

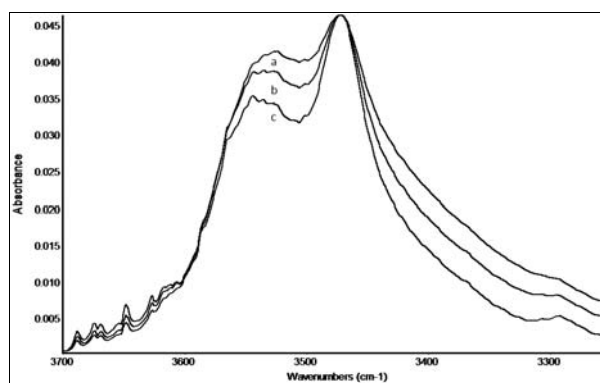
FTIR SPECTROSCOPIC EFFICIENCY OF EUGENOL METHYLEETHER ADDITIVE AS FREE RADICAL SCAVENGER AGENT TO SUNFLOWER COOKING OIL AT HIGH TEMPERATURE AND DIFFERENT CONCENTRATIONS

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A rapid and precise method for the evaluation efficiency of Eugenol methylether additives as free radical scavenger agent at three different concentrations to heated sunflower oil at 160°C for ten hours using Fourier transform infrared (FTIR) spectroscopy for analysis was carried out. Before any additives, three characteristic absorption bands were observed at 3544, 3473 and 3290 cm^{-1} . The recorded bands were assigned to the unbounded, bounded hydroperoxide or free alcohols groups and to the O-H stretching alcohol associated group, respectively. After the addition of Eugenol methylether at 0.8 mL/L concentration, the hydroperoxide formation content as the result of thermal oxidation is reduced. It has been shown that the free radical scavenger agent plays an acceptable role in reducing the hydroperoxide formation content as the result of thermal oxidation.



INTRODUCTION

Cooking oils become deleterious and unhealthy as a result of many uses at high temperature causing an oxidation to these oils; this oxidative process is the major reason of cooking oils deterioration. Lipids cooking oils contains mainly the following three carboxylic acids with these percentages presented in the studied sunflower oil: oleic acid (14-35%), linoleic acid (55-75%) and linolenic acid (<0.3%) which is considered the most important oil crops.

Lipids are susceptible to oxidative processes in the presence of catalytic systems such as light, heat, enzymes, metals, metalloproteins, and micro-organisms, giving rise to the development of off-

flavors and loss of essential amino acids, fat-soluble vitamins, and other bioactives. Lipids may undergo autoxidation, photo-oxidation, thermal oxidation, and enzymatic oxidation under different conditions, most of which involve some type of free radical or oxygen species formation.¹⁻³ Autoxidation is a spontaneous reaction between atmospheric oxygen and lipids which can be accelerated at high temperature during deep-fat frying. This process reaction is called thermal oxidation leading to the increase of free fatty acids and polar matter contents, foaming, color changing and viscosity.⁴ Thermal oxidation of unsaturated fatty acids occurs via a free radical chain reaction that proceeds through three steps of initiation, propagation, and termination, full details and the

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scheme explaining the mechanism can be seen in the following references.^{1,5} Numerous analytical methods are routinely used for measuring lipid oxidation in foods. The available methods to monitor lipid oxidation in foods can be classified into five groups based on what sort of measurement: the absorption of oxygen, the loss of initial substrates, the formation of free radicals, and the formation of primary and secondary oxidation products.⁶ Depending on the instrumental analysis used, many lipid oxidation parameters can be obtained. Over the past 20 years, as a result of the development of Fourier transform infrared (FT-IR) spectroscopic instrumentation, the application of this technique expanded in food research and particularly has become a powerful analytical tool in the study of edible oils and fats. Fourier transform infrared (FTIR) method is a finger print spectroscopy and usually used for the determination of peroxide value, PV frequency very rapidly.

