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THE EFFECT OF STORAGE ON QUALITY OF HERBS GENUS *ORIGANUM*

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ABSTRACT

Herbs of *Origanum* genus are rich in essential oils and contain large amounts of phenols, lipids, fatty acids, flavonoids and anthocyanins. Antioxidant activity of these herbs depends on many factors, including the type herbs, post-harvest processing and subsequent processing. The aim of this study was therefore to confirm the hypothesis that the composition of oils of these two herbs of the *Origanum* genus depends on the post-harvest treatment of herbs and that the dried herb antioxidant activity is higher for fresh than that of frozen herbs. *Lamiaceae* family herbs: oregano (*Origanum vulgare* L.) and Greek oregano (*Origanum heracleoticum* L.) were planted and analyzed. Herb samples were extracted by hot demineralised water. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method was used for antioxidant activity assessment. The total phenolic content was determined spectrophotometrically by using Folin-Ciocalteu reagent. Steam distillation of essential oils was carried out via Clevenger Apparatus. The obtained essential oils were analysed by GC-MS technique. Results of tested fresh, dried and frozen herbs showed a considerable potential for quenching the free DPPH radical. Significantly higher antioxidant activity was found in dried herbs comparing to fresh and frozen, but only in case of values calculated per 100 g of the sample. However, the differences were not statistically significant after recalculation when expressed on dry matter content. There was no difference between fresh and frozen samples. The content of total phenols was significantly higher in dried than in frozen herbs in values recalculated per 100 g of sample. A strong correlation between the results of DPPH and TPC was found again only for values expressed per 100 g of the sample. Post-harvest treatment of herbs affects the composition of their essential oils. The dominant essential oil component of Greek oregano is carvacrol with a proportion of 60% or more. On the contrary, there is no such dominating component in oregano essential oil but there are more components with a share of 10 to 20%.

Keywords: Oregano; Greek oregano; DPPH method; total phenolics contents; essential oils composition

INTRODUCTION

Herbs of the *Origanum* genus are found mainly in the Mediterranean region and Asia. This genus is rich in essential oils where the quantity and quality vary considerably **(Asensio et al., 2015)**. Herbs of *Origanum* genus contain large amounts of phenols, lipids, fatty acids, flavonoids and anthocyanins **(Kintzios, 2002)**.

Oregano is a perennial plant of *Lamiaceae* family groving in Asia, Europe and North Africa. This aromatic herb is widely used in many world cuisines **(Teixeira et al., 2013)**, and in traditional medicine **(Kintzios, 2002)**. There are many studies describing the effect of oregano for food preservation **(Chorianopoulos et al., 2004; Chouliara et al., 2007; Carmo et al., 2008)**, as well as its antioxidant activity **(Sahin et al., 2004; Capecka et al., 2005; Ličina et al., 2013)**. Biologically active substances of oregano include phenols, phenolic acids, flavonoids, glycosides and their esters and steroids. According to **Crocoll et al., (2010)**, the dominant components of essential oils of this herb are monoterpenes and sesquiterpenes showing significant antioxidant activity **(Kinzitos et al., 2002; Grassmann, 2005; Beena et al., 2013)**. The main compounds with antifungal properties include thymol and carvacrol **(Rao et al., 2011)**.

Origanum vulgare L. has been used in Persian traditional medicine for its anti-inflamantory **(Javadian et al., 2016)** and other effects such as diuretics, stomachics, antineuralgics, antitussives and expectorans **(Afsharypour et al., 1997)**.

Greek oregano is widely used in gastronomy. Its typical flavour is produced by the presence of essential oils **(Stamenic et al., 2014)**. Antimicrobial **(De Martino et al., 2009; Govaris et al., 2010; Stamenic et al. 2014)**, antifungal **(Adam et al., 1998)** and antioxidant **(Kulisic et al., 2004; Zheng et al., 2009)** effects were described in this plant. **Charles (2013)** indicates that the most significant secondary metabolites in Greek oregano are tannins, resins, flavonoids, bitter substances, sterols, phenols, and essential oils. **Kikuzaki et al., (1989)** and **Koukoulitsa et al., (2006)** found a variety of phenolic compounds with antioxidant activity in Greek oregano such as rosmarinic acid and its derivatives, caffeic acid, protocatechuic acid and phenyl glucoside. In a report by **Zheng et al., (2009)**, the essential oil of Greek oregano consists of 78.28% of phenolic compounds carvacrol and thymol followed by γ-terpinene (5.54%) and p-cymene (7.35%). The antioxidant activity of essential oil of Greek

oregano and its use in the food industry was also discussed by **Kulisic et al., (2004)**.

