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ARTICLE

Preservation effect of cinnamon and clove essential oil vapors on shelled walnut

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ABSTRACT Shelled walnut (*Juglans regia*) kernels are prone to rancidity during storage. In this study we examined the preservation effect of cinnamon and clove essential oil (EO) vapors compared to cold storage and vacuum packaging by measuring the hexanal content, indicating rancidity, in stored walnut kernels. Odor and taste of stored shelled walnut was investigated by sensory evaluation and by measuring residues of the main EO components in the kernels. During storage under EO vapors, cinnamaldehyde and eugenol were absorbed on the surface of walnuts in a time-dependent manner changing the odor and taste of the kernels. Clove (*Syzygium aromaticum*) EO prevented rancidity and EO treated kernels were rated as acceptable by the sensory panel while cinnamon EO treatment increased rancidity compared to the other treatments and the control samples. **Acta Biol Szeged 62(2):141-145 (2018)**

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Introduction

Walnut (*Juglans regia*) has a valuable kernel rich in oils, vitamins, antioxidants and numerous other metabolites (Rao et al. 2016). The composition of walnuts can show slight differences by region and by variety, but proteins and fats account for more than 70% of the walnut kernel weight (Pereira et al. 2008; Tapia et al. 2013). According the study of Abdallah et al. (2015) the main volatile compounds of walnut flavors are pentanal (0.07-0.12%), hexanal (0.26-0.8%), nonanal (0.34-0.89%), 2-decenal 0.25-0.68%) and hexanol (0.21-1.58%).

High hexanal content together with the presence of 1-octen-3-ol, octanal, and 2-octenal can be found in rancid walnuts causing the characteristic odor and taste (Vidrih et al. 2012). During storage in air the unsaturated oils, especially linoleic acid, of the walnuts will be oxidized leading to compounds that decrease the chemical, nutritional and organoleptic properties of the nuts and reduce shelf life (Vaidya and Eun 2013). Auto-oxidation of oils is influenced by various factors such as oxygen, temperature, light and metal ions (Varga and Őrsi 2005). Different methods are known to prevent nuts from auto-oxidation: cooling (Laczay 2013) using antioxidant preservatives (Atarés et al. 2011; Varga and Őrsi 2013), modified atmosphere packaging or combined preservation methods (Mexis et al. 2009).

Essential oils (EOs) are originated from plant materials and their antimicrobial and antioxidant properties are well-known. About 300 EOs are commercially important (Van de Braak et al. 1999; Speranza and Corbo 2010) and some of them are used in food industry as flavorings and natural food preservatives (Hyldgaard et al. 2012). As plant-based preservatives EOs can enhance the shelf life of foods by protecting them from microbial contamination or oxidative degradation during storage and post-harvest processing (Prakash et al. 2014). Ruberto and Baratta (2000) tested about 100 pure EO compounds and have found that phenols possessed the highest antioxidant activity followed by some monoterpene hydrocarbons, namely, terpinolene, α- and γ-terpinene. The EOs used in our study had benzene ring containing compounds, cinnamaldehyde (CIN) and eugenol (EUG, a phenol), as main components (Bakkali et al. 2008). CIN is thought to be responsible for the excellent antimicrobial effect of cinnamon EO (Souza et al. 2013), and EUG, for clove EO (Vijayalakshmi et al. 2014). Both EOs were examined for their effect on retarding lipid oxidation of crude hazelnut and poppy seed oils (Özcan and Arslan 2011) and both showed significant antioxidant effect.

In this study, walnut kernels were stored refrigerated at 4 °C or at ambient temperature in vacuum packages or in containers vaporized with cinnamon or clove essential oils. Signs of rancidity were detected by sensory evaluation and by measuring hexanal content during storage. Our goals included also the determination of adsorbed quantity of the two main components of the used EOs, CIN and EUG, and their effect on the sensory properties of walnut kernels.

Materials and Methods

Materials

Walnuts (variety Milotai 10) were obtained at a local market in Szeged, Hungary, in November and were shelled. Kernels were left to dry for 3 days. Damaged and crushed kernels or kernels with signs of mold contamination were discarded.