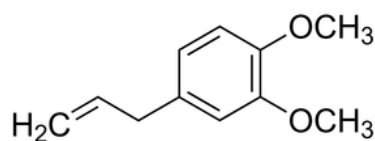
The advantages of this method are: rapid and non-destructive technique, no sample preparation, reduces or eliminates solvents and chemical reagents which are hazardous to human health or to environment; therefore, FTIR spectroscopy can be classified as green analytical chemistry technique. Hydroperoxides can quantitatively be determined by FTIR spectroscopy via the measurement of their characteristic O-H stretching absorption band.⁷ An absorption band at about 2.93 μm (3412 cm^{-1}) indicates the generation of hydroperoxides, whereas the replacement of a hydrogen atom on a double bond or polymerization accounts for the disappearance of a band at 3.20 μm (3125 cm^{-1}). The formation of aldehydes, ketones, or acids gives rise to an extra band at 5.72 μm (1748 cm^{-1}). Furthermore, *cis*-, *trans*-isomerization and formation of conjugated dienes can be detected through the changes in the absorption band in the range of 10 μm to 11 μm ($910\text{-}1000\text{ cm}^{-1}$).⁸ The determination of peroxide value, PV by Fourier transform infrared spectroscopy was carried out first by van de Voort and co-workers.^{9, 10} It seems that FTIR spectroscopic method is an efficient technique to determine the peroxide value as well as the antioxidant efficiency in edible oil.

Van de Voort and co-workers developed a spectral reconstitution (SR) technique to facilitate mid-FTIR transmission analysis of inherently viscous samples and simplified the determination of peroxide value of edible oils.¹¹ Very recently,

Rohman and Che Man reported the application of FTIR spectroscopy for monitoring the stabilities of selected vegetable oils including sunflower oil during thermal oxidation at 160°C for 120 hr.¹² It has been found that there were several absorbance changes between non-oxidized and oxidized vegetable oils during thermal oxidation. The peak intensities at 3470, 1655 and 967 cm^{-1} were increased; meanwhile peak intensities at 3008 and 722 cm^{-1} were decreased.¹²

Hamed and Allam reported the application of FTIR spectroscopy in the determination of antioxidant efficiency in sunflower oil.¹³ They found that the intensity of the hydroperoxide absorption band in the infrared spectra was increased proportionally with the increase of hydroperoxide concentration. The Fourier transforms infrared spectroscopy for authentication of functional food oils has been carried out and proved to be an efficient method for authentication food oils.¹⁴

FT-IR spectroscopy is used also as an effective analytical tool for the determination of extra virgin olive oil adulteration with lower priced vegetable oils (sunflower oil, soya bean oil, sesame oil, corn oil).^{15,16} Eugenol methylether, $\text{C}_{11}\text{H}_{14}\text{O}_2$ (1,2-dimethoxy-4-prop-2-enylbenzene, IUPAC name) is an antioxidant species and widely used as food flavoring agent and found naturally in the essential oil of *ocimum sanctum L.* plant which is belonging to Lamiaceae family.¹⁷ It has the following chemical structure.



Scheme 1 – Chemical structure of Eugenol methylether.

This paper reports an attempt to investigate reducing the thermal oxidation of sunflower cooking oil heated at 160°C and the efficiency of adding Eugenol methylether as free radical scavenger agent at three different concentrations using FTIR spectroscopy technique for analysis.

EXPERIMENTAL

1. Chemicals and Sample Preparation

Chemicals used in the analysis and cleaning of the infrared cells were of spectroscopic grades and were purchased from

Merck. Eugenol methylether was purchased from Aldrich as food grade additives and used as received. The investigated sunflower oil samples applied in this study were purchased from local shops which are usually used in food preparation for frying chips, chicken, fish and falafel, etc.

2. FTIR spectra acquisition

The infrared spectra were recorded on Nicolet 6700 in the range 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1} at 64 scans as a liquid cell. The instrument is equipped with DTGS detector and KBr beam splitter. The operating system used was the OMNIC software (Version 7.3, Thermo Nicolet, USA). The samples were injected between two well-polished KBr windows separated by Teflon thin layer spacer in order to increase the path-length of the beam which leads to enhance the peroxide absorption bands obviously according to Beer-Lambert law. The thickness of Teflon spacer is 0.0911 mm and was calculated by the following equation:

$$L(\text{in mm}) = \frac{10n}{2(\nu_1 - \nu_2)}$$

Where n presents the number of fringes counted, ν_1, ν_2 presents the starting and ending fringes frequency in wavenumbers (cm^{-1}), while L is cell thickness (mm). The constant in the equation 10, converts the unit from centimeter to millimeter.