Antioxidant activity of herbs depends on many factors, including the type herbs, the methods and conditions of cultivation, harvest, post-harvest processing and subsequent processing. The extraction method, the manner of extraction and the type of solvent used also affect the level of antioxidant activity **(Škrovánková et al., 2012)**. The effect of different growing conditions on the chemical composition and biological activity of the essential oil of oregano was studied **De Falco et al., (2013)**. **Kouřimská et al., (2014)** investigated the antioxidant activity of plants of the *Lamiaceae* family grown under organic and conventional conditions. **Ozkan et al., (2010) and Baranauskienė et al., (2013)** focused on the impact of harvest on essential oil composition and antioxidant activity of oregano. They found the highest antioxidant activity was found in herbs harvested during their flowering.

The water content in fresh *Lamiaceae* herbs is typically in the range of 75 – 80%. For the preservation of the herbs, it is necessary to reduce the amount of water to less than 15% **(Diaz-Maroto et al., 2002)**. Lowering the water activity inactivates the enzymes, which in its active form may be cause degradation of antioxidant ingredients of fresh herbs **(Hossain et al., 2010)**. Water activity may be reduced via different methods. The most commonly used method is drying. This method results in an increase in the content of some substances. Cell tissues of herbs are damaged during the drying process which leads to the release of phenolic compounds and increase of antioxidant activity. Changes in appearance and flavour are due to loss or development of volatile compounds because of oxidation and esterification reactions **(Hossain et al., 2010)**.

Air drying is the simplest and cheapest method of drying. Low temperature prevents the degradation of the active ingredients of herbs during this type of conservation method, but the drying is relatively slow and thus the metabolic processes may continue and cause changes in quality of herbs **(Keinänen and Julkunen-Tiitto, 1996)**. Slow loss of water can act as a stressor and the defensive mechanism of most plants is the production of phenolic compounds **(Dixon and Paiva, 1995; Hossain et al., 2010)** which may contribute to a higher antioxidant activity of dried herbs. **Hossain et al., (2010)** found significantly higher amounts of rosmarinic acid in the extracts of herbs dried at room temperature than in fresh samples. Higher amounts of carvacrol can be obtained during drying at lower temperatures (below 40 °C) **(Novák et al., 2011)**.

Freezing is another method to reduce the activity of water. Crystals are formed during freezing which causes destruction of plant cells enabling better extraction of the active substances **(Keinänen and Julkunen-Tiitto, 1996)**. **Tomsone and Kruma (2014)** considered this phenomenon as a possible explanation of higher phenol content of frozen herbs. **Chan et al., (2014)** argues that the effect of freezing and other methods on the antioxidant activity varies depending on the particular herb. **Tomsone and Kruma (2014)** investigated the effect of drying and freezing on the phenol content and antioxidant activity of lovage and horseradish. Both these parameters were highest in frozen herbs and therefore the authors evaluated this processing method as the most suitable for preserving the antioxidant activity and the content of phenols.

Different extraction methods can be employed for the isolation of antioxidants from herbs. Extraction from the solid phase into the liquid and steam distillation are frequently used methods. Extraction using non-toxic solvents, such as supercritical fluid extraction with carbon dioxide and subcritical water extraction are increasingly applied **(Rodríguez-Meizoso et al., 2006)**. **Škrovánková et al., (2012)** reported that polar solvents (ethanol, methanol, water, etc.) and non-polar (hexane, etc.) as the most commonly used extraction liquids. Different solvents for the isolation of antioxidative components were used for Greek oregano by **Tsimogiannis et al., (2006)**. The highest antioxidant activity determined by DPPH was found in diethyl ether and ethanol extract, the lowest in petroleum extract.

