

The main quality concern related to walnuts is the development of off-flavours due to lipid oxidation, and the consequent formation of oxidative degradation products (*13,14*). Fungal growth and the consequent production of aflatoxins is another food-safety issue worldwide. All nuts are susceptible to aflatoxin contamination, and rigorous limits to aflatoxin B1 were set in 1998.

Numerous volatiles are involved in the off-flavour in walnuts. Aldehydes and ketones are important contributors to the typical walnut aroma (*15,16*). 1-Pentanol, 1-hexanol and hexanal are present at the highest levels, and they are considered to be the degradation products of the decomposition of linoleic acid. Crowe *et al.* (*17*) showed increased levels of hexanal when the sensory qualities of walnuts deteriorate. Hexanal has been shown to greatly increase in oxidised walnuts, and is an important marker of oxidative flavour (*18*). With over 100 compounds having been identified in walnuts of different origins (*18*), their major degradation compounds are hexanal, followed by 1-pentanol, pentanal, 1-hexanol and 1--penten-3-ol. Hexanal has always been found at the highest concentrations, as compared to these other compounds.

Formation of oxidative degradation products depends on a number of factors. Among the intrinsic factors, endogenous lipoxygenases have a role in the formation of some of the lipid-derived volatile compounds (*19*). Pershern *et al.* (*19*) showed that the shelf-life of hazelnuts is inversely proportional to the lipoxygenase levels. The levels of antioxidants and prooxidants, such as minerals, temperature and atmospheric composition are important in the development of the walnut aroma.

In this study, the influence of the storage atmosphere on the development of aroma volatiles in ground walnut is investigated, with emphasis on the aroma deterioration markers. We also determined the antioxidative potential (AOP) and the concentrations of total phenols following the storage of ground walnut over a 10-month period.

Materials and Methods

Walnuts

Seven cultivars of air-dried walnuts were used: Rasna, Fernette, Marbot, Lara, Fernor, Chandler and Franquette. Approximately 100 walnuts were deshelled and ground in a kitchen mixer to obtain approx. 400 g of homogenous powder. A mass of 10 g of each of these ground walnuts was put into 20-mL vials, after which the headspace comprised approx. 10 mL. The vials were then purged with pure O_2 or N_2, closed tightly, and stored in the dark at 20 °C for 10 months. The experiment was designed in triplicates, 3 vials prepared for each cultivar×storage condition.

Total phenols

The levels of total phenolics in the walnut extracts were determined according to the Folin-Ciocalteu procedure (*20*). All of the samples were analysed in three parallel analyses.

Fatty acid analysis

The fatty acid composition was determined by gas--liquid chromatography, using *in-situ* transesterification (*21*), as modified by Polak *et al.* (*22*). The samples were analysed in three parallel experiments.

Working conditions on GC

The composition of the fatty acid methyl esters (FAME) was determined by gas-liquid chromatography on an Agilent Technologies 6890 gas chromatograph (Palo Alto, CA, USA) equipped with a flame ionisation detector and a Supelco SPB–PUFA capillary column (Supelco, Sigma--Aldrich, St. Louis, MO, USA, Cat. no. 24314; 30 m×0.25 mm×0.2 μm). The column temperature was set to 210 °C, injector temperature to 220 °C and detector temperature to 280 °C. The flow rate of the carrier gas (He, 99.9999 %; Istrabenz, Koper, Slovenia) was 1.0 mL/min.

The FAMEs were identified through their retention times in comparison with certified standard, Supelco 37 Component FAME mix (Supelco, Sigma-Aldrich). The mass fractions of each of the FAs in the samples were determined using the response factor and the factor of transformation of FA content from the FAME content. The results were expressed as g of FA per 100 g of total FAs.

Antioxidant activity

Free radical scavenging activity was determined according to the method described by Nakajima *et al.* (*23*). The samples were analysed in three parallel experiments and the results were expressed as Trolox equivalent antioxidant capacity (TEAC) on a dry mass basis (μmol of TEAC per g of dm).