RESULTS AND DISCUSSION

Fig. 1 shows the FTIR absorption spectrum of virgin sunflower oil taken at room temperature

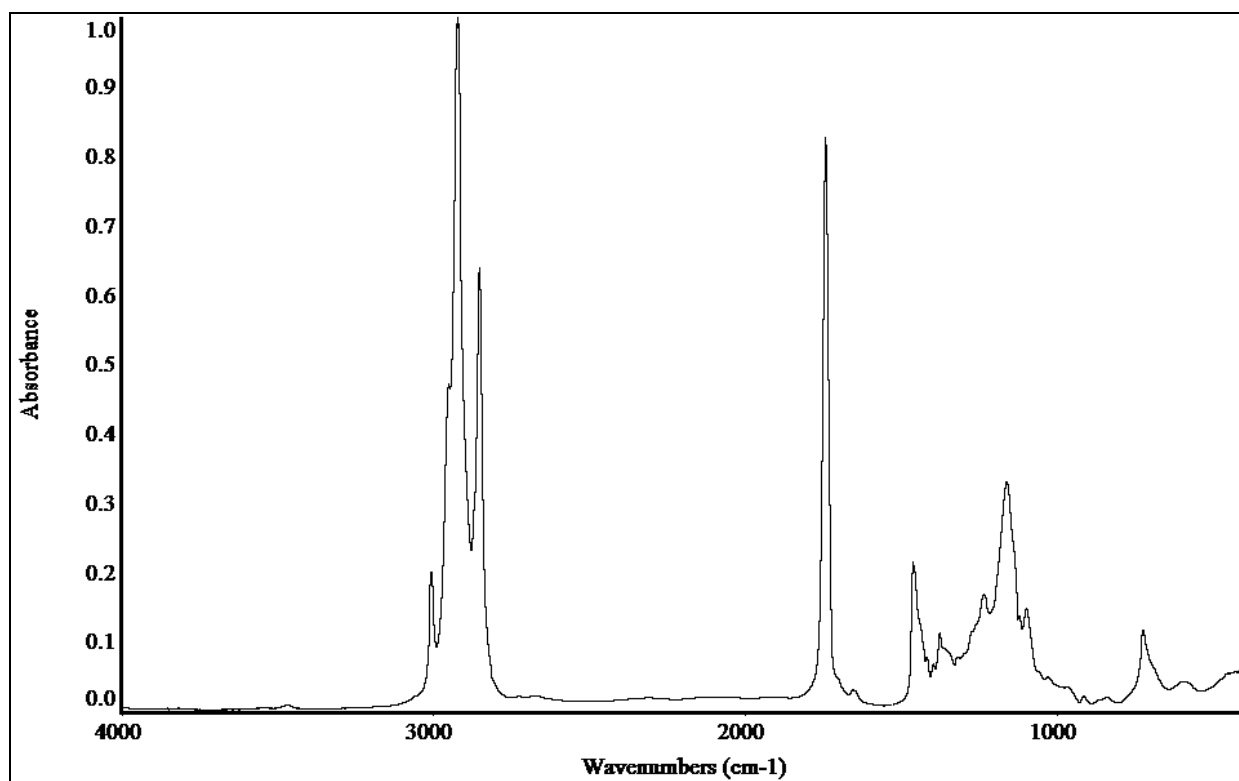


Fig. 1 – FTIR absorption spectrum of virgin sunflower oil at room temperature.

without any additives in the range 500-4000 cm^{-1} . The recorded spectrum shows a typical characteristic of absorption bands for common triglyceride substances; it is the main component composed edible fats and oils. The observed frequencies are in consistent with the earlier reported literature.^{15,18} The recorded spectrum has been assigned as follows: The band at about 3009 cm^{-1} is attributed to the stretching vibration of *cis*-double bond =C-H presented in the investigated oil. By the way, oils with high proportion of linolenic or linoleic acyl groups as in sunflower oil show higher frequency intensity at 3009 cm^{-1} than oils with high proportion of oleic acyl groups.