Although there is a lot of scientific literature focussed on the effects of drying or freezing on the antioxidant activity and composition of certain medicinal and aromatic herbs, there is still lack of a comprehensive study comparing these two conservation practices both at the same time in the case of oregano and Greek oregano. The aim of this study was therefore to confirm the hypothesis that the composition of oils of these two herbs of the *Origanum* genus depends on the post-harvest treatment of herbs and that the dried herb antioxidant activity is higher for fresh than that of frozen herbs.

MATERIAL AND METHODOLOGY

Herbs

Lamiaceae family herbs: oregano (*Origanum vulgare L.*) and Greek oregano (*Origanum heracleoticum L.*) were planted and analyzed. The seeds were sown on 20th April to the sunny, unfertilized plot of sandy loam medium soil in the Jirny locality (50° 6' 56'' N, 14° 41' 57'' E, district Prague-East). The seeds were purchased from Kiepenkerl company. Plant parts were harvested before flowering on 30th July and divided into three parts. One part was spread on papers in the laboratory and dried at 25 °C for one week. Another part was placed in plastic bags and frozen at -18 °C. The last third was analysed immediately.

Chemicals

All chemicals, methanol (Lachner, CR), sodium carbonate anhydrous (Lachner, CR), DPPH 2,2-difenyl-1-pikrylhydrazyl (Sigma Aldrich, USA), Folin & Ciocalteu´s phenol reagent (Merck, Germany), ascorbic acid (Penta, CR), Gallic acid (Sigma Aldrich, USA), n-hexane (Lachner, CR) and sodium sulphate anhydrous (Lachema, CR) were of analytical grade purity.

Determination of dry matter content

Balances with infrared dryer, Precisa HA 300 (Precisa Instruments, Swirzerland) were used for dry mater content determination. Samples of herbs (1 g) were ground and spread on aluminium foil and dried at a maximum temperature 105 °C to the constant weight (the weight difference less than 2 mg for 30 s). All samples were measured in triplicate and the average was calculated.

Herb extraction

Fresh herbs (6 g) or the equivalent amount of dried herbs (calculated from total dry matter of individual herbs) were taken for the preparation of water extracts. Herb samples were extracted twice by 50 mL of hot demineralised water in the ultrasonic bath for 10 min. Samples were then filtered into 100 mL volumetric flasks and filled up to the mark after cooling. The extracts were analysed on the same day.

Determination of antioxidant activity by the DPPH method

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method taken from **Adámková et al., (2015)** and **Chrpová et al., (2010)** was used for antioxidant activity assessment. The intensity of the violet DPPH radical solution was measured at 522 nm. As the reaction equilibrium is usually reached after two hours for most compounds, the absorbance of samples was measured after 1, 2 and 3 hours and its minimum was used for the antioxidant activity calculation. The method was calibrated with ascorbic acid and the results were expressed as equivalents of ascorbic acid per unit mass of sample.

Determination of total phenolic compounds (TPC)

The total phenolic content was determined spectrophotometrically (spectrophotometer UV-2900, Tsingtao Unicom-Optics Instruments Co., Ltd., China) at 760 nm by using Folin-Ciocalteu reagent. The method was previously reported by **Dorman et al., (2003)** and **Stratil et al., (2008)**. Results were expressed as the content of Gallic acid per unit mass of the sample.

Steam distillation of essential oils

Steam distillation of essential oils was carried out via Clevenger Apparatus (Wilmad-LabGlass, USA) for 4 hours **(Memarzadeh et al., 2015)**. The apparatus was also used for the determination of extracted volume of essential oil.

GC analysis of essential oils

The obtained essential oils (10 μL) were placed into the vials with 500 μL of n-hexane (with a few crystals of anhydrous sodium sulphate) and analysed by GC-MS using Agilent 7890A GC coupled to Agilent 5975C single-quadrupole mass detector equipped with a HP-5MS column (30 m × 0.25 mm ID, 0.25 μm film). The sample volume of 1 μL was injected in split mode (ratio 12:1) into the injector heated to 250 °C. The initial oven temperature was 60 °C (hold 3 min), ramp to 250 °C at 3 °C . min^{-1} (hold 10 min). Helium was used as carrier gas with the flow rate of 1 $mL.min^{-1}$. The MS analysis was carried out in full scan mode, the electron ionization energy was set at 70 eV. The analytes were identified according to their relative retention times and by the comparison of their mass spectra with the National Institute of Standards and Technology Library (NIST, USA). The results were calculated by area normalisation method.

