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ORIGINAL PAPER

Antimicrobial and antioxidant capacity of biodegradable gelatin film forming solutions incorporated with different essential oils

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Abstract Biodegradable film forming solutions prepared by gelatin (4% w/v) and different concentrations of thyme, orange, sage, peppermint and clove essential oils (EOs) were investigated for their antioxidant and antimicrobial activities. Total phenolic contents and the antioxidant activity of EOs and gelatin film forming solutions incorporated with EOs were determined by Folin–Ciocalteau and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays, respectively. Antimicrobial activity of gelatin film forming solutions incorporated with different EOs were tested on yeast *Candida albicans*, Gram (+) bacteria *Staphylococcus aureus* and Gram (−) bacteria *Escherichia coli*. Among the EOs studied, thyme and clove EOs showed the highest values in total phenolic content (279,797 and 251,663 mg/L gallic acid) while sage EO had the lowest total phenolic content (14,533 mg/L gallic acid). Total phenolic content of the gelatin film forming solution combined with EOs increased proportional to the EO concentration. Antioxidant capacities of the EOs were found to be high which is supposed to be directly related to the active chemical substances of the EOs. The antioxidant properties of the tested EOs were correlated with total phenolic content. EOs showed potential antimicrobial activity against tested microorganisms. Antimicrobial capacity of the combination of gelatin film forming solution with EOs increased depending on the EO concentration. The study results revealed out that biological activities of biodegradable gelatin film forming solution may be effectively enhanced by using herbal essential oils that have strong biochemical properties.

Keywords Biodegradable film · Gelatin · Essential oil · Antioxidant · Antimicrobial · Phenolic content

Introduction

Edible food packaging is gaining much importance with its enhanced functional properties. As microorganisms are the biological threats for food spoilage, it is important to prevent the food with natural agents without hazardous constituents. Biopolymers are the most preferred materials for packaging industry. Edible coating and film technology which is recently one of the most popular technique of active packaging is mainly used for preserving the food and prolonging its shelf-life. Edible films and coatings that are made from polysaccharides, proteins and lipids can extend the shelf-life of foods by acting as moisture, oxygen, carbon dioxide or vapor barriers and as enhancers of mechanical properties [1]. Gelatin is a valuable bio compound for the production of biodegradable packaging film which is produced by partial hydrolysis and physical, chemical or biochemical degradation of collagen obtained from mammalian bones and connective tissues [2]. Gelatin-based edible films and coatings have already been proposed to extend the shelf-life of various meat products [3]. Films including antimicrobial, antioxidant and aromatic agents can enhance the mechanical and biological features of the food [4]. Essential oils (EOs) are liquid mixtures of volatile compounds obtained from aromatic plants. Essential oils are considered generally recognized as safe (GRAS) so they can be used in foods, as long as their maximum protective effects is attained with the minimum change in the sensorial and organoleptic

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properties of the food [5]. EOs have been shown to possess antibacterial and antifungal activities against several microorganisms associated with meat and meat products, including Gram-negative and Gram-positive bacteria [6, 7]. In general terms, essential oils are composed of > 70 components, principally polyphenols, terpenes, monoterpenes and sesquiterpenes, some of which may represent more than 85% of the total content [8]. Essential oils have been widely used as natural additives in food, especially in combination with other forms of preservation such as refrigeration [9]. Addition of EOs to edible products, either by direct mixing or in active packaging and edible coatings, may therefore represent a valid alternative to prevent autoxidation and prolong shelf life of food [10]. Due to their antioxidant and antibacterial properties, the essential oils can be used in coating and packaging materials [11, 12].

Although there are studies about biological activities of the essential oil-incorporated gelatin films, to the best of our knowledge, there is no study about the concentration-dependent activities of gelatin film forming solutions incorporated with different herbal essential oils. The aim of the present study is to evaluate the total phenolic contents, antioxidant and antimicrobial activities of gelatin film solutions incorporated with different essential oils widely used in Mediterranean countries.

Material and method

Gelatin and essential oils

In this study, food grade gelatin powder (Doğa Drug and Raw Material Co. Ltd., Ankara, Turkey) was used. Five different essential oils [Thyme (T), Orange (O), Sage leaf (S), Peppermint (P) and Clove (C)] were purchased from a local market in Muğla province of Turkey.