Cinnamon (*Cinnamomum zeylanicum*) and clove (*Syzygium aromaticum*) EOs were purchased from Aromax (Budapest, Hungary).

Sample preparation

150 g walnut kernels were placed in 720 ml sealed jars (3 jars/ treatment) with approximately 1.5 - 2 cm left between the cap of the jar and the walnuts. Storage was done at room temperature except cool storage at 4 °C. Types of treatments: unshelled walnuts (kept in textile bag at room temperature; control 1), kernels without any treatment (control 2), vacuum-packed kernels, and kernels in cinnamon or clove EO vapor. For EO vapor treatment sterile paper discs were fixed on the inside surface of jar caps with a drop of agar-agar (1.5%). The discs were impregnated with 12.5 or 25 mg essential oils giving the vapor concentration of 17 or 35 mg/l air in the closed jars.

Samples (ten pieces of kernels) were taken immediately and after 6, 13 and 18 weeks of storage and were placed in 50 ml sterile test tubes and stored at -20 °C until GC analysis. Before the sensory analysis, the samples were left on the table for 1 hour to attain room temperature.

Detection of cinnamaldehyde and eugenol residues in walnut kernels

The walnut samples (2 g) were powdered with liquid nitrogen, then suspended in 12 ml water and incubated at 4 °C for 2 hours. Then 100 µl of 10 mg/ml methyl-benzoate (Sigma, Hungary) in ethyl-acetate (VWR, Hungary) was added as internal standard and was vortexed for 0.5 min. The essential oils were extracted with 8 ml cold ethyl-acetate and the upper layer was collected after the centrifugation (3000 rpm, 4 °C, 10 min). The organic phase was dried with sodium-sulfate (Reanal, Hungary) and evaporated by IKA rotation evaporator (VWR, Hungary) approximately to 5 ml, which was followed under slight nitrogen stream up to 1 ml. After centrifugation (13 000 rpm, 4 °C, 10 min) samples were injected to an Agilent 6890N GC-FID system (Agilent, USA), using HP-Innowax (60 m x 0.25 mm x 0.5 µm) column (Agilent, USA). For carrier gas, helium was applied in constant pressure mode (32 psi), while the oven temperature was programmed from 110 °C to 250 °C with a rate of 15 °C/min, and held at the final temperature for 15 min. The injector temperature was 250 °C in split mode with 25:1 split ratio and FID detector temperature was also 250 °C. For the detector, the H_2 and air flow rates were 30 ml/min and 300 ml/min, respectively. The injection volume was 2 µl. For the quantitation, six-point calibration curves were applied for both essential oil components in the range of 40.0–1000.0 µg/ml and 33.4–834.2 µg/ml, where the y = 1.0517 x -0.001 and y = 0.8717 x -0.003 equations (r^2>0.999) were used with the 7.81 µg/ml / 20 µg/ml limit of detection (LOD) and 15.62 µg/ml / 40 µg/ml limit of quantification (LOQ) values in the case of CIN and EUG, respectively.

Detection of hexanal level in walnut kernels

Powdered walnut samples (1 g) were suspended in 2.5 ml water directly in 20 ml head space vials. Then the samples were measured using a Perkin Elmer 8500 GC-FID system equipped with a HS-101 head space autosampler (Perkin Elmer, USA), using a TG-WAXMS (60 m x 0.25 mm x 0.25 µm) column (VWR, Hungary). The initial temperature of the oven was 30 °C for 5 min, which was increased up to 200 °C with 20 °C/min and held for 2 min. The FID temperature was 210 °C, while the pressures of H_2 and air detector gases were 100 and 150 kPa, respectively. The HS-101 syringe and transfer line temperature were 110 °C, while the autosampler pretreatment program included 30 min sample incubation at 90 °C, 1 min pressurization time, 0.2 min injection time and 0.2 withdrawal time. For the hexanal calibration a six-level calibration curve (0.256 – 8.2 µg/ml) was used with the y=7.5297x-915.52 equation, where the LOD and LOQ were 48 ng/ml and 144 ng/ml, respectively.