Statistical evaluation

The data obtained were analysed using statistical software Statistica 12.0 (StatSoft Inc.). Analysis of variance (one-way ANOVA) was performed and the significant differences in the means were separated using the Scheffé's test. The data were expressed as an average of triplicates ± standard deviation. For all statistical tests, a 5% level of significance was used.

RESULTS AND DISCUSSION

Comparison of dry matter content, antioxidant activity and phenolics content

Dry matter content results of analysed herbs are presented in Table 1. The results correspond with the works of other authors, for example **Kouřimská et al., (2014) and Adámková et al., (2015)**. It can be seen that there are considerable losses of water content during drying compared to freezing.

Results of antioxidant activity of tested herbs analysed by DPPH method expressed in mg of ascorbic acid (AA) per 100 g of sample or per 100 g of dry matter are shown in

Table 1 Dry matter (DM) content of tested herbs in % (w/w).

Herb	DM (% w/w)		
	fresh	dried	frozen
Oregano (*Origanum vulgare L.*)	32.73 ±0.97	85.27 ±0.34	26.73 ±1.12
Greek oregano (*Origanum heracleoticum L.*)	24.04 ±1.41	83.62 ±0.12	21.35 ±0.96

Table 2 Antioxidant activity of tested herbs analysed by DPPH method expressed in mg of ascorbic acid (AA) per 100 g of sample or per 100 g of dry matter (DM).

Herb	DPPH (mg AA/100 g)	DPPH (mg AA/100 g DM))
Oregano – fresh	1156 ±160	3532 ±450
Oregano – dried	3572 ±481	4189 ±560
Oregano - frozen	1034 ±162	3867 ±607
Greek oregano - fresh	463 ±9	1926 ±39
Greek oregano - dried	2608 ±563	3119 ±674
Greek oregano - frozen	566 ±63	2652 ±294

Table 3 Total phenolics content (TPC) results of tested herbs expressed in mg of Gallic acid (GA) per 100 g of sample or per 100 g of dry matter (DM).

Herb	TPC (mg GA/100 g)	TPC (mg GA/100 g DM))
Oregano – fresh	1248 ±14	3812 ±44
Oregano – dried	2166 ±5	2540 ±6
Oregano - frozen	425 ±6	1588 ±23
Greek oregano - fresh	795 ±6	3309 ±27
Greek oregano - dried	3067 ±43	3668 ±52
Greek oregano - frozen	390 ±1	1825 ±5

Table 2. The results show that both herbs possess significant potential to quench the free radical DPPH which confirmed the work carried out by **Chrpová et al., (2010)**; **Ličina et al., (2013)** or **Skotti et al., (2014)**. Dried herbs had the highest antioxidant activity. The values of frozen and fresh herbs expressed per 100 g of sample were not very different. There can be seen, only a slight difference between fresh, dried and frozen samples of oregano expressed on dry matter content. When comparing oregano and Greek oregano there was always higher antioxidant activity in case of oregano samples.

Table 3 shows the total phenolics content of tested herbs analysed by Folin-Ciocalteu reagent and expressed in mg of Gallic acid (GA) per 100 g of sample or per 100 g of dry matter. It can be observed that fresh, dried and frozen herbs contain reasonable amounts of phenols, which also corresponds to the results of **Ivanova et al., (2005)** and **Skotti et al., (2014)**. The highest amount of phenols per 100 g of the plant was determined on the dried herbs, which correlates with the highest antioxidant activity. After recalculating the results based on dry matter content, fresh oregano samples had the highest phenol content. The lowest amount of total phenols showed frozen herbs even after recalculating to dry matter content. Higher phenol content in dried herbs corresponds with the conclusion of **Ahmad-Qasem et al., (2013)**, who found lower levels of phenols in the frozen sample of olive-tree than in the dried sample. The authors explained this lower content of phenols in a frozen sample as a result of the temporary inactivation of the enzymes during freezing.

