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Preserving quality of fresh walnuts using plant extracts

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ABSTRACT

Fresh walnut kernel is considered as a way of walnut consumption, particularly in Asia, even though its market is less commercial than the dried kernel. There is a little knowledge about the storage of this commodity. Postharvest treatments including plant extracts either of *Thymus vulgaris* (ET) or walnut green husk (EWGH) in four concentrations (25, 50, 75 and 100 mg/L) and distilled water (as control) were assayed to increase fresh walnut kernel's shelf life at ambient temperature in the aqueous environment. Compared with untreated walnuts, the content of saturated fatty acids and linolenic was almost constant. Oleic acid concentration did not change considerably during storage in treated samples, while it was decreased in untreated kernels. The level of linoleic acid also decreased except for samples treated with the three higher EWGH concentrations. All treatments delayed lipid peroxidation and increment of acid value (AV). In addition, total antioxidant activity (TAA) and total phenolics (TPs) losses were observed by advanced time. After 28 days, the greatest losses of TAA and TPs were observed in untreated kernels. These results illustrate that ET and EWGH have potential to maintain fresh walnut quality during storage at 25 °C in the aqueous environment for 28 d.

1. Introduction

Walnut is one of the nuts generally found in many countries diets. Among species of walnuts, the Persian walnut (*Juglans regia* L.) is the most cultivated species and the most commercially important) Vahdati, 2000). In addition, it has a high nutritional value because its kernel rich in protein, minerals, and lipids contains about 70% polyunsaturated fatty acids (Abbey, Noakes, Belling, & Nestel, 1994; Pribis et al., 2012), however the oxidation of these polyunsaturated fatty acids is linked to the emersion of unpleasant odors and flavors. Walnut is customarily eaten as a dried nut, but since the 1990s, it has been reported that fresh walnuts are more nutritious than dried ones (Cannella & Dernini, 2005; Jiang et al., 2015) and compared to dried walnuts, antioxidant and phenolic compounds are more abundant in fresh walnuts (Arcan & Yemenicioglu, 2009; Christopoulos & Tsantili, 2012; Manzocco, Calligaris, Mastrocola, Nicoli, & Lerici, 2001). Also, the particular flavor of the fresh nut is respected in some countries (Cannella & Dernini, 2005); however, this product is less widespread with little information about its storage (Christopoulos & Tsantili, 2012).

Fresh walnuts are only available for a short period of time. The traditional way to store fresh walnuts in Iran is to keep them in water up to 3 days due to lack of proper packaging. On the other hand, dry walnuts can be stored for several months even at room temperature. To investigate postharvest behavior of fresh walnuts, some methods have been used, including the evaluation of the storage temperature effect (Christopoulos & Tsantili. 2011, 2012), ^{60}Co γ-irradiation (Ma, Lu, Liu, & Ma, 2013), 1-methylcyclopropene (1-MCP), chlorine dioxide (ClO_2) (Jiang et al., 2015) and cold plasma (Amini & Ghoranneviss, 2016).

Recently, using the natural compounds to preserve the various products after harvest is getting more common (Di Venere, Gatto, Ippolito, & Bianco, 2016). The bioactivity of plant extracts can be ascribed to the presence of different phenolic compounds (Di Venere et al., 2016; Gatto et al., 2011). Thyme (*Thymus vulgaris* L.) is a perennial plant with a strong flavor. The effective antimicrobial compounds in the extracts of thyme contained eugenol and carvacrol (Roby, Sarhan, Selim, & Khalel, 2013). Furthermore, the green husk of walnut is one of the major useless products of walnut production industries. Recent studies revealed the potential of such low price natural substances as a source of phenolic compounds with antiradical and antimicrobial activities and source of phytochemicals (Fernandez-Agullo et al., 2013). Since green walnut husk contains high concentrations of chloregenic acid, caffeic acid, ferulic acid, sinapic acid, gallic acid, ellagic acid, protocatechuic acid, syringic acid, vanillic acid, catechin, and juglone (Stampar, Solar, Hudina, Veberic, & Colaric, 2006), the aqueous extract of walnut green husk could prevent the growth of the gram positive bacteria (Oliveira et al., 2008). Also, it is proved that essential oils and their phenolics are able to disintegrate the outer membrane of gram-negative bacteria, releasing lipopolysaccharides

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(LPS) and increasing the permeability of the cytoplasmic membrane to ATP potassium and hydrogen ions (Burt, 2004).

The objective of this study was to evaluate the influence of thyme and walnut green husk extracts on inhibition of biochemical changes in fresh walnut kernels quality in term of lipid oxidation, fatty acids profile, antioxidant activity and the total phenolic content during storage life in aqueous environment.

2. Materials and methods

2.1. Samples and treatment

Fresh walnuts (*Juglans regia* L.) were collected in summer of 2016 once the packing tissue surrounding the kernel nuts turned completely brown from a promising genotype (Bavanat) located in Bavanat, Fars, Iran. Immediately after picking the fruits, they were transported to the laboratory. The green husk of walnuts and their hard shells were manually removed. Concentrations of 25, 50, 75, 100 mg/L from of the extract of *Thymus vulgaris* (ET) and extract of walnut green husk (EWGH) were prepared in distilled water. Distilled water was used as untreated control. A total of 15 kernels were sampled for immediate analysis to monitor fruit characteristics at harvest before application of treatments (day 0). Also, 1080 whole kernels were randomly distributed into 9 groups and kernels of each group were randomly distributed into 12 plastic bags contains 0.7 L of each treatment (each bag contains 10 replicates) and stored at 25 °C in the dark. A sampling of either treated or control was carried out at 0, 7, 14, 21 and 28 days. Three bags were sampled at each sampling times as three replications.