Preparation of film solutions from gelatin incorporated with different essential oils

Preparation of gelatin film forming solutions was slightly modified from Gomez-Estaca et al. [13]. For preliminary experiments; 2, 4, 6, 8 and 10 g of gelatin in 100 mL distilled water were tried to determine the film forming capacities. After casting the film forming solutions of above concentrations, 4 g/100 mL gelatin concentration was decided to use throughout the study. Food grade gelatin powder (4 g) was dissolved in 100 mL of distilled water (at room temperature) and the mixture was stirred until the gelatin completely dissolved (approx. 15 min). Glycerol (Merck) (0.15 mL per g of gelatin) and D-sorbitol (Merck) (0.15 g per g of gelatin) were then added to the gelatin film forming solutions, which were kept at 45 °C for additional 15 min. 0.5, 1, 2, 5 and 10% (v/w gelatin) of each EOs were then added to the gelatin film forming solutions. To stabilize the emulsion, Tween-80 was also added to the gelatin film solutions with a ratio of 0.2% of the essential oil.

Control group was prepared without the addition of EO (0%). Then the gelatin film forming solutions with EOs were homogenized with an Ultraturrax T25 basic blender (21,500 rpm, position 5, for 1 min; IKA-Werke GMBH & Co. KG, Staufen, Germany).

Total phenol content

Total phenolic content (TPC) was estimated by the Folin–Ciocalteu colorimetric method using Gallic acid as standard [14]. A 20-µL sample aliquot of essential oil or Gallic acid standard (50–500 mg/L) was mixed with 1.58 mL water followed by 100 µL Folin–Ciocalteau's reagent. After vortexing and incubating at room temperature for 8 min, 300 µL of 20% aqueous sodium carbonate solution were added. Samples were vortexed and held at room temperature for 2 h. Absorbance of the blue-color solution was recorded at 765 nm on a UV visible spectrophotometer (T80+ Model, PG Instruments, Leicestershire, UK). The concentration of the total phenolic content was calculated as mg of Gallic acid equivalent by using an equation obtained from Gallic acid calibration curve. The determination of total phenol compounds in the fractions was carried out in triplicate and the results were averaged.

Antioxidant activity of gelatin film forming solutions

The percentage of antioxidant activity (AA%) of each EO and gelatin film forming solutions incorporated with different concentration of EOs were assessed by DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical assay. The measurement of the DPPH radical scavenging activity was performed according to methodology described by Brand-Williams et al. [15]. The samples were reacted with the stable DPPH radical in an ethanol solution. The reaction mixture consisted of adding 0.5 mL of sample, 3 mL of absolute ethanol and 0.3 mL of DPPH radical solution 0.5 mM in ethanol. The changes in color (from deep violet to light yellow) were read at 517 nm after 100 min of reaction using a UV–Vis spectrophotometer (T80+ Model, PG Instruments, Leicestershire, UK). The mixture of ethanol (3.3 mL) and sample (0.5 mL) serve as blank. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). The scavenging activity percentage (AA%) was determined according to Mensor et al. [16]:

$$AA\% = 100 - \left(\frac{(\text{Abssample} - \text{Absblank}) \times 100}{\text{Abscontrol}} \right)$$

Antimicrobial activity of gelatin film forming solutions

The antimicrobial activity of each EO and gelatin film forming solutions incorporated with different concentrations of EOs was tested using agar well diffusion assay over three food pathogen microorganisms; *Candida albicans* ATCC 10239, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 [17]. The above mentioned microorganisms were cultured in Nutrient Broth (NB) at appropriate temperatures. Inoculums were prepared by adjusting the turbidity of the medium to match the 0.5 McFarland Standard Dilutions. 20 mL of Mueller Hinton Agar (Difco) were sterilized in separated flasks and cooled to 45–50 °C. After injecting the microorganism cultures to sterile plates (1000 µL), media was distributed and mixed homogenously. 20 µL of test solutions were injected to the wells of 6 mm in diameter. Four different concentrations of gelatin + essential oil combination were evaluated for antimicrobial activity; 1.25, 2.5, 5 and 10%. Plates inoculated with *E. coli* and *S. aureus* strains were incubated at 37 ± 0.1 °C for 24–48 h while *C. albicans* was incubated at 30 ± 0.1 °C for 24–48 h. After the proper incubation period for each microorganism, antimicrobial activity was evaluated by measuring the zone of inhibition against the tested microorganisms. Gelatin film forming solution without EO combination was used as control group.