Bands in the region of 2954–2926 cm^{-1} are due to C-H asymmetric and symmetric stretching vibrations, respectively. The vibrations frequencies of methylene ($-\text{CH}_2-$) and methyl ($-\text{CH}_3$) groups can be observed at 2925 and 2855 cm^{-1} , respectively. The bending frequencies of Methylene and methyl groups are observed at 1465 cm^{-1} and 1378 cm^{-1} . The band at 1747 cm^{-1} is due to stretching vibration frequency of the carbonyl group, C=O. Deformation and rocking of C-H and stretching vibration of C-O bands are in the range 1500–650 cm^{-1} .

In order to clarify the infrared hydroperoxide bands, Teflon spacer was used in the infrared cell for increasing the path length.

Triplicate measurements of about 1-2 mL of each sunflower oil sample were injected between the two KBr windows in the percent of Teflon spacer. Fig. 2 shows a set of six spectra of sunflower oil including the reference unheated oil (0 h) and the five remaining spectra were the result of heating constantly the oil at 160°C for ten hours with continuous stirring using magnetic stirrer and the spectra were recorded after cooling down the samples to room temperature after every two hours up to 10 hours of heating under the same conditions in the range 3250-3700 cm^{-1} . Three characteristic absorption bands were observed at 3544, 3473 and 3290 cm^{-1} .

The formation of first two bands indicates the generation of hydroperoxide groups as the result of oxidation. The first two frequencies at 3544, 3473 cm^{-1} were assigned to the unbounded and bounded hydroperoxide groups, respectively while the third frequency at 3290 cm^{-1} assigned to the O-H stretching alcohol associated group. The first observed band at 3544 cm^{-1} is consistent with the

earlier reported work which was detected for the unbounded hydroperoxides groups at 3550 cm^{-1} .¹⁸ The second band at 3473 cm^{-1} is very close to the band observed at 3511 cm^{-1} which was assigned to free alcohols and bounded hydroperoxides groups.¹⁹ Some reporters assigned the band at 3473 cm^{-1} to ester linkage carbonyl overtone band which has a frequency at 3475 cm^{-1} .

After six hours of heating, the hydroperoxide absorption bands increase proportionally with the increasing heating time duration.

The O-H stretching associated alcohol group decreases after six hours of heating and the band centre was shifted slightly to higher frequency. There is a reciprocal relationship in the term of intensity between the hydroperoxide absorptions and the O-H stretching alcohol group band. The decreasing in the alcohol associated group intensity could be attributed to further oxidation state and transferring either to ketones or aldehydes compounds causing further oxidation to the oil as a result of increasing time heating duration. Note that the band at 3544 cm^{-1} is resolved after ten hours of heating and could be supporting the last suggestion.