Sensory evaluation

For sensory evaluation, a panel of five untrained judges was asked to evaluate odor and taste intensities of all samples. Taste and odor of walnut kernels was evaluated according to the following description: 5 – fresh walnut odor or taste; 4 – matured walnut odor or taste; 3 – foreign taste or odor, harmonizing with walnut; 2 - foreign taste or odor, undesirable; 1- rancid taste or odor. Evaluation was made immediately after beginning the treatments, and after 6, 13 and 18 weeks of storage. Panelists were served with fresh table water to cleanse the palate after every sample. Samples were served on white plates and identified by a number. Samples were regarded acceptable at mark 2.5 or above.

RESULTS

Cinnamaldehyde and eugenol content of samples

The untreated samples contained, as expected, no CIN or EUG. It could be observed that EUG and CIN concentration increased with the amount of oil added (Fig 1 and 2).

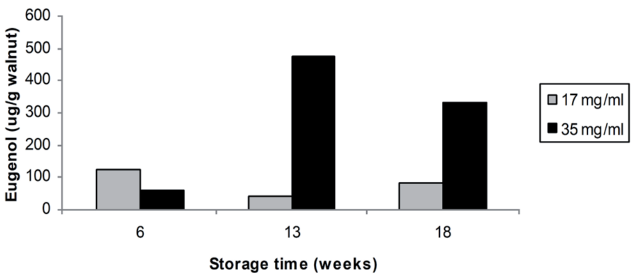

Figure 1. Eugenol content of walnut kernels treated with clove EO vapor.

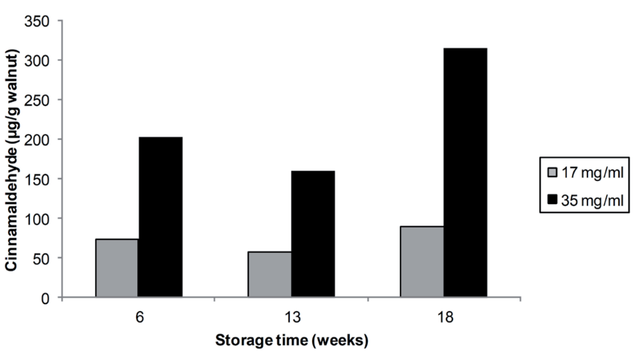

Figure 2. Cinnamaldehyde content of samples treated with cinnamon EO vapor

Hexanal (HEX) content of walnut kernels

Hexanal content of walnut samples increased in a time-dependent manner (Table 1) and reached LOQ after 18 weeks of storage. Among the non-EO treated samples, the highest amount of HEX was found in the kernels stored at room temperature, and the lowest in the ones stored in unshelled form. Using clove EO vapor, HEX content was under the LOQ during the whole storage time (Table 1). In contrast to that, cinnamon EO treated samples contained high amounts of HEX after 13 - 18 weeks of storage; higher than in any other sample (Fig. 5). It seems that in this case the EO promoted rancidity. This could be since in high concentrations cinnamon EO can work as a pro-oxidant (Bakkali et al. 2008). According to Bakkalbasi et al. (2012) and Mexis et al. (2009) the sensory limit for HEX concentration for rancid taste is 1 - 2 mg/kg walnut (1000-2000 ng/g). In kernels, treated with 35 mg/ml cinnamon EO vapor, the HEX concentration reached this value after 13 weeks and most members of the sensory panel evaluated the walnut kernels as rancid.

Sensory evaluation

At the beginning, walnut kernels were found to have a fresh, bittersweet taste and odor which during storage matured to a sweet but oilier taste. Regarding whole walnuts which were shelled just before sensory analysis, the taste at the end of the experiment was defined as undesirable although HEX content was below the sensory detection limit. This was possibly due to mold contamination vis-

Table 1. Hexanal content in the various walnut samples during storage.