Statistical analysis

The data was statistically performed using the SPSS® computer program (SPSS Statistical Software, Inc., Chicago, IL, USA). One-way analyses of variance (ANOVA) were carried out, differences between pairs of means being assessed on the basis of confidence intervals using the Tukey-b test with a level of significance of $P \leq 0.05$.

Results and discussion

The results of antioxidant capacity, total phenolic content and antimicrobial activity of different EOs incorporated gelatin were given in Table 1, collectively.

Phenolic compounds are the most important antioxidant plant components and are generally studied in many medicinal plants and vegetables for screening their antioxidant behaviors [18]. Folin–Ciocalteu phenol reagent was used to determination of the phenolic groups present in the gelatin film forming solutions incorporated with EOs. Total polyphenol contents of the gelatin film forming solutions with plant EOs are shown in Fig. 1. Gelatin solution without EO was found to have no phenolic contents. Total phenolic contents of thyme, orange, sage leaf, peppermint and clove EOs alone were found to be 2798.0, 1279.6, 145.3, 1008.3 and 2516.6 mg/L gallic acid equivalents (GAE), respectively. The lowest total phenolic content was observed for sage EO while the highest levels were obtained for thyme and clove EOs. As the EO concentration increased, total phenolic content of the gelatin film forming solutions incorporated with EOs reached the phenol levels of EOs analyzed alone. Similarly, Reyes Mendez [19] reported that the addition of essential oils in the gelatin matrix caused an increase in the content of total phenol in films.

Phenol compounds are often correlated to the antioxidant activity due to their capability to act as electron donors in free radical reactions. The phenolic content could be used as an important indicator of the antioxidant capacity, which may be used as a preliminary screen for essential oils when intended as natural sources of antioxidants in functional foods [20]. Many studies over recent years have demonstrated that the antioxidant activity of plants is caused mainly by phenolic compounds [21]. In this study, antioxidant activity of the gelatin film forming solutions incorporated with EOs increased in parallel with the phenolic content.

The DPPH radical scavenging activities of gelatin film forming solutions incorporated with EOs are shown in Fig. 2. Gelatin solution without EO was found to have no antioxidant activity. The highest activity was observed for thyme EO (98.49%) which is followed by clove EO (98.26%). Among the gelatin film forming solutions incorporated with EOs, the lowest antioxidant activity was seen for the solution containing 0.5% sage EO. Clove and orange EO incorporation resulted in the highest antioxidant activity values among all gelatin film forming solutions. In general, phenolic compounds, both natural (e.g., α-tocopherol) or synthetic (e.g., BHA), act as antioxidants due to their high reactivity with peroxyl radicals [22]. Reyes Mendez [19] concluded that clove and basil essential oils added to the gelatin films presented higher antioxidant activity than mint essential oil. Viuda-Martos et al. [5] also reported that clove essential oil had the highest amount of total phenols (898.89 mg/L GAE) and showed the highest percentage inhibition of DPPH radical (98.74%). Alparslan et al. [3] presented that orange peel essential oil (2%) incorporated with gelatin film forming solutions was found to have higher free radical scavenging activity than other concentrations (0.5 and 1%).

The antimicrobial activities of gelatin film forming solutions with plant essential oils are shown in Fig. 3. The results of this in vitro study show that the control films (gelatin film forming solution without EO) did not inhibit the growth of the three pathogenic microorganisms. All tested EOs were found to be active against the microorganisms. The highest antimicrobial activity was seen for orange EO against *S. aureus* (31.5 mm). Plant EOs have been reported to possess high antimicrobial activity [11]. Sung et al. [23] reported that antimicrobial packaging is

Table 1 Total phenolic content, antioxidant capacity and antimicrobial activity of gelatin film forming solutions incorporated with EOs