2.2. Extraction and preparation of treatments

Green walnut husk and thyme were dried at room temperature and 50 g of each sample was powdered in the grinder. The extract were prepared by Soxhlet method with ethanol. Then the solvent was removed by using a rotary evaporation device.

2.3. HPLC analysis of phenolic compounds in ET and EWGH

The HPLC analysis of phenolic compounds present in ET and EWGH, was performed using an Agilent 1200 series liquid chromatograph (Agilent Technologies, Germany) equipped with binary pump (Agilent P/N G1312A) and spectrophotometric photodiode array detector (DAD) (Agilent P/N G1328A). The Agilent ChemStation (Rev. A.06.03) software was used for spectra and data processing. An analytical Phenomenex (Torrance, California, USA) Zorbax eclipse (XDB) C_{18}, 5 μm (ID), 4.6 × 150 mm (FT), column at 30 °C in a thermostatic oven was used for peak separation. The mobile phase was a mixture of methanol (A) and 1% (v/v) formic acid in deionized water (B) and the established elution gradient was as follows: 10% A for 0 min, 10–25% A over 10 min, 25–60% A over 20 min, 60–70% A over 30 min, and column re-equilibration (40 min), using a flow rate of 1 mL/min. Solutions of available pure known compounds, such as caffeic acid, routine, quercetin, p-coumaric acid, carvacrol, trans-phenolic acids, hesperidin, rosmarinic acid, eugenol and hesperetin were chromatographed as external standards (Sigma Chemical Co. St. Louis, MO, USA). Their concentrations ranged from 0.7 to 15.0 mg/L. The results were expressed as mg/g DW and % of each compound from the total phenolic compounds.

2.4. Properties of walnut oils

Three g of ground kernels were extracted with pure hexane (100 ml) for 48 h at 4 °C in dark. Then, the samples were centrifuged for 20 min at 4000 g. The supernatant was filtered through filter paper and the solvent was removed by using a rotary evaporation device. The peroxide value was determined by the standard AOCS Cd 8–53 method and calculated in terms of meq of oxygen per kg of extracted oil. The acid value was determined by the standard AOAC 3d 63 method (AOAC, 1997).

2.5. Fatty acids

Walnut fat components were extracted by diethyl ether at 4 °C. Methyl esters were prepared by trans-methylation using 2 mol/L KOH in methanol and n-hexane according to the method described by Ichihara, Shibahara, Yamamoto, and Nakayama (1996) with minor modification; 10 mg of extracted oil was dissolved in 2 mL hexane, followed by 4 mL of 2 mol/L methanolic KOH. The tube was then vortexed for 2 min at room temperature. After centrifugation at 4000 rpm for 10 min, the hexane layer was taken for gas chromatography (GC) analyses. To evaluate fatty acid composition of walnut oil, all three replications were mixed thoroughly and two injections were done. To do this, a 100 m fused silica capillary column (0.25 mm inner diameter, 0.2-μm film thickness; CPSIL 88, Chrompack 7489, Varian Iberica S.A., Madrid, Spain) was connected to an Agilent-7890 gas chromatograph, equipped with a flame ionization detector (FID) and split/split less injector. N_2 was used as the carrier gas at a velocity of 23 mL/s, and as the make-up gas at a rate of 30 mL/min. A temperature program of 150 °C for 2 min rising to 220 °C at a rate of 5 °C/min was used. The fatty acid methyl ester (FAME) dissolved in hexane was injected (1 μL) in a split mode of injection at a split ratio of 1:20. The injector and detector temperatures were 230 °C and 260 °C, respectively. Agilent-7890A integrator was used for recording the peak areas. Fatty acids were identified and quantified by comparing sample peak retention times and areas with those of mixed standards (Sigma Chemical Co. St. Louis, MO, USA) of known composition and concentrations.

2.6. Total phenolic concentration and total antioxidant activity

Total phenolics (TPs) were measured by homogenizing 0.5 g of two frozen kernels from each of the three replicates with 10 mL methanol and then centrifuged at 10 °C for 3 min at 12000 g. The supernatant was removed and used for phenol determination. The TPs concentration was measured by a Folin-Ciocalteu reagent and results were declared as mg/g of gallic acid on a dry weight basis. For this purpose, on each sampling day, part of each sample (control and treated) was used to estimate the dry weight. Drying was carried out at 70 °C for 3 d.

Total antioxidant activity (TAA) was determined by the 2, 2-diphenyl-1- picryl-hidrazil (DPPH) radical-scavenging method according to (Zhang, Liao, Moore, Wu, & Wang, 2009). The absorbance was calculated at 517 nm, using a spectrophotometer (Model Varian 220, Australia). Total antioxidant activity was calculated as the percentage inhibition of the DPPH according to the following formula:

$$\text{TAA}\ (\%) = \frac{\text{Abs sample} \pm \text{Abs control}}{\text{Abs sample}} \times 100$$

2.7. Sensory evaluations

Sensory evaluation (shell color, interior color, taste, fragility and fat) was performed at the end of storage period. A 9-point scale was used for each factor, and the test was carried out with a consumer panel of 15 members to detect. In the case of pellicle color and interior color, the rank 9, showing the highest intensity of brown color, corresponded to 1 very bright. For overall taste, the taste of fat and crispiness, the rank 9 corresponded to the highest intensity and vice versa.

2.8. Statistical analysis

SPSS software version 22.0 (IBM SPSS Statistics 22) was used to perform statistical analysis. Data were expressed as the mean ± standard error from triplicate samples. Symmetric factorial experiment

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