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 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 & Yemencioglu, 2009; Christopoulos & Tsantili, 2012; Manzocco, Calligaris, Mastrocola, Nicoll, & 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), 60Co γ-irradiation (Ma, Lu, Liu, & Ma, 2013), 1-methycyclopropene (1-MCP), chlorine dioxide (ClO2) (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-Aguillo et al., 2013). Since green walnut husk contains high concentrations of chlorogenic acid, caffic 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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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. N2 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-di-phenyl-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
Table 1

Major phenolics compounds (mg/g DW or % of the total) identified in thyme (ET) and walnut green husk (EWGH) ethanolic extract by HPLC (n = 2).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Approximate Rt (min)</th>
<th>Thyme (mg/g DW) (%)</th>
<th>Thyme (mg/g DW) (%)</th>
<th>Thyme (mg/g DW) (%)</th>
<th>Thyme (mg/g DW) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeic acid</td>
<td>11.6</td>
<td>–</td>
<td>10.7</td>
<td>17.4</td>
<td></td>
</tr>
<tr>
<td>Routine</td>
<td>12.6</td>
<td>0.29</td>
<td>2.2</td>
<td>2.87</td>
<td>4.7</td>
</tr>
<tr>
<td>Quercetin</td>
<td>21.6</td>
<td>4.08</td>
<td>31.6</td>
<td>18.06</td>
<td>29.4</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>15.6</td>
<td>0.02</td>
<td>0.2</td>
<td>0.60</td>
<td>1.0</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>28.4</td>
<td>6.09</td>
<td>47.2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Trans-phenolic acids</td>
<td>16.3</td>
<td>0.24</td>
<td>1.9</td>
<td>1.54</td>
<td>2.5</td>
</tr>
<tr>
<td>Hesperidin</td>
<td>18.5</td>
<td>–</td>
<td>3.65</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>Rosmarinic acid</td>
<td>19.2</td>
<td>0.98</td>
<td>7.6</td>
<td>20.61</td>
<td>33.6</td>
</tr>
<tr>
<td>Eugenol</td>
<td>23.7</td>
<td>0.75</td>
<td>5.8</td>
<td>3.37</td>
<td>5.5</td>
</tr>
<tr>
<td>Hesperetin</td>
<td>22.4</td>
<td>0.35</td>
<td>3.5</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

with completely randomized design (CRD) was performed. The statistical significance of differences between mean values were calculated at $P < .05$ with the LSD test in the general model of the SPSS statistical package. The sensory evaluation of the kernels was analyzed through one-way analysis of variance taking into account the effect of the different treatments on the quality attributes.

3. Results and discussion

3.1. Phenolic content and composition of plant extracts

Phenolic concentrations of plant extracts used in this experiment are shown in Table 1. The HPLC analysis of EWGH and ET showed a rich phenolic pattern. In particular, rosmarinic acid, quercetin, eugenol, trans-phenolic acids, caffeic acid, carvacrol, routine and p-coumaric acid, besides many other unidentified compounds, were discovered (Fig. 1(a) and (b)). Cosmulescu et al. (2010) reported six phenolic compounds in extracts of walnut green husk named juglone, ferulic acid, vanillic acid, coumaric acid, syringic acid and myricetin. Lee, Koo, and Min (2004) reported that the most abundant phenolic compounds in thyme were eugenol, thymol and carvacrol possibly which somehow differed from those reported in this study, possibly as a result of different experimental procedures and environmental conditions.

Based on the HPLC results the total recognized phenolic compounds in different concentrations of EWGH (25, 50, 75 and 100 mg/L) were 1.54, 3.07, 4.61 and 6.14 mg/L and in the case of ET, the total recognized phenolic compounds were 0.32, 0.64, 0.97 and 1.29 mg/L, respectively (Table 1).

3.2. Peroxide value

The initial peroxide value (PV), which is an important index of the degree of rancidity in nuts, of walnut kernel oil was 0.56 ± 0.06 meq O₂/kg oil. PV increased in all treatments during the storage, but it was greater in untreated than treated nuts (Fig. 2(a) and (b)). By the end of the storage period, the increment of PV in untreated nuts was 435%, compared with 216% in 100 mg/L of EWGH and 272% in 75 mg/L of ET-treated fresh walnuts. In addition, after two weeks of storage, a significant sharp increase in PV levels was observed in untreated samples only.