Analysis of volatiles

Analyses of volatile content of the samples were carried out using a gas chromatography system (6890N, Agilent Technologies) equipped with an autosampler (MPS2, Multipurpose Sampler, Gerstel, Germany) and an ion-selective mass detector (Hewlett-Packard 5971A; Palo Alto, CA, USA). The gas chromatography system was fitted with a ZB-WAX capillary column (60 m×0.32 mm×0.5 μm; Phenomenex, Torrance, CA, USA). Helium 6.0 was used as the carrier gas, with a flow rate of 1.2 mL/min at 40 °C. The aroma deterioration volatiles were sampled for 30 min at 30 °C using a solid-phase microextraction fibre, with a Supelco Carbowax/polydimethylsiloxane coating (85-μm thickness StableFlex™, Sigma-Aldrich). For thermal desorption, the solid-phase microextraction fibre remained in the injector for 5 min. The temperature of the injector was set to 270 °C. The oven temperature programme was set for 5 min to 40 °C, from 40 to 230 °C at 4 °C/min, and for 5 min to 230 °C. The mass selective detector was operated at 70 eV, with electron impact ionisation. The transfer line was set to a temperature of 280 °C. Mass spectra were acquired in fullscan mode (30–300 *m/z*). The peaks were identified by comparisons with experimental spectra of the National Institute for Standards and Technology (Gaithersburg, MD, USA) database. Relative concentrations of the volatiles in the headspace were estimated by comparison of the peak areas of the

volatiles with that of the external standard, 6-methyl-5--hepten-2-one. A volume of 10 mL of external standard solution (0.109 μg/mL) was placed in a 20-mL vial. The fibre was exposed to the standard headspace for 30 min at 30 °C.

Statistical analyses

The results were analysed by the method of least squares using GLM procedure (*24*). All of the data are presented as least square mean (LSM) values and were compared at the 5 % probability level.

Results and Discussion

As well as from a nutritional point of view, antioxidants are crucial compounds in quality maintenance of many food products. They have roles in preventing discolouration of food and in changes in their sensory characteristics by preventing many deterioration reactions that can take place. The AOP is an important parameter that provides information on the overall levels of antioxidants. In the present study, only cultivar showed a significant influence on the AOP, while atmosphere and the cultivar×atmosphere interaction showed no statistically significant dependencies (Table 1).

Table 1. Source of variability for antioxidative potential, and statistical significance of the influence of cultivar, atmosphere and cultivar×atmosphere interaction

Parameter	Source of variability (p-value)		
	Cultivar	Atmosphere	Cultivar×atmosphere
Antioxidative potential	<0.0001	0.2822	0.6250

Walnuts are known to be a rich source of antioxidants, with the main antioxidants being polyphenols (*25*) and tocopherols. In general, polyphenols are potent antioxidants and show even higher AOP than other antioxidants. The results of the present study show that Marbot cultivar had the highest AOP in both storage atmospheres, while Fernette cultivar showed only half the AOP of Marbot cultivar (Table 2). Thus the cultivar significantly influenced AOP in both of these atmospheres.

The presence of O_2 results in the deterioration of food quality, so atmospheres that are low in O_2 are beneficial for the maintenance of food quality. In the present study, the most vulnerable substrates were the unsaturated fatty acids, which are prone to oxidation in the presence of O_2. As expected, walnut stored in the N_2 atmosphere showed higher AOP in the majority of the cultivars, with the exception of Fernette and Chandler, although these differences were not statistically significant. Duration of storage has been shown to be influenced by AOP, with higher AOP in fresh walnuts than in dry ones (*25*).