Statistical comparison of the results of the fresh, dried and frozen herbs expressed per dry matter content did not show any significant differences in antioxidant activity including the content of total phenols (pDPPH = 0.6509, pTPC = 0.0731). However, significant differences were found comparing the values per 100 g of herbs for both antioxidant activity (p = 0.0339) and in the content of total phenols (p = 0.0266). A more detailed analysis using Scheffé test showed a statistically significant difference in antioxidant activity between fresh and dried herbs (p = 0.0498) and between frozen and dried herbs (p = 0.0493). In the case of TPC, there was only a statistically significant difference between frozen and dried herbs (p = 0.0290). A strong correlation (r = 0.83) was observed between the values of DPPH and TPC expressed per 100 g of herbs.

Higher antioxidant activity by DPPH method in the dried sample may be due to stress of plants accompanied by the formation of phenolic compounds. Also the disruption of tissues during drying could result in the release of phenolics and increasing their content in the extraction process. This is partly confirmed by the results of total phenols, where a strong correlation between the values of TPC and DPPH was found, but only for the results expressed per 100 g of herbs. A significant correlation between the total content of phenols and antioxidant

Table 4 GC-MS analysis of major components of essential oil extracted from oregano.

Component	Content in fresh (%)	Content in dried (%)	Content in frozen (%)
Sabinene	ND	4.30	3.49
β-Phellandrene	1.51	ND	ND
1-Octen-3-ol	0.23	3.09	3.32
β-Myrcene	0.95	ND	1.92
α-Terpinene	ND	2.38	2.36
(E)-β-Ocimene	5.77	5.44	ND
(Z)-β-Ocimene	6.86	ND	5.41
α-Pinen	ND	8.38	8.77
3-Carene	2.54	3.03	0.07
γ-Terpinene	12.20	16.40	15.47
1,6-Octadien-3-ol, 3,7-dimethyl-	0.33	1.71	0.80
Benzene, 1-methoxy-4-methyl-2-(1-methylethyl)-	5.38	3.36	3.59
Germacene D	10.16	24.44	29.18
Caryophyllene	14.39	5.07	3.86
α-Caryophyllene	2.10	0.77	ND
Cubebol	ND	6.60	5.14
β-Cubebene	16.82	ND	ND
β-Bisabolene	8.34	2.92	2.12

ND = not detected

Table 5 GC-MS analysis of major components of essential oil extracted from Greek oregano.

Component	Content in fresh (%)	Content in dried (%)	Content in frozen (%)
Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	1.27	ND	1.76
Bicyclo[3.1.0]hexane, 4-methyl-1-(1-methylethyl)-, didehydro deriv.	ND	1.42	ND
Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	ND	ND	1.23
β-Myrcene	1.47	1.73	1.92
Cyclohexene, 1-methyl-4-(1-methylethylidene)-	1.20	0.09	0.08
1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	ND	1.70	ND
Bicyclo[4.1.0]hept-2-ene, 3,7,7-trimethyl-	ND	ND	1.93
(E)-β-Ocimene	4.61	4.21	4.36
1,3,6-Octatriene, 3,7-dimethyl-, (E)-	0.97	1.41	1.70
γ-Terpinene	6.21	9.33	9.73
Benzene, 2-methoxy-4-methyl-1-(1-methylethyl)-	0.97	ND	1.08
Phenol, 5-methyl-2-(1-methylethyl)-	ND	ND	7.19
Carvacrol	73.09	67.92	59.66
Caryophyllene	1.00	1.46	1.24

ND = not detected

activity of plants has been demonstrated in several studies **(Ivanova et al., 2005; Wojdyło et al., 2007; Chrpová, et al., 2010)**.

Higher antioxidant activity of frozen herbs (values expressed on dry matter) was found compared to fresh herbs. The reason could be damage of herb tissues due to the formation of crystals during freezing and subsequently increased release of secondary metabolites into the solvent **(Keinänen and Julkunen-Tiitto, 1996)**. It should be noted that the results and conclusions of studies investigating the effect of freezing on the antioxidant activity of plants are not always unambiguous and conclusive as mentioned for example by **Chan et al., (2014)**.

