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

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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 O2 or N2 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 N2 atmosphere. The mass fraction of unsaturated linolenic acid tended to decrease during storage under the O2 atmosphere; statistically significant differences were only found between individual varieties. The O2 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 O2 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).
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 1,3-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: Rasa, Bernette, 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 O₂ or N₂, 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, antioxi-
dants are crucial compounds in quality maintenance of
many food products. They have roles in preventing dis-
colouration of food and in changes in their sensory char-
acteristics by preventing many deterioration reactions
that can take place. The AOP is an important parameter
that provides information on the overall levels of anti-
oxidants. In the present study, only cultivar showed a
significant influence on the AOP, while atmosphere and
the cultivar×atmosphere interaction showed no statisti-
cally 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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Source of variability (p-value)</th>
<th>Antioxidative potential</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cultivar</td>
<td>Atmosphere</td>
</tr>
<tr>
<td>Antioxidative potential</td>
<td>&lt;0.0001</td>
<td>0.2822</td>
</tr>
</tbody>
</table>

Walnuts are known to be a rich source of antioxi-
dants, with the main antioxidants being polyphenols (25)
and tocopherols. In general, polyphenols are potent anti-
oxidants and show even higher AOP than other antioxi-
dants. 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 Mar-
bot cultivar (Table 2). Thus the cultivar significantly in-
fluenced AOP in both of these atmospheres.

The presence of O2 results in the deterioration of food
quality, so atmospheres that are low in O2 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
O2. As expected, walnut stored in the N2 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 antioxi-
dant 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
hydrolysable tannins (28). According to the analyses of
total phenols, significant differences among walnut va-
rieties 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, al-
though more polyphenols were found in the samples
stored in the N2 atmosphere, as compared to the O2 at-
mosphere, for most of the cultivars, with the exception
of Fernor cultivar. The O2 atmosphere obviously pro-
voked oxidation of the polyphenols into products that
are not susceptible to Folin-Ciocalteu analysis. In agree-
ment 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 oxida-
tion and can be oxidised easily under certain condi-
tions. The availability of O2 is the most important extrin-
sic factor required for oxidation, although further factors
such as temperature and the presence of prooxidants (mi-
erals) and light can also accelerate the oxidation pro-
cess. In the first step, fatty acids are oxidised to hydro-
peroxides, which are not stable, and which form aldehydes
and ketones as their main degradation products. Other
degradation products include aliphatic and aromatic hy-
drocarbons, monoterpenes, alcohols, furans, esters and
lactones. As seen in Table 4, cultivar significantly in-
fluenced 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×atmo-
sphere 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 inter-
action) significantly influenced the content of the degra-

Table 2. Antioxidative potential of ground walnut stored in N2 or O2 atmospheres for 10 months

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Antioxidative potential</th>
<th>Significance (atmosphere)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol of TEAC per g of dm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N2 atmosphere</td>
<td>O2 atmosphere</td>
</tr>
<tr>
<td>Rasna</td>
<td>(113±17)ab</td>
<td>(106±17)bc</td>
</tr>
<tr>
<td>Fernette</td>
<td>(77±23)b</td>
<td>(90±7)c</td>
</tr>
<tr>
<td>Marbot</td>
<td>(157±13)a</td>
<td>(150±27)a</td>
</tr>
<tr>
<td>Lara</td>
<td>(117±3)ab</td>
<td>(97±10)bc</td>
</tr>
<tr>
<td>Fernor</td>
<td>(107±10)b</td>
<td>(97±7)bc</td>
</tr>
<tr>
<td>Chandler</td>
<td>(87±20)b</td>
<td>(93±27)c</td>
</tr>
<tr>
<td>Franquette</td>
<td>(150±40)a</td>
<td>(124±30)ab</td>
</tr>
</tbody>
</table>

TEAC=Trolox equivalent antioxidant capacity, dm=dry mass, ns=statistically not significant (p>0.05)
et al.