Several studies have shown that walnuts are a rich source of antioxidants (*26*) and have a greater antioxidant capacity than other nuts (*27*). As indicated above, polyphenols are potent antioxidants and they contribute significantly to the total AOP of food. Walnut is a rich source of polyphenols, which are mainly in the form of

Table 2. Antioxidative potential of ground walnut stored in N_2 or O_2 atmospheres for 10 months

Cultivar	Antioxidative potential μmol of TEAC per g of dm		Significance (atmosphere)
	N_2 atmosphere	O_2 atmosphere	
Rasna	$(113\pm17)^{ab}$	$(106\pm7)^{bc}$	ns
Fernette	$(77\pm23)^{b}$	$(90\pm7)^{c}$	ns
Marbot	$(157\pm13)^{a}$	$(150\pm27)^{a}$	ns
Lara	$(117\pm3)^{ab}$	$(97\pm10)^{bc}$	ns
Fernor	$(107\pm10)^{b}$	$(97\pm7)^{bc}$	ns
Chandler	$(87\pm20)^{b}$	$(93\pm27)^{c}$	ns
Franquette	$(150\pm40)^{a}$	$(124\pm30)^{ab}$	ns
Significance (cultivar)	0.0058	0.0076	

TEAC=Trolox equivalent antioxidant capacity, dm=dry mass, ns=statistically not significant ($p>0.05$)
groups with a different letter in superscript within a column differ significantly ($p\leq0.05$)
($\bar{x}\pm$so, Duncan's test, $\alpha=0.05$)

hydrolysable tannins (*28*). According to the analyses of total phenols, significant differences among walnut varieties can be found. As seen in Table 3, the amounts of total polyphenols ranged from 10 to 26 mg/g, which is in a range also found by Christopoulos and Tsantili (*26*). Cultivar significantly influenced the polyphenol content, while atmosphere did not influence it significantly, although more polyphenols were found in the samples stored in the N_2 atmosphere, as compared to the O_2 atmosphere, for most of the cultivars, with the exception of Fernor cultivar. The O_2 atmosphere obviously provoked oxidation of the polyphenols into products that are not susceptible to Folin-Ciocalteu analysis. In agreement with this, other studies have clearly demonstrated that nuts with a seed coat (intact nuts) have a higher content of phenolic compounds, and consequently, higher AOP (*25*).

Fatty acids, and especially PUFAs, are prone to oxidation and can be oxidised easily under certain conditions. The availability of O_2 is the most important extrinsic factor required for oxidation, although further factors such as temperature and the presence of prooxidants (minerals) and light can also accelerate the oxidation process. In the first step, fatty acids are oxidised to hydroperoxides, which are not stable, and which form aldehydes and ketones as their main degradation products. Other degradation products include aliphatic and aromatic hydrocarbons, monoterpenes, alcohols, furans, esters and lactones. As seen in Table 4, cultivar significantly influenced the fatty acid composition of the walnut during storage, which was also observed by Pereira *et al.* (*29*). On the other hand, atmosphere had no significant effect on the fatty acid composition, while the cultivar×atmosphere interaction significantly influenced only the levels of palmitic, linolenic and arachidic acid (Table 4).

For the degradation products, none of the parameters tested (cultivar, atmosphere, cultivar×atmosphere interaction) significantly influenced the content of the degra-

Table 3. Content of total phenols in ground walnut stored in N_2 or O_2 atmospheres for 10 months

Cultivar	Total phenols/(mg of GAE per g of dm)			Significance (atmosphere)
	Initial sample	N_2 atmosphere	O_2 atmosphere	
Rasna	$(17.2\pm0.0)^{c}$	$(15.6\pm1.5)^{c}$	$(13.5\pm2.4)^{b,c,d}$	ns
Fernette	$(14.5\pm0.0)^{x,f}$	$(10.9\pm0.7)^{y,e}$	$(10.9\pm0.8)^{y,c,d}$	ns
Marbot	$(14.6\pm0.0)^{e}$	$(19.1\pm1.4)^{b}$	$(18.7\pm3.6)^{a,b}$	ns
Lara	$(23.3\pm0.0)^{x,b}$	$(11.8\pm1.3)^{y,d,e}$	$(9.9\pm2.0)^{y,d}$	***
Fernor	$(16.8\pm0.0)^{d}$	$(13\pm1.6)^{d,e}$	$(16.6\pm6.3)^{a,b,c}$	ns
Chandler	$(11\pm0.0)^{y,g}$	$(14.1\pm1.3)^{x,c,d}$	$(13.5\pm2.0)^{x,b,c,d}$	*
Franquette	$(26.5\pm1.5)^{x,a}$	$(26.1\pm1.3)^{x,a}$	$(20.9\pm1.1)^{y,a}$	**
Significance (cultivar)	***	***	*	