A statistically significant difference in antioxidant activity of fresh, dried and frozen herbs determined by DPPH in values calculated per 100 g of plant corresponds with the results of many studies **(Hossain et al., 2010; Kouřimská et al., 2014)**, but they also show a statistically significant difference even after recalculation to dry matter content. **Hossain et al., (2010)** explain the lower antioxidant activity of fresh herbs by the presence of active enzymes that may cause degradation of the antioxidants. Antioxidant activity determined by DPPH was in all cases higher in oregano samples then in Greek oregano samples, concurring with the findings of **Chrpová et al., (2010)**.

The highest content of total phenols was in most cases determined in samples of dried herbs, as reported by **Hossain et al., (2010)** and **Kouřimská et al., (2014)**. It is caused by the release of phenolic compounds and increase of antioxidant activity when the cell tissues of herbs are damaged during the drying process. Comparing the total phenolic content of fresh and dried herbs there was no statistically significant difference which is in line with **Adámková et al., (2015)**.

Comparison of extracted volume of essential oil and its composition

Extraction of the essential oils from the fresh, dried and frozen samples gave significantly higher yields from samples of Greek oregano (6.3, 14.8 and 8.2 $mL.kg^{-1}$ respectively) than for oregano (0.2, 3.2 and 1.7 $mL.kg^{-1}$ resp.). The lowest yield was in case of fresh samples. **Baranauskienė et al., (2013)** also found a higher yield at the Greek oregano compared with oregano and all samples that they harvested in different periods.

The main components of fresh oregano essential oil analysed by GC-MS were β-cubebene, caryophyllene, γ-terpinene and germacene D (Table 4). **Mockutë et al., (2004)** and **Şahin et al., (2004)** also determined germacene D and caryophyllene as the major components of oregano essential oil. The high content of γ-terpinene is consistent with results of **Ličina et al., (2013)**. Other studies found additional components which is influenced by many factors such as growing conditions, locality, time of harvest, extraction method etc. **(Ozkan et al., 2010; Tibaldi et al., 2011; De Falco et al., 2013; Kawase et al., 2013)**. Cubebol, sabinene and α-terpinene were found only in the essential oils of dried and frozen herbs, while β-cubebene and β-phellandrene were only in the samples of fresh oregano.

Carvacrol was the main component of Greek oregano essential oil samples (Table 5), its highest content was in the fresh sample. The second major component was γ-terpinene and (E)-β-ocimene. **Zheng et al., (2009)** and **Stefanakis et al., (2013)** also reported carvacrol and γ-terpinene as major compounds. The results show that the various post-harvest treatments cause the changes in the composition and the content of essential oils components. This corresponds to the studies of **Novák et al., (2011)** who described the changes in the composition of the Greek oregano essential oil treated by various types of drying.

Najafian (2014) observed different representation of ingredients of lemon balm essential oil as a result of the storage of herbs in the freezer, refrigerator and room temperature.

The smallest changes in the composition of essential oils were found during the storage of herbs in the freezer and refrigerator compared to storage at room temperature. The highest level of carvacrol was found in the essential oil of fresh oregano Greek, while the lowest amount was in the frozen sample. Reduced proportion of carvacrol was always associated with increased content of its precursor γ-terpinene. This phenomenon was also highlighted by **Novák et al., (2011)**. High concentration of carvacrol in the essential oil of Greek oregano is responsible for its antioxidant activity.

CONCLUSION

The antioxidant activity and the composition of essential oils of selected plants of the genus *Origanum* were affected by post-harvest treatment of plants. All fresh, dried and frozen herbs showed a considerable potential for quenching the free DPPH radical. Significantly higher antioxidant activity was found in dried herbs comparing to fresh and frozen, but only in case of values calculated per 100 g of the sample. However, the differences were not statistically significant after recalculation when expressed on dry matter content. There was no difference between fresh and frozen samples. The content of total phenols was significantly higher in dried than in frozen herbs in values recalculated per 100 g of sample. A strong correlation between the results of DPPH and TPC was found again only for values expressed per 100 g of the sample. Post-harvest treatment of herbs affects the composition of their essential oils. The dominant essential oil component of Greek oregano is carvacrol with a proportion of 60% or more. On the contrary, there is no such dominating component in oregano essential oil but there are more components with a share of 10 to 20%.

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