hexanol 0.0859 0.0001 0.5475
2-pentenal 0.4565 0.3742 0.4352
octanal 0.4482 0.3722 0.4463
1-octen-3-ol 0.4727 0.1460 0.4299
1-pentanol 0.4021 0.8947 0.4242
2-pentylfuran 0.5097 0.8858 0.3505
2-pentenal 0.5360 0.5371 0.3727
hexanal 0.4967 0.6652 0.3906
pentanal 0.5005 0.4957 0.4273
Degradation product
C20:0 <0.0001 0.5446 0.0015
C18:3 <0.0001 0.6707 0.0595
C18:2 <0.0001 0.8888 0.1665
C18:1 <0.0001 0.6682 0.0928
C16:0 <0.0001 0.1776 0.0103
Fatty acid

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 O2, although again, these differences were not statistically significant due to the high associated standard deviations. Hexanal content has been shown to negatively correlate with O2 levels, where it was also shown that there was less hexanal in packages with an O2 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 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 N2 or O2 atmospheres for 10 months

<table>
<thead>
<tr>
<th>Degradation product</th>
<th>Cultivar</th>
<th>Stored under N2 atmosphere</th>
<th>Stored under O2 atmosphere</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rasna</td>
<td>Fernette</td>
<td>Marbot</td>
</tr>
<tr>
<td>C18:2 (61.57)</td>
<td>35±34</td>
<td>4092±7088</td>
<td>(62±53)Y</td>
</tr>
<tr>
<td>hexanal (3899)</td>
<td>254±255</td>
<td>27275±46793</td>
<td>(96±60)Y</td>
</tr>
<tr>
<td>2-pentenal (7)</td>
<td>(2±6)Y</td>
<td>338±580</td>
<td>(8±3)Y</td>
</tr>
<tr>
<td>1-octen-3-ol (21)</td>
<td>114±65</td>
<td>4843±7921</td>
<td>(51±18)Y</td>
</tr>
<tr>
<td>2-pentylfuran (36)</td>
<td>(184±11)Y</td>
<td>3709±5817</td>
<td>(238±115)Y</td>
</tr>
<tr>
<td>trans-2-heptenal</td>
<td>7±12</td>
<td>3344±8632</td>
<td>0Y</td>
</tr>
<tr>
<td>octanal (335)</td>
<td>0</td>
<td>23181±40150</td>
<td>0</td>
</tr>
<tr>
<td>2-pentenal+1-octen-3-ol</td>
<td>337±6</td>
<td>1212±258</td>
<td>508±243</td>
</tr>
</tbody>
</table>

a,b groups with a different superscript letter within rows differ significantly (p<0.05), x,y groups with a different superscript letter within a column (N2 or O2 atmosphere) differ significantly (p<0.05) (F15,59, Duncan’s test, α=0.05)

Table 6. Fatty acid composition of ground walnut stored in N2 or O2 atmospheres for 10 months

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Cultivar</th>
<th>w(fatty acid)/%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rasna</td>
<td>Fernette</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:0</td>
<td>(6.59±0.07)Y</td>
<td>(7.68±0.12)a</td>
</tr>
<tr>
<td>C18:0</td>
<td>(2.96±0.11)b</td>
<td>(3.33±0.06)a</td>
</tr>
<tr>
<td>C18:1</td>
<td>18.35±0.01</td>
<td>19.41±0.03</td>
</tr>
<tr>
<td>C18:2</td>
<td>(61.57±0.19)Y</td>
<td>(59.66±0.22)ba</td>
</tr>
<tr>
<td>C18:3</td>
<td>(10.37±0.25) yc,b</td>
<td>(9.75±0.31)c</td>
</tr>
<tr>
<td>C20:0</td>
<td>(0.13±0.01)c</td>
<td>(0.15±0.01)ba</td>
</tr>
</tbody>
</table>

a,b,c groups with a different superscript letter within rows differ significantly (p<0.05), x,y groups with a different superscript letter within a column (N2 or O2 atmosphere) differ significantly (p<0.05) (F15,59, Duncan’s test, α=0.05)
significant changes were observed in the content of linolenic acid only. In the N₂ atmosphere, this highly unstable fatty acid was preserved, with no significant effects of the N₂ 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 O₂.

Oxidation processes affected the total AOP, and here the AOP proved to be cultivar dependent and tended to be higher in the N₂ atmosphere, although with no significant differences observed. As expected, the O₂ 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 different from the AOP in most cases. The O₂ atmosphere tended to bring the oxidation forward to a more advanced stage.

References