GAE=gallic acid equivalent, dm=dry mass; ns=statistically not significant ($p>0.05$), *statistically significant ($p<0.05$), **highly statistically significant ($p<0.01$), ***very highly statistically significant ($p<0.001$)
$^{a–g}$groups with a different superscript letter within rows differ significantly ($p\leq0.05$), ^{x,y}groups with a different superscript letter within a column (initial samples, N_2 or O_2 atmosphere) differ significantly ($p\leq0.05$)
($\bar{x}\pm so$, Duncan's test, $\alpha=0.05$)

Table 4. Source of variability and statistical significance of the influence on cultivar, atmosphere and the cultivar×atmosphere interaction

Parameter	Source of variability (p-value)		
	Cultivar	Atmosphere	Cultivar× atmosphere
Fatty acid			
C16:0	<0.0001	0.1776	0.0103
C18:0	<0.0001	0.5530	0.0958
C18:1	<0.001	0.6682	0.0928
C18:2	<0.001	0.8888	0.1665
C18:3	<0.0001	0.6707	0.0595
C20:0	<0.0001	0.5446	0.0015
Degradation product			
pentanal	0.5005	0.4957	0.4273
hexanal	0.4967	0.6652	0.3906
2-pentenal	0.5360	0.5371	0.3727
2-pentylfuran	0.5097	0.8858	0.3505
1-pentanol	0.4021	0.8947	0.4242
1-octen-3-ol	0.4727	0.1460	0.4299
octanal	0.4482	0.3722	0.4463
2-pentenal	0.4565	0.3742	0.4352
hexanol	0.0859	0.0001	0.5475
2-octenal+1-octen-3-ol	0.4585	0.3845	0.4388

dation products. The only exception to this was hexanol, which is not a very typical rancid-like substance. Relative concentrations of degradation products expressed as peak area are presented in Table 5. These data show high variability among the individual analyses for all degradation products. Nevertheless, most of the degradation products were found at higher concentrations in samples stored under the O_2 atmosphere, as compared to the N_2 atmosphere. With regard to O_2 and N_2 atmospheres, only few differences in the concentration of degradation products appeared to be statistically significant for individual cultivars. Among these products, the yield of pentanal, hexanal, 1-octen-3-ol, octanal, 1-pentanol and hexanol was statistically significant for one cultivar, of 2-pentylfuran for two cultivars, and of 2-pentenal for four cultivars. Differences between the cultivars were statistically significant for 2-pentenal only. Degradation products are present at low concentrations, and they can also represent markers of rancidity. Human senses of smell and taste are extremely sensitive to these compounds, which can be detected in the range of parts per million; therefore, relatively low concentrations of some degradation products can provoke rancid taste, which is detrimental to food quality. Hence the rancid smell and taste are easily detected by the consumer, and are not acceptable in food.

The substance with the most rancid-like taste is hexanal, which is usually found at higher concentrations compared to other similar compounds (*30*). As reported by Elmore *et al.* (*18*), hexanal content can account for 80 % of all aldehydes. As seen for other parameters, hexanal content was higher in walnut stored in O_2, although again, these differences were not statistically significant due to the high associated standard deviations. Hexanal content has been shown to negatively correlate with O_2 levels, where it was also shown that there was less hexanal in packages with an O_2 absorber (*31*). Mexis and Kontominas (*13*) reported higher hexanal content with increased gamma irradiation dose.