	Total phenolic content (mg gallic acid/L)	Antioxidant activity (%)	Antimicrobial activity [inhibition zone (mm)]		
			S. aureus	*E. coli*	*C. albicans*
Gelatin	*	*	*	*	*
Thyme (T)	2798.0^{a}	98.5^{a}	29^{a}	27^{a}	29.5^{a}
$T_{0.5}$	1328.0^{e}	49.0^{e}	*	*	*
T_{1}	2078.0^{d}	60.2^{d}	*	*	7.5^{d}
T_{2}	2260.6^{c}	63.1^{d}	*	12.5^{c}	14.5^{c}
T_{5}	2350.3^{b}	74.0^{c}	13.5^{c}	19.5^{b}	16.5^{c}
T_{10}	2361.6^{b}	87.5^{b}	18^{b}	23.5^{a}	22^{b}
Orange (O)	1279.6^{a}	92.6^{a}	31.5^{a}	30^{a}	25^{a}
$O_{0.5}$	476.0^{e}	47.6^{d}	*	*	*
O_{1}	561.6^{d}	50.0^{d}	*	*	13^{d}
O_{2}	682.6^{c}	62.5^{c}	*	13.5^{d}	15.5^{c}
O_{5}	943.0^{b}	73.3^{b}	9.5^{c}	16.5^{c}	21.5^{b}
O_{10}	1208.0^{a}	76.4^{b}	12.5^{b}	21^{b}	23^{ab}
Sage (S)	145.3^{a}	64.8^{a}	24^{a}	21^{a}	9^{a}
$S_{0.5}$	65.6^{c}	14.6^{f}	*	*	*
S_{1}	105.6^{b}	26.3^{e}	*	*	*
S_{2}	108.0^{b}	32.9^{d}	*	*	*
S_{5}	132.3^{a}	45.3^{c}	13.5^{c}	11^{c}	*
S_{10}	135.3^{a}	50.0^{b}	19.5^{b}	16^{b}	7^{a}
Peppermint (P)	1008.3^{a}	89.8^{a}	10^{a}	11.5^{a}	18.5^{a}
$P_{0.5}$	528.3^{e}	24.2^{e}	*	*	*
P_{1}	528.0^{e}	20.6^{e}	*	*	*
P_{2}	721.3^{d}	40.9^{d}	*	*	*
P_{5}	852.3^{c}	53.5^{c}	9^{a}	*	10^{b}
P_{10}	977.6^{b}	73.5^{b}	10^{a}	8.5^{b}	12^{b}
Clove (C)	2516.6^{a}	98.3^{a}	16^{a}	23.5^{a}	24.5^{a}
$C_{0.5}$	2320.6^{c}	67.9^{d}	*	*	*
C_{1}	2330.3^{c}	74.2^{c}	*	*	*
C_{2}	2377.3^{b}	76.4^{c}	*	*	*
C_{5}	2394.0^{b}	79.1^{c}	*	10^{c}	11^{c}
C_{10}	2474.3^{ab}	86.6^{b}	8^{b}	14^{b}	16^{b}

*No antimicrobial effect. Different small letters indicate significant difference among means in the same column ($P < 0.05$)

a multifunctional application by reducing harmful microbial activity in food. This technique helps to increase food safety and reduces food wastage so improves the food shelf life. They also concluded that bio-based antimicrobial agents in packaging material provide extra safety for health. Edible films of chitosan incorporated with thyme EO showed good antibacterial effect [24]. Reyes Mendez [19] reported that clove and basil essential oils added to the gelatin films presented antimicrobial activity higher than mint essential oil. Muthaiyan et al. [25] figured out that cold-pressed Valencia orange essential oil inhibited the growth of antibiotic-resistant *S. aureus*, caused gene expression changes consistent with the inhibition of cell wall synthesis, and triggered cell lysis. Anti-candidal effect of thyme EO was obviously higher when used alone (29.5 mm). Omran and Esmailzadeh [26] evaluated the antimicrobial activity of thyme (Thymus vulgaris L.), pennyroyal (Mentha pulegium L.) and lemon (Citrus aurantifolia Christm.) against different species of Candida, including C. albicans, and found that thyme essential oil had the highest inhibitory effect against various Candida species. Among the gelatin film forming solutions with EOs, the solution with 10% clove EO showed the highest anti-candidal effect. Gomez-Estaca et al. [13] reported that gelatin + chitosan film incorporated clove essential oil presented antimicrobial activity and decreased total bacteria count. As expected, all EOs combined with gelatin were