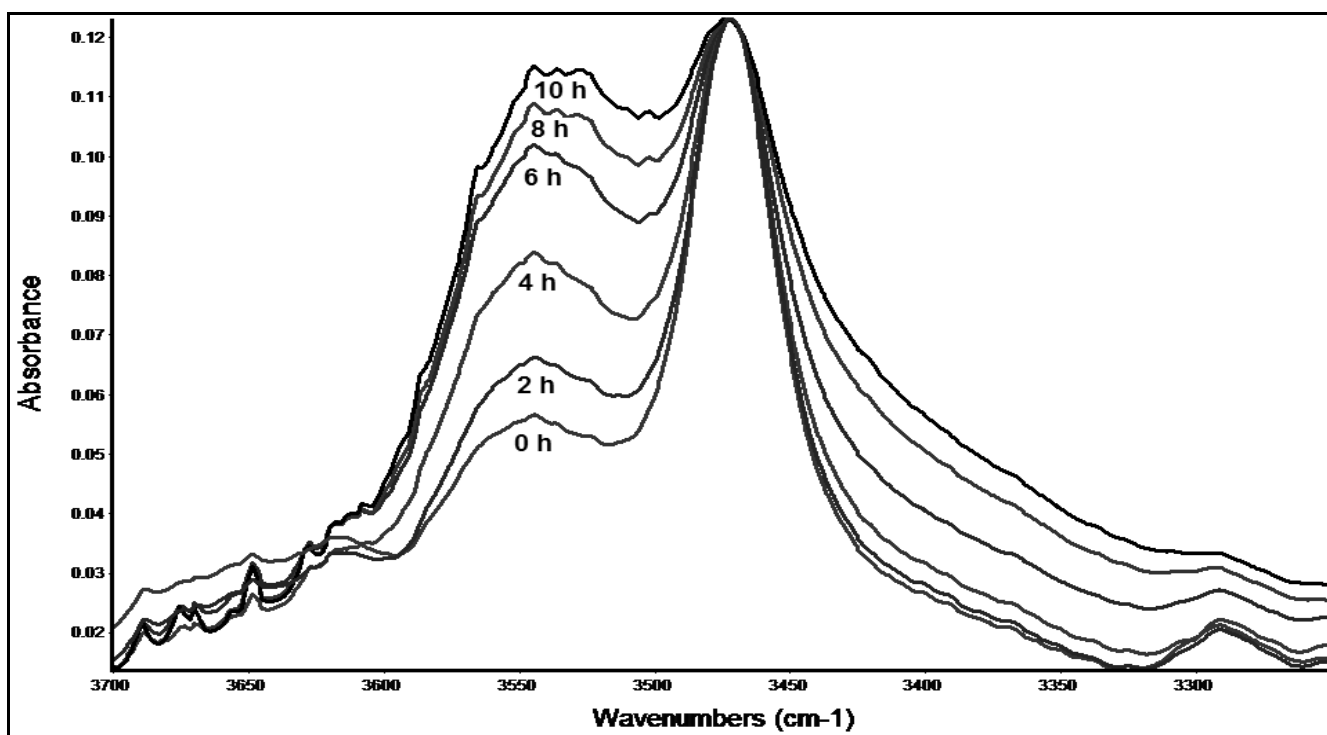


Fig. 2 – A set of six spectra of sunflower oil heated constantly at 160°C for 10 hours including the reference spectrum (0 h).