As walnut is a good source of PUFAs, it can also contain appreciable amounts of linolenic acid, which is classified as an ω-3 fatty acid. The ratio of ω-6:ω-3 is of great importance, and in walnut it is around 5:1, which meets the World Health Organisation recommendations. As indicated above, PUFAs are important from the nutritional point of view, although on the other hand, they are also unstable and prone to oxidation.

The fatty acid composition was cultivar dependent, with significant differences between cultivars for all of the fatty acids. The greatest difference was seen for linolenic acid, the content of which ranged from 9.7 to 13.2 %. We expected the atmosphere to significantly influence the PUFA content, but according to the data in Table 6,

Table 5. Concentration of oxidation degradation products expressed as peak areas in ground walnut stored in N_2 or O_2 atmospheres for 10 months

Degradation product	Cultivar						
	Rasna	Fernette	Marbot	Lara	Fernor	Chandler	Franquette
	Peak area						
	Stored under N_2 atmosphere						
pentanal	35±34	4092±7088	(62±53)^{y}	42±38	104±33	0	185±187
hexanal	254±255	27275±46793	(96±60)^{y}	172±174	38±13	71±29	1799±2149
2-pentenal	(7±6)^{y}	338±580	(8±3)^{y}	(3±2)^{y}	1±2	2±3	(14±2)^{y}
2-pentylfuran	(184±11)^{y}	3709±5817	363±295	(238±115)^{y}	317±72	249±60	427±399
1-pentanol	114±65	4843±7921	(51±18)^{y}	125±134	118±29	60±37	615±719
1-octen-3-ol	17±10	234±346	64±38	42±43	42±29	52±32	(8±2)^{y}
octanal	0	23181±40150	0	0	0	0	(338±435)^{y}
trans-2-heptenal	7±12	3344±8632	0^{y}	(5±9)^{y}	0^{y}	0	33±24
hexanol	3770±2535	10451±19777	1259±1133	(3986±4878)^{y}	2609±1358	1104±1129	19794±19261
2-octenal+1-octen-3-ol	337±6	1212±258	508±243	528±244	261±93	527±316	414±247
	Stored under O_2 atmosphere						
pentanal	338±274	130±143	(195±37)^{x}	308±200	93±98	140±126	485±148
hexanal	3899±3159	1328±1446	(1772±416)^{x}	3017±2533	816±739	1563±1488	5411±1666
2-pentenal	(36±5)^{x,a,b}	(4±6)^{e}	(27±2)^{x,b,c}	(23±2)^{x,d,c}	(15±8)^{d}	(19±10)^{d,c}	(41±1)^{x,a}
2-pentylfuran	(1058±347)^{x}	523±350	868±135	(651±184)^{x}	679±383	525±268	688±206
1-pentanol	1056±810	748±571	(333±67)^{x}	1088±657	282±277	662±605	1135±133
1-octen-3-ol	21±8	17±3	23±21	17±2	22±7	35±28	(16±2)^{x}
octanal	619±579	339±394	162±280	881±193	0	65±113	(424±388)^{x}
trans-2-heptenal	59±335	28±26	(61±3)^{x}	(63±27)^{x}	(43±20)^{x}	37±36	81±19
hexanol	35392±24137	25233±19290	9419±5973	(32803±16138)^{x}	8956±11619	23856±23647	33703±1452
2-octenal+1-octen-3-ol	605±313	278±180	903±541	528±463	261±230	526±248	414±148

$^{a–e}$groups with a different superscript letter within rows differ significantly ($p \leq 0.05$), ^{x,y}groups with a different superscript letter within a column (N_2 or O_2 atmosphere) differ significantly ($p \leq 0.05$)
($\bar{x}$±so, Duncan's test, α=0.05)

Table 6. Fatty acid composition of ground walnut stored in N_2 or O_2 atmospheres for 10 months