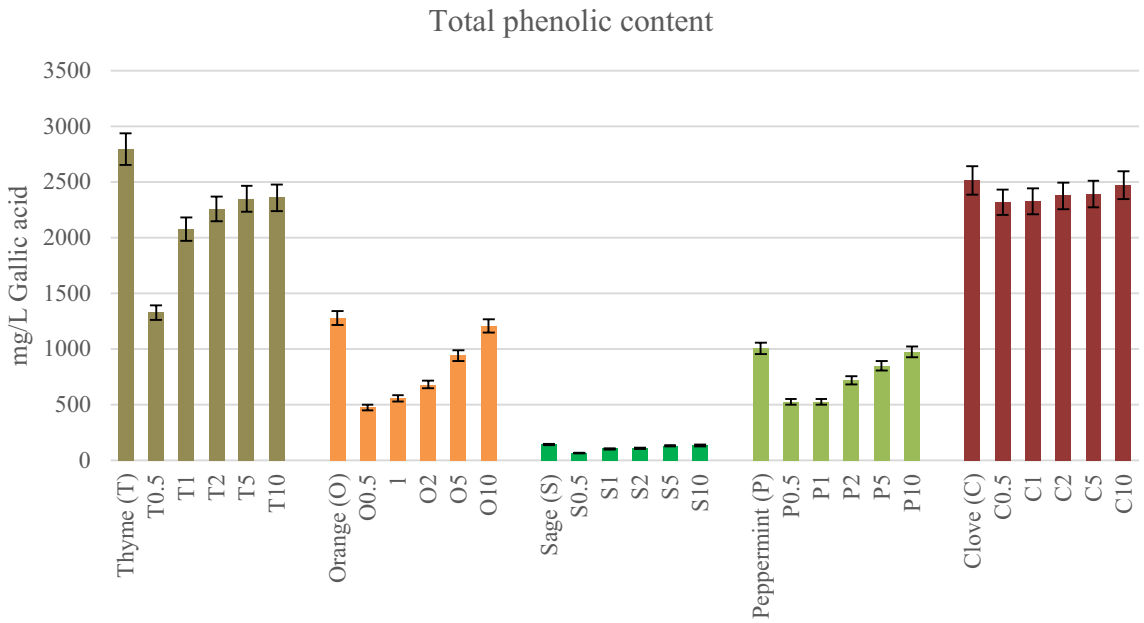

Fig. 1 Total phenolic contents of gelatin film forming solutions incorporated with essential oils