A similar range of PV in fresh walnut oil has been reported previously (Sabaghi, Maghsoudi, Khomeiri, & Ziaiifar, 2015; Wang, Liang, Ma, Zhang, & Shi, 2016). Our treatments were rich in terms of phenolics (Table 1). Phenolics are effective in preventing the oxidation of walnut oil. This could be due to high levels of rosmarinic acid and quercetin in the EWGH and carvacrol and quercetin in the ET which could lead to high level of antioxidant activities. Antioxidants can prevent lipid peroxidation by two significant mechanisms of action. First, by reducing the concentration of oxygen and second, by preventing the initiation chain of free radicals.

3.3. Acid value

The initial acid value (AV) of kernels was 1.75 ± 0.07 mg/g fresh weight. AV increased in untreated kernels during storage but in ET and EWGH treated nuts was approximately stable by 14 days of storage and slightly increased thereafter. AV in untreated samples was higher than treated kernels after 7 days of storage and reached to the highest final concentration (2.25 ± 0.17 mg/g fresh weight), while there was no difference among EWGH treatments at the end of the storage (Fig. 2(c) and (d)). Also, treatment with ET led to a lower AV throughout the storage time. The AV of all treatments and the control increased during the storage and these increments were faster during the two final weeks of storage. In addition, the least final AV was obtained in 100 and 75 mg/L ET treatments.

Acid values symbolize the amount of free fatty acid in the walnut kernel and its increment during storage is due to the enzymatic hydrolysis of lipids, which may adversely affect the flavor of the walnut kernel. Also, AV and PV are important indices for evaluating kernel quality. Health standard GB 2716-2005 proclaims that the AV of edible oil must be less than 3 mg/g and a PV greater than 1.0 mg/kg (the upper limit for good-quality walnuts) are associated with the onset of oxidative rancidity. In our study, although AV values were under 3 mg/g during storage for both treated and untreated nuts, PV values in treated and untreated kernels were higher than 1.0 mg/kg in last two weeks of storage. However, much higher values were observed in controls.

3.4. Fatty acids profile of the walnut

The results for changes in the palmitic (16:0), oleic (18:1n-9), linoleic (18:2n-6) and linolenic (18:3n-3) acids in walnut oil among the different samples of this study are shown in Table 2. The most fatty acids of walnut are formed of unsaturated fatty acids (USFA), whereas saturated fatty acids (SFA) did not beyond than 8% of total fatty acids and it was 7.51 (g/100 g) at harvest. Also, saturated fatty acids remained stable during storage. In addition, oleic acid did not have considerable changes during storage in treated samples, but in untreated kernels significantly decreased during storage. The level of linoleic acid as the most abundant fatty acid in walnut oil also decreased after storage period except for 50, 75 and 100 mg/L EWGH, whereas linolenic acid content was constant.

The decline in USFAs could not only be largely attributed to their oxidation, but also to a transformation (polymerization, isomerization, cyclization) process (Hanus, Goldshlag, & Dembitsky, 2008), while peroxides could be formed in intermediate stages of USFA antioxidation. The amount of fatty acids was in agreement with those reported in the literature for dry kernel walnut in which the saturated fatty acids remained stable during storage, whereas the unsaturated decreased (Christopoulos & Tsantili, 2015b). Furthermore, the present linoleic and oleic content in fresh walnuts were negatively correlated to PV values. In particular, for untreated walnuts PV value and rate of loss of these fatty acids were very high, while this trend was restricted by ET and EWGH. The same inhibition of USFA losses was shown in hazelnuts and walnut when refrigerated and/or stored at N₂ storage (Christopoulos & Tsantili, 2015b; Ghirardello et al., 2013).

3.5. Total phenolics and total antioxidant activity

The TP concentrations of the fresh walnuts at harvest date was 1.32 mg GAE/g dry weight and decreased rapidly during storage in untreated fruit. TP in ET-treated kernels also decreased during 28 days of storage; however, 100 mg/L of ET to some extent could decelerate
this process (Fig. 3(b)). The highest TPs were observed in 75 and 100 mg/L EWGH during first two weeks of storage then decreased. Also, these two mentioned treatments were the most effective treatments to preserve phenolic compounds at the end of the storage period (Fig. 3(a)).