Fatty acid	Cultivar						
	Rasna	Fernette	Marbot	Lara	Fernor	Chandler	Franquette
	w(fatty acid)/%						
	N_2 atmosphere						
C16:0	(6.59±0.07)^{c}	(7.68±0.12)^{a}	(7.61±0.12)^{y,a}	(7.31±0.42)^{b}	(7.79±0.07)^{a}	(6.87±0.55)^{c}	(7.71±0.17)^{a}
C18:0	(2.96±0.11)^{b}	(3.33±0.06)^{a}	(2.55±0.06)^{c}	(2.68±0.30)^{c}	(2.93±0.02)^{y,b}	(2.41±0.35)^{d}	(2.52±0.10)^{c}
C18:1	18.35±0.01	19.41±0.03	20.33±0.02	20.19±5.92	19.75±0.01	(16.96±0.03	20.84±0.02
C18:2	(61.57±0.19)^{a}	(59.66±0.22)^{b,a}	(59.44±0.20)^{b,a}	(59.40±4.22)^{b}	(59.39±0.17b)^{a}	(60.62±0.34)^{b}	(58.60±0.21)^{y,b,a}
C18:3	(10.37±0.25)^{x,b}	(9.75±0.31)^{c}	(9.91±0.33)^{c,b}	(10.24±1.03)^{c,b}	(9.98±0.18)^{x,c,b}	(13.00±1.23)^{a}	(10.17±0.17)^{x,b}
C20:0	(0.13±0.01)^{c}	(0.15±0.01)^{b,a}	(0.14±0.01)^{b,a}	(0.16±0.02)^{a}	(0.14±0.01)^{y,b,c}	(0.12±0.01)^{d}	(0.13±0.01)^{y,c}
	O_2 atmosphere						
C16:0	(6.71±0.31)^{d}	(7.54±0.46)^{b}	(7.70±0.06)^{x,a}	(7.00±0.36)^{c}	(7.79±0.18)^{a}	(6.79±0.05)^{d}	(7.72±0.04)^{a}
C18:0	(2.99±0.33)^{b,c}	(3.23±0.49)^{a}	(2.58±0.09)^{d}	(2.83±0.20)^{c}	(3.05±0.10)^{x,b,a}	(2.31±0.05)^{e}	(2.50±0.05)^{d}
C18:1	18.63±6.50	19.01±0.02	20.49±0.04	20.44±0.06	20.38±0.00	16.38±0.02	20.82±0.02
C18:2	61.47±5.19	59.75±0.20	59.37±0.41	59.27±0.58	59.88±0.24	61.17±0.19	(58.87±0.10)^{x}
C18:3	(10.03±0.74)^{y,d}	(10.29±1.10)^{c,b}	(9.70±0.43)^{c,d}	(10.29±0.46)^{b}	(9.72±0.12)^{y,c,d}	(13.20±0.19)^{a}	(9.92±0.15)^{y,c,b,d}
C20:0	(0.14±0.02)^{b}	(0.14±0.01)^{b}	(0.14±0.02)^{b}	(0.15±0.01)^{b,a}	(0.15±0.01)^{x,a}	(0.12±0.00)^{c}	(0.14±0.01)^{x,b}

$^{a–e}$groups with a different superscript letter within rows differ significantly ($p \leq 0.05$), ^{x,y}groups with a different superscript letter within a column (N_2 or O_2 atmosphere) differ significantly ($p \leq 0.05$)
($\bar{x}$±so, Duncan's test, α=0.05)

significant changes were observed in the content of linolenic acid only. In the N_2 atmosphere, this highly unstable fatty acid was preserved, with no significant effects of the N_2 atmosphere seen on either linoleic or oleic acid. Mexis and Kontominas (*13*) used gamma irradiation to suppress the growth of microorganisms on walnuts; however, one of the side effects was oxidation of fatty acids, with a decrease in monounsaturated fatty acids and a slight decrease in the content of linolenic acid.

Conclusions

In the present study, the mass fraction of the most unstable linolenic acid decreased due to its oxidation, while the other fatty acids remained largely unaffected. Besides fatty acid oxidation, other changes can take place in the storage period of 10 months. Polyphenols, the content of which was cultivar dependent, were also subjected to oxidation. Their concentrations decreased more in the presence of O_2.