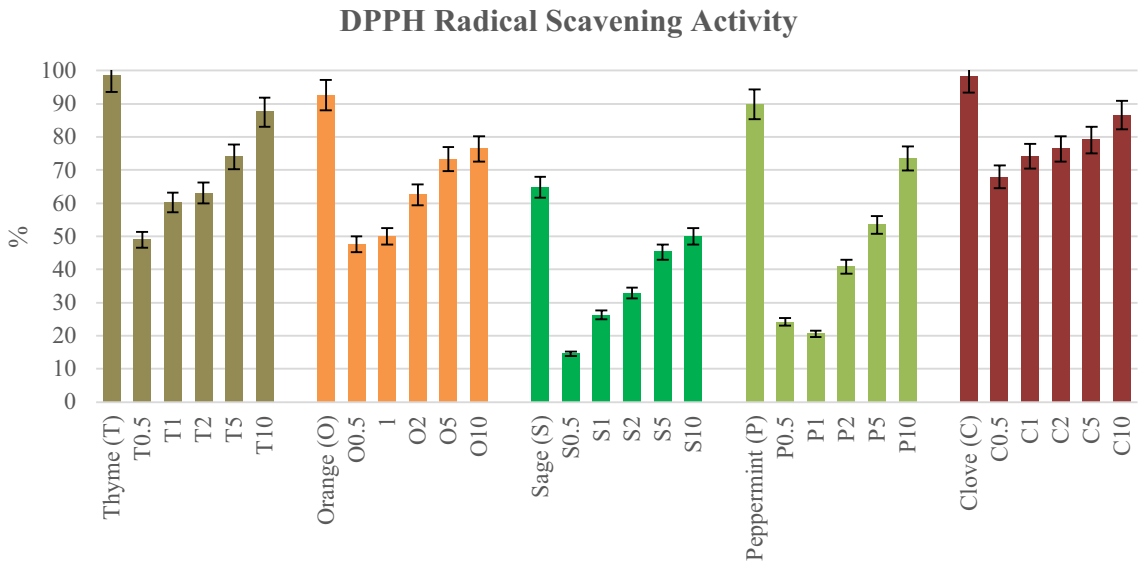

Fig. 2 Antioxidant activity of gelatin film forming solutions incorporated with essential oils

found to be active at 10% concentration. Inhibition was increased with increasing concentration of essential oil. None of the gelatin film forming solution showed antimicrobial effect at 0.5% EO concentration. As the amount of essential oil added to the gelatin films increased, the antimicrobial effect on all microorganisms was also increased. It is supposed that the different performances offered by EOs can be related to essentially different chemical compositions and other factors such as biological properties, geographical regions, etc. [27].

Conclusion

In the present study, 4% (w/v) gelatin film forming solution found to have no antimicrobial characteristics itself. Incorporating 10% (v/v) natural antioxidant and antimicrobial

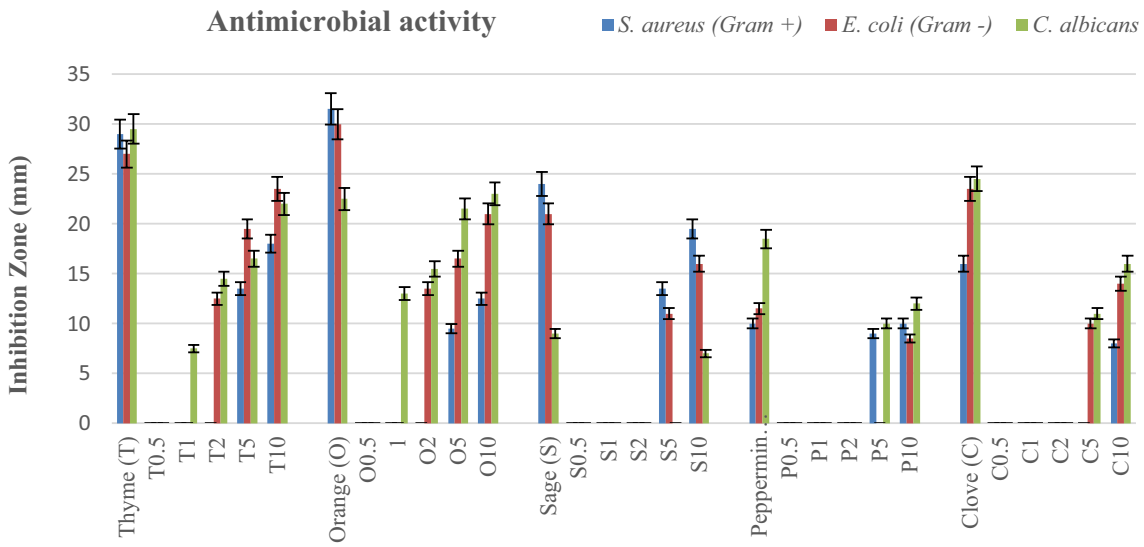

Fig. 3 Antimicrobial activity of different essential oils incorporated gelatin

agents as thyme, orange, sage, peppermint and clove essential oils increased the activities of gelatin film forming solutions. It is concluded that with plant-derived essential oils, it is possible to gain antimicrobial activity to polymer-based film forming solutions against pathogenic microorganism. Among the studied essential oils, thyme and orange essential oils are found to be highly active against tested microorganisms. This study presented important antioxidant and antimicrobial properties of essential oils from thyme, orange, sage, peppermint and clove when used in conjunction with gelatin coating that could be interesting to the food industry. It can be concluded that edible essential oils of aromatic plants can be effectively used for antimicrobial food packaging industry.

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