The results are not in agreement with the range reported for dry walnuts in some literature. For instance, Christopoulos and Tsantili (2011) reported that the concentration of TPs approximately ranged from 22 mg GAE/g DW to 13 mg GAE/g DW in ‘Chandler’ and ‘Hartley’ walnuts. In contrast, it was reported that the TPs in fresh and dry walnuts were 2.4 and 1.7 mg GAE/g DW, respectively (Arcan & Yemencioglu, 2009) which are similar to phenolics content in our study. Christopoulos and Tsantili (2011) reported that the presented differences in TP content among cultivars could be attributed to background genetics. In addition, thyme oil could be a contributory factor in increasing the phenolic compounds by increasing the activity of phenylalanine ammonia lyase (PAL) (Sellamuthu, Mafune, Sivakumar, & Soundy, 2013). Some phenolic compounds could reduce the loss of other phenolic compounds during storage, due to the revival of antioxidant compounds and inhibiting several types of oxidative enzymes (Dugas et al., 2000). High level of phenolics in ET and EWGH could have the same role in our study.

During the storage, TAA decreased in either untreated or ET and EWGH treatments. TAA was relatively stable during 14 days of storage in 100 mg/L EWGH and 100 mg/L ET and the activity in either 75 or 100 mg/L EWGH and 75 or 100 mg/L ET were greater than the other treatments after 7 days of storage. Also, the highest level of TAA was recorded in the first day of the experiment (95%) and interestingly, a sharp decline in TAA of untreated fresh walnuts was observed after 7 days of storage at 25°C (Fig. 3(c) and (d)).

On the contrary, current research findings by Amini and Ghoranneviss (2016) showed that cold storage of fresh walnuts for one month increases the concentration of total phenolic compounds and TAA in fresh and dried walnuts. Also, previous studies displayed that total phenolic content of walnuts increased after 20 d of cold storage (Christopoulos & Tsantili, 2015a). In particular, increasing the methylated phenolic acids, such as vanillic and syringic during cold storage have been considered to be responsible for increment of TAA (Christopoulos & Tsantili, 2012). Here, there are two main differences with previous studies. In all mentioned reports, fresh walnuts have been stored in both dry and cold conditions, while we stored fresh kernels at 25°C in aqueous environment. Increment in methylated phenolic acids could be related to plant defense mechanisms (Wildermuth, 2006). Therefore, the absence of chilling effect at the higher storage temperature could indicate a possible reason why the TPs and TAA have not increased during storage. Furthermore, thermal processing is responsible of antioxidant losses (Manzocco et al., 2001). At harvest, fresh ‘Franquette’ kernels exhibited TAA levels higher than thermal dried ones (Christopoulos, Tsantili, Papageorgiou, Komaitis, & Rouskas, 2010). However, there are no studies on effects of ambient temperature in fresh walnuts stored at the aqueous environment. Therefore, the physiological function of this condition on TPs and TAA remains unclear.

Several studies have shown that walnut is an appropriate source of antioxidants (Christopoulos et al., 2010; Zhang et al., 2009). Plant phenolic compounds present in herbal extracts increased phenolic content in avocado fruit (Sellamuthu et al., 2013). Similarly, eugenol increased flavonoids and free radical scavenging capability in strawberry (Wang, Wang, Yin, Parry, & Yu, 2007).

In the current study, the higher concentration of EWGH and ET was more successful to delay decrease of antioxidant activity (Fig. 3(a) and (b)). Decline in antioxidant activity of the samples might be due to senescence and deterioration during the storage period. These effects could be the result of the capacity of high amount of phenolics in EWGH and ET to retain fresh walnut quality attributes. In addition, in the present study, there is a similar pattern of TPs and TAA changes.

![Fig. 1. HPLC chromatograms of thyme (A) and walnut green husk (B) ethanoic extract, n = 2.](image-url)
similarity of these patterns of changes (Fig. 3(a–d)) could be explained by the fact that phenolics contributed significantly to TAA in walnuts similar to those reported previously (Christopoulos & Tsantili, 2015a; Li et al., 2006).

3.6. Sensory properties

The effect of different treatments on sensorial properties of fresh walnut kernels showed in Fig. 4. After 28 days storage, the darkest color in pellicle and inside the kernel were observed in untreated kernels. Also, in relation to overall taste, kernels treated with 100 mg/L EWGH had the highest score, while the highest one for crispiness was recorded in 100 mg/L ET. The scores for taste of fat were below 4 in controls and most of treated kernels. In contrast, the highest scores (4) were given to those kernels treated with the 100 mg/L EWGH.