Oxidation processes affected the total AOP, and here the AOP proved to be cultivar dependent and tended to be higher in the N_2 atmosphere, although with no significant differences observed. As expected, the O_2 atmosphere influenced the oxidation of the above-mentioned parameters, which was in most cases significant; however, the situation was not quite so clear with regard to the degradation products. Their levels increased during storage, but none of the degradation products was significantly affected by either variety or atmosphere, except for hexanol, which was significantly affected by atmosphere only. Most of the studied degradation products provoked a rancid taste and smell, with hexanal usually having the pivotal role. The explanation for this behaviour could be that even though the walnut was in an O_2 atmosphere, there might not have been enough O_2 to take the oxidation forward to a more advanced stage.

References

1. P.M. Kris-Etherton, S. Yu-Poth, J. Sabaté, H.E. Ratcliffe, G. Zhao, T.D. Etherton, Nuts and their bioactive constituents: Effects on serum lipids and other factors that affect disease risk, *Am. J. Clin. Nutr.* (Suppl.), *70* (1999) 504–511.
2. G.P. Savage, P.C. Dutta, D.L. McNeil, Fatty acid and tocopherol contents and oxidative stability of walnut oils, *J. Am. Oil Chem. Soc. 76* (1999) 1059–1063.
3. J.S. Amaral, S. Casal, J.A. Pereira, R.M. Seabra, B.P.P. Oliveira, Determination of sterol and fatty acid compositions, oxidative stability, and nutritional value of six walnut (*Juglans regia* L.) cultivars grown in Portugal, *J. Agric. Food Chem. 51* (2003) 7698–7702.
4. A.P. Simopoulos, Omega-3 fatty acids in health and disease and in growth and development, *Am. J. Clin. Nutr. 54* (1991) 438–463.
5. R.U. Almario, V. Vonghavaravat, R. Wong, S.E. Kasim-Karakas, Effects of walnut consumption on plasma fatty acids and lipoproteins in combined hyperlipidemia, *Am. J. Clin. Nutr. 74* (2001) 72–79.
6. M. Carvalho, P.J. Ferreira, V.S. Mendes, R. Silva, J.A. Pereira, C. Jerónimo, B.M. Silva, Human cancer cell antiproliferative and antioxidant activities of *Juglans regia* L., *Food. Chem. Toxicol. 48* (2010) 441–447.
7. B.L. Halvorsen, K. Holte, M.C.W. Myhrstad, I. Barikmo, E. Hvattum, S.F. Remberg *et al.*, A systematic screening of total antioxidants in dietary plants, *J. Nutr. 132* (2002) 461–471.
8. B.L. Halvorsen, M.H. Carlsen, K.M. Phillips, S.K. Bøhn, K. Holte, D.R. Jacobs Jr., R. Blomhoff, Content of redox-active compounds (ie, antioxidants) in foods consumed in the United States, *Am. J. Clin. Nutr. 84* (2006) 95–135.
9. Z. Zhang, L. Liao, J. Moore, T. Wu, Z. Wang, Antioxidant phenolic compounds from walnut kernels (*Juglans regia* L.), *Food Chem. 113* (2009) 160–165.
10. I.F. Almeida, E. Fernandes, J. Lima, P.C. Costa, M.F. Bahia, Walnut (*Juglans regia*) leaf extracts are strong scavengers of pro-oxidant reactive species, *Food. Chem. 106* (2008) 1014–1020.
11. L. Zwarts, G.P. Savage, D.L. McNeil, Fatty acid content of New Zealand-grown walnuts (*Juglans regia* L.), *Int. J. Food Sci. Nutr. 50* (1999) 189–194.
12. P.N. Jensen, G. Sørensen, S.B. Engelsen, G. Bertelsen, Evaluation of quality changes in walnut kernels (*Juglans regia* L.) by Vis/NIR spectroscopy, *J. Agric. Food Chem. 49* (2001) 5790–5796.