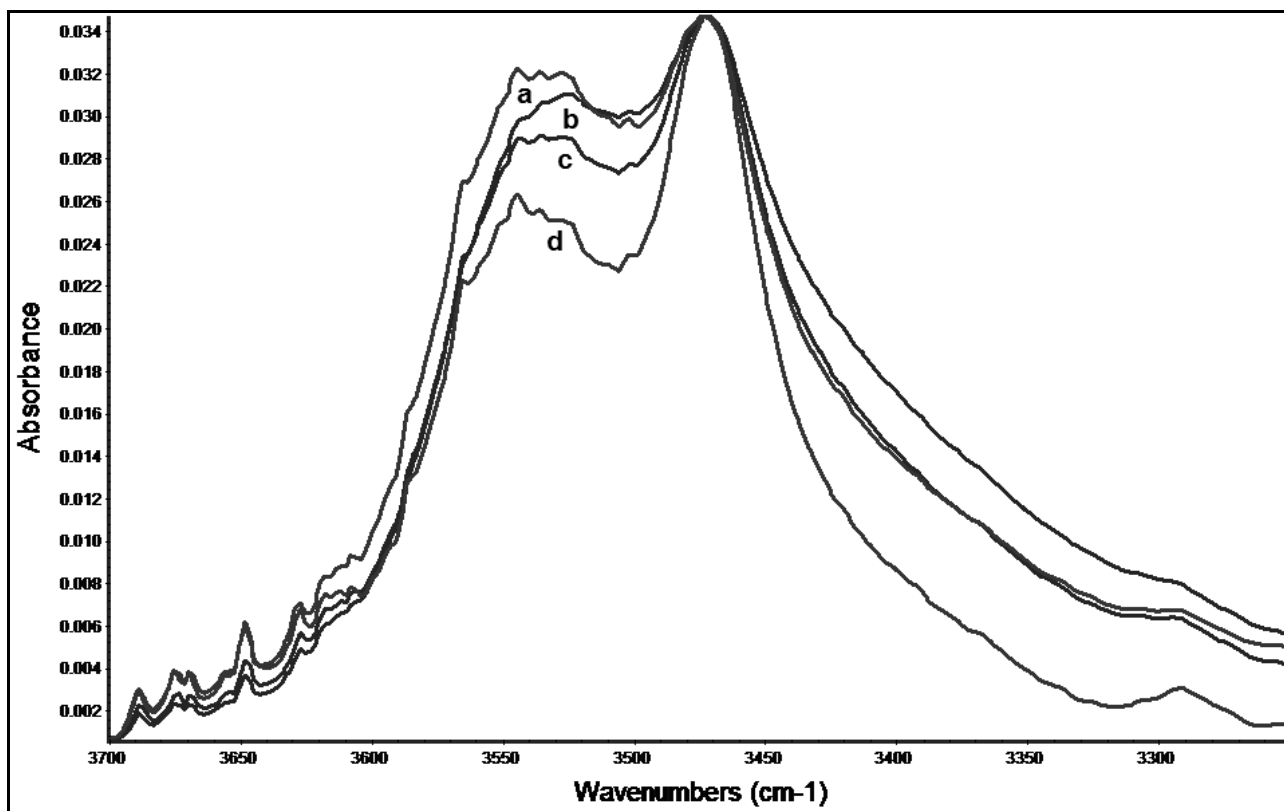


Fig. 3 – A set of four spectra of sunflower oil heated constantly at 160°C for 10 hours without any additive (a) and with three different concentration additives of Eugenol methylether at 0.8 mL/L (b), 1.2 mL/L (c) and 1.6 mL/L (d).

Having confirmed the formation of hydroperoxide groups, the next step was to use Eugenol methylether as free radical scavenger agent and to investigate the effect of this additives compound on the heated oil. By the way, as it was stated before, one of the aims of this work was to eliminate or reduce the thermal oxidation process using synthetic or natural antioxidants additives. Fig. 3 shows a set of four different spectra in the range 3250-3700 cm^{-1} with three different added concentrations of Eugenol methylether to the sunflower oil at 0.8 mL/L, 1.2 mL/L and 1.6 mL/L, including the reference unheated oil. The mixture of 250 mL of sunflower oil with the added concentration was heated separately into three different beakers at 160°C for ten hours with stirring using magnetic stirrer and the spectra were recorded after cooling down the samples to room temperature.

Some changes in the FT-IR spectra were observed. The spectral region between 3250 and 3700 cm^{-1} undergoes some changes after the addition of Eugenol methylether at three different concentrations. The band frequency centre of the unbounded hydroperoxides groups at 3544 cm^{-1} is shifted generally to lower frequencies after the

addition of free radical scavenger agent. The band at 0.8 mL/L (a) and 1.2 mL/L (b) concentrations was shifted to 3536 and 3540 cm^{-1} , respectively and the band feature is different from that of 10 hours of heating without any addition. While at 1.6 mL/L (c) concentration, the bounded hydroperoxide frequency has no changes. Also, the shape and the intensity of the band for the first two added concentrations are varied and decreased in comparison with the recorded spectrum in Fig. 2. It is known that there is a relationship between intensity and concentration, by decreasing the intensity of unbounded hydroperoxides groups leading to reduce the concentration of hydroperoxide content and that can be seen clearly in the recorded FTIR spectra proving that this technique is efficient, simple and fast and can be applied to evaluate the thermal oxidation of edible oils including the efficiency of adding free radical scavengers. At all three added concentrations, the intensity of the free alcohols or bounded hydroperoxides group at 3473 cm^{-1} was reduced substantially and leading to reduce of the hydroperoxide formation content after the addition of free radical scavenger agent. The O-H stretching associated alcohol group feature is almost

demolished after the addition of 0.8 mL/L concentration of Eugenol methylether. It can be deduced that the added free radical scavenger agent plays a very good part in reducing the hydroperoxide formation content as the result of thermal oxidation. It can be pointed out here that the reducing of the hydroperoxide formation content as the result of thermal oxidation using free radical scavenger agent, Eugenol methylether, at 0.8 mL/L is efficient and there is no need for extra addition of free radical scavenger since the reducing of hydroperoxide formation is matched with this concentration. It is recommended that biological and toxicological investigations should be taken prior to consider Eugenol methylether as free radical scavenger into heated edible oils.

CONCLUSIONS

It is proved that FTIR spectroscopy is an effective analytical tool for the investigation and evaluation of food additives and also very useful to evaluate the thermal oxidation of edible oils in a simple and fast way. It can be deduced that the added free radical scavenger agent plays a very good part in reducing the hydroperoxide formation content as the result of thermal oxidation and the concentration of the free radical scavenger agent, Eugenol methylether, at 0.8 mL/L is enough and efficient and has the potential for further investigations.

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