Storage	Essential oil concentration (mg/l)	Storage time (weeks)	Hexanal (ng/g kernel)
Unshelled walnuts (Control 1)	0	0	-
		6	-
		13	-
		18	172.4
Kernels at room temperature (Control 2)	0	0	-
		6	-
		13	+
		18	329.4
Kernels at 4 °C	0	0	-
		6	-
		13	+
		18	325.7
Vacuum packaged kernels	0	0	-
		6	+
		13	+
		18	274.1
Kernels in clove essential oil vapor	17	0	-
		6	+
		13	+
		18	+
	35	0	-
		6	+
		13	+
		18	+
Kernels in cinnamon essential oil vapor	17	0	-
		6	+
		13	+
		18	337.0
	35	0	-
		6	+
		13	1524.7
		18	3636.4

-: below limit of detection (LOD); +: below limit of quantification (LOQ).

ible on a lot of whole walnuts and mold metabolites being responsible for the undesirable taste. Vacuum-packed and refrigerated samples were rated as having good or acceptable taste and odor throughout the experiment.

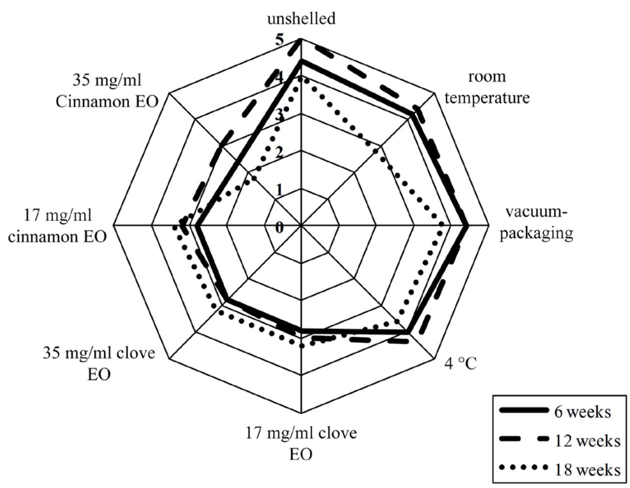

Figure 3. Average scores of the sensory evaluation (odor) of walnut kernel samples after treatment.

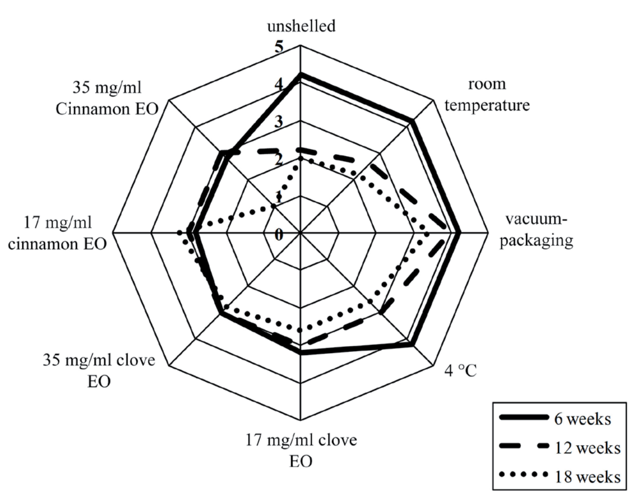

Figure 4. Average scores of the sensory evaluation (taste) of walnut kernel samples after treatment.

EO vapor treated samples had the characteristic cinnamon and clove odor but it was rated as harmonic to the walnuts' own odor. It was said to be "cake-like" odor. Taste was also rated as foreign but harmonic except samples with the highest cinnamon vapor concentration which had a rancid taste. As seen in Table 1, in this case HEX concentration reached the sensory detection limit. In general, the panelists stated that clove EO gave a pleasant taste to the walnut samples (Fig. 3 and 4).

Conclusions

Several studies demonstrated that EOs have antimicrobial, and/or antioxidant properties. An advantage of EOs is their activity in the vapor-phase: it makes them attractive for stored product protection (Krisch et al. 2013). In the recent study, cinnamon EO vapor at high concentration is not a good candidate for walnut preservation, supporting rancidity by the possible pro-oxidant effect. On the other hand, clove EO vapor can be used as potential walnut preservative at 17 mg/l concentration, giving a harmonic "cake-like" taste and odor.

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