The use of plant extracts and their compounds such as eugenol and carvacrol were used in different studies on different products. Chen et al. (2017) reported treatment with eugenol prevented browning in lettuce. Also, Sellamuthu et al. (2013) showed the extract of thyme makes a durability crispiness, taste and texture of the avocado fruit. Further, Salejda, Janieczek, Konrzeniowska, Kolniak-Ostek, and Krasnowska (2016) reported the use of green walnut husk powder improves the sensory characteristics of feed products. Here, the taste and color of kernels were more desired in green husk extracts at higher concentration.

Table 2

<table>
<thead>
<tr>
<th>Fatty acids (g/100 g)</th>
<th>Before storage</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Palmitic (C16:0) **</td>
<td>7.5 ± 0.2</td>
<td>7.1 ± 0.3</td>
</tr>
<tr>
<td>Oleic (C18:1) **</td>
<td>15.1 ± 0.9a</td>
<td>11 ± 0.8b</td>
</tr>
<tr>
<td>Linolenic (C18:2) **</td>
<td>63.7 ± 1.8a</td>
<td>47 ± 2.6c</td>
</tr>
<tr>
<td>Linolenic (C18:3) **</td>
<td>11.3 ± 0.9</td>
<td>10.9 ± 1.1</td>
</tr>
<tr>
<td>USFA 2</td>
<td>90.1 ± 0.8</td>
<td>68.9 ± 7.7</td>
</tr>
<tr>
<td>USFA/SFA</td>
<td>75 ± 0.8</td>
<td>57.9 ± 6.5</td>
</tr>
<tr>
<td>PUFAs</td>
<td>10.0 ± 0.8</td>
<td>7.9 ± 0.7</td>
</tr>
<tr>
<td>USFA/PUFA</td>
<td>12.0 ± 0.9</td>
<td>9.7 ± 1.1</td>
</tr>
</tbody>
</table>

1. The symbol (ns and **) showed no significant or significant difference (P < .01) for each fatty acid.
Each value represents mean ± standard deviation of three replicates, different letters in the same line mean significant difference (P < .05).
2. SFA: Saturated fatty acid, USFA: unsaturated fatty acid, PUFAs: poly unsaturated fatty acid.
Fig. 3. Total phenolics (mg gallic acid equivalents per 100 g of dry weight ((a and b) and antioxidant activity (c and d) of fresh walnuts treated with different concentrations of extract of walnut green husk (EWGH) (a and c) and extract of Thymus vulgaris (ET) (b and d) during 28 days of storage at 25 °C (25 mg/L EWGH; 50 mg/L EWGH; 75 mg/L EWGH; 100 mg/L EWGH; 25 mg/L ET; 50 mg/L ET; 75 mg/L ET; 100 mg/L ET; Control). Each value represents mean ± standard deviation of three replicates (** represents P < .01).

Fig. 4. Sensory attributes (overall taste, crispiness, taste of fat, pellicle brown color and interior brown) of control and treated walnut kernels with extract Thymus vulgaris (ET) and walnut green husk extract (EWGH) at different concentrations after 28 days of storage (25 mg/L EWGH; 50 mg/L EWGH; 75 mg/L EWGH; 100 mg/L EWGH; 25 mg/L ET; 50 mg/L ET; 75 mg/L ET; 100 mg/L ET; Control). Data are the mean of estimation performed by 15 people in 3 replicates (* represents P < .05). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
concentrations, and the crispiness was better in thyme extract treated fresh kernels.

4. Conclusions

In conclusion, EWGH which is a quite cheap and available product in walnut growing regions could be considered as a useful chemical for maintaining fresh walnut kernels quality and extending its postharvest life. In particular, considering peroxide values, EWGH 100 mg/L could prolong postharvest life of fresh walnut kernels for one week. Similarly, use of EWGH and ET is contributed to a slower loss of linoleic acid and total antioxidants. Potential modes of action may include high levels of phenolics in the EWGH and the ET treatments which could lead to a high level of antioxidant capacity. However, further studies are necessary to fully understand the mechanism by which these extracts may affect characteristics of fresh walnut kernels and also, to decrease the risk of microbial contamination employ a disinfection treatment would be useful in further studies.

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