13. S.F. Mexis, M.G. Kontominas, Effect of γ-irradiation on the physicochemical and sensory properties of hazelnuts (*Corylus avellana* L.), *Radiat. Phys. Chem. 78* (2009) 407–413.
14. G. Wilson-Kakashita, D.L. Gerdes, W.R. Hall, The effect of gamma irradiation on the quality of english walnuts (*Juglans regia*), *LWT-Food Sci. Technol. 28* (1995) 17–20.
15. R.G. Clark, H.E. Nursten, Volatile flavour components of walnuts (*Juglans regia* L.), *J. Sci. Food Agric. 27* (1976) 902–908.
16. R.G. Clark, H.E. Nursten, The sensory analysis and identification of volatiles from walnut (*Juglans regia* L.) headspace, *J. Sci. Food. Agric. 28* (1977) 69–77.
17. T.D. Crowe, T.W. Crowe, L.A. Johnson, P.J. White, Impact of extraction method on yield of lipid oxidation products from oxidized and unoxidized walnuts, *JAOCS, 79* (2002) 453–456.
18. J.S. Elmore, I. Nisyrios, D.S. Mottram, Analysis of the headspace aroma compounds of walnuts (*Juglans regia* L.), *Flavour Fragr. J. 20* (2005) 501–506.
19. A.S. Pershern, W.M. Breene, E.C. Lulai, Analysis of factors influencing lipid oxidation in hazelnuts (*Corylus* spp.), *J. Food Process. Preserv. 19* (1995) 9–26.
20. K. Slinkard, V.L. Singleton, Total phenol analyses: Automation and comparison with manual methods, *Am. J. Enol. Vitic. 28* (1977) 49–55.
21. P.W. Park, R.E. Goins, *In-situ* preparation of fatty acid methyl esters for analysis of fatty acid composition in foods, *J. Food Sci. 59* (1994) 1262–1266.
22. T. Polak, A. Rajar, L. Gašperlin, B. Žlender, Cholesterol concentration and fatty acid profile of red deer (*Cervus elaphus*) meat, *Meat Sci. 80* (2008) 864–869.
23. J.J. Nakajima, I. Tanaka, S. Seo, M. Yamazaki, K. Saito, LC/PDA/ESI-MS profiling and radical scavenging activity of anthocyanins in various berries, *J. Biomed. Biotechnol. 5* (2004) 241–247.
24. SAS Software v. 8.01., SAS Institute, Cary, NC, USA (1999).
25. I. Arcan, A. Yemenicioğlu, Antioxidant activity and phenolic content of fresh and dry nuts with or without the seed coat, *J. Food Compos. Anal. 22* (2009) 184–188.
26. M.V. Christopoulos, E. Tsantili, Effects of temperature and packaging atmosphere on total antioxidants and colour of walnut (*Juglans regia* L.) kernels during storage, *Sci. Hortic. 131* (2011) 49–57.
27. S. Arranz, J. Pérez-Jiménez, F. Saura-Calixto, Antioxidant capacity of walnut (*Juglans regia* L.): Contribution of oil and defatted matter, *Eur. Food Res. Technol. 227* (2008) 425–431.

28. T. Fukuda, H. Ito, T. Yoshida, Antioxidative polyphenols from walnuts (*Juglans regia* L.), *Phytochemistry, 63* (2003) 795–801.

29. J.A. Pereira, I. Oliveira, A. Sousa, I.C.F.R. Ferreira, A. Bento, L. Estevinho, Bioactive properties and chemical composition of six walnut (*Juglans regia* L.) cultivars, *Food Chem. Toxicol. 46* (2008) 2103–2111.

30. S.F. Mexis, A.V. Badeka, K.A. Riganakos, K.X. Karakostas, M.G. Kontominas, Effect of packaging and storage conditions on quality of shelled walnuts, *Food Control, 20* (2009) 743–751.

31. P.N. Jensen, G. Sørensen, P. Brockhoff, G. Bertelsen, Investigation of packaging systems for shelled walnuts based on oxygen absorbers, *J. Agric. Food Chem. 51* (2003) 4941–4947.