

# Effects of an antibiotic and two phytochemical substances (cinnamaldehyde and 1,8-cineole) on yolk fatty acid profile and storage period-associated egg lipid peroxidation level

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**ABSTRACT** - This study was aimed at determining the effects of two phytochemical antioxidants, namely, cinnamaldehyde and 1,8-cineole, and an antibiotic added to laying hen feed on the fatty acid profile of egg yolk and the weight loss and lipid peroxidation levels of eggs stored for different periods. Ninety-six 48-week-old Bovans White hens were randomly assigned to four groups, each with four replicates of six hens per replicate. The four groups were provided with the following feeds: maize and soybean-based laying hen feed, basal ration (control group); basal ration added 500 mg/kg of an antibiotic; basal ration added 100 mg/kg of cinnamaldehyde; and basal ration added 100 mg/kg of 1,8-cineole. At the end of an eight-week feeding schedule, 48 eggs, including 12 from each group, were used for yolk fatty acid analysis. In total, 240 eggs, including 48 eggs for each of the five different storage periods tested (1, 14, 28, 42, and 56 days), were collected for the detection of egg weight loss and yolk malondialdehyde (MDA) levels. The feed supplements cinnamaldehyde and 1,8-cineole were determined to have significantly reduced lipid peroxidation in the yolk of eggs stored for 14, 28, 42, and 56 days, when compared with the results of the control group and antibiotic-treated group. Furthermore, dietary cinnamaldehyde supplementation was determined to have decreased the yolk level of myristic acid, a saturated fatty acid, and to have increased the yolk level of oleic acid, the major unsaturated fatty acid found in egg yolk (46.28%) in comparison with the levels measured in the other three groups. Cinnamaldehyde and 1,8-cineole were determined to extend the shelf life of eggs by providing protection against free oxygen radicals. Cinnamaldehyde could be used as an alternative feed supplement to enrich the yolk fatty acid profile in unsaturated fatty acids.

**Keywords:** egg weight loss, malondialdehyde, myristic acid, oleic acid, oxidative stability

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## 1. Introduction

Eggs, which have an important place in the human diet, may undergo quality deterioration, depending on the storage temperature and length of the storage period (Lee et al., 2016; Yenilmez et al., 2017; Cimrin et al., 2019). Egg spoilage is explained by various mechanisms. Accordingly, one of these

mechanisms involves the increased susceptibility of eggs to bacterial infections as a result of the rapid degeneration of gallin, otherwise known as ovodefensin, a major component of the egg defence system, in the albumen (Liu et al., 2018). Another common mechanism is lipid peroxidation. The unsaturated bonds of the yolk fatty acids easily react with free radicals and generate peroxidation products, such that the rate at which peroxidation occurs is defined by the storage temperature and the length of the storage period of the eggs. The resulting peroxidation products not only deteriorate lipids, particularly fat-soluble vitamins and essential fatty acids, but also cause a foul odour and taste in eggs, and eventually decrease the quality and safety of eggs (Ramalho and Jorge, 2006). Eggs, which are rich in essential fatty acids, are reported to be more prone to oxidation due to their high level of unsaturated fatty acids (Hayat et al., 2010).

Previous research has shown that antioxidants added to feed are transferred to the eggs laid by hens that consume this feed, and thereby, prevent the oxidation of yolk fatty acids (Botsoglou et al., 2005; Ahmad et al., 2015). One of these antioxidants is cinnamaldehyde, which is the main component of the cinnamon plant and is found at a level of 90% in cinnamon essential oil (Kahraman, 2009; Friedman, 2017). Phenyl terpenes, such as cinnamaldehyde, thymol, menthol, and vanillin, show a strong antioxidant effect against lipid oxidation (Bizzo et al., 2009; Gouda et al., 2017). It has been reported that, in chickens fed rations supplemented with antioxidant herbal compounds, blood and intestinal malondialdehyde (MDA) levels decrease, and both immunity and performance status improve (Fascina et al., 2017). Haripriya and Vijayalakshmi (2014) reported that dietary cinnamaldehyde supplementation activates the anti-lipidemic defence mechanism and produces an antihypercholesterolaemic effect, which prevents the development of cardiovascular disease. Another phytogetic substance known for its strong antioxidant effect is 1,8-cineole (Estévez et al., 2007; Juergens et al., 2018). Being the main component of various herbal essential oils (i.e. eucalyptus, rosemary, sage, and daphne), the level of 1,8-cineole varies greatly with the plant species, harvest season, and geographical region (Karık et al., 2015; Cimrin and Demirel, 2016a).

Previous studies have shown that plant extracts added to feed improve antioxidant enzyme activity in laying hens exposed to heat stress (Torki et al., 2018) and increase the lipid oxidation stability of eggs stored at 25 °C (Batista et al., 2017). It has also been suggested that the supplementation of laying hen feed with such extracts would enable the use of eggs as a major source of unsaturated fatty acids in the human diet (Hayat et al., 2010; Yi et al., 2014; Cimrin and Demirel, 2016b; Kutlu and Şahin, 2017; Batista et al., 2017). Similar feed supplementation practices would also increase the level of unsaturated fatty acids in muscle tissue (Aghwan et al., 2014). Diets enriched in unsaturated fatty acids would directly affect the blood lipid profile (Chilliard et al., 2007). In this context, it is considered that such functional food products would allow some flexibility to strict diets designed for the prevention of cardiovascular diseases. In fact, Shinn et al. (2018) reported that the new dietary guidelines for Americans suggested not to reduce or cancel out the level of dietary fat, but to optimize the types of fat consumed.

This study was aimed at determining the effects of an antibiotic and two phytogetic antioxidants [cinnamaldehyde (99%), 1,8-cineole (98.63%)] on the weight loss, MDA levels, and yolk fatty acid profile of eggs observed with different egg storage periods.

## 2. Material and Methods

The study material comprised eggs laid by 48-week-old 96 Bovans White hens during an eight-week feeding schedule. The feeding trial was conducted in an experimental hall of poultry company in Mucur, Kırşehir, Turkey (39°04'02.4" N and 39°19'17.4" E).

The hens were randomly allocated to four groups, each with four replicates of six hens per replicate. A laying hen feed mixture, prepared in accordance with the nutrient recommendations of the National Research Council (NRC, 1994) was used as the basal ration. Group I, which was maintained for control purposes, fed the basal ration alone. The other three groups fed the basal ration supplemented with 500 mg/kg of an antibiotic (chlortetracycline), 100 mg/kg of cinnamaldehyde (99% purity), and

100 mg/kg of 1,8-cineole (98.63% purity), respectively, for a period of eight weeks. The antibiotic (chlortetracycline) (Vimar Food Agriculture and Livestock Inc.), cinnamaldehyde, and 1,8-cineole (Agromiks Feed Additives, Livestock and Food Industry and Trade Limited Company) were purchased from business firms. While the cinnamaldehyde feed additive contained 99.00% of cinnamaldehyde, 0.44% of benzaldehyde, and 0.19% of thymol, the 1,8-cineole supplement contained 98.63% of 1,8-cineole, 0.48% of limonene, 0.43% of cinnamaldehyde, 0.17% of para-cymene, 0.11% of alpha-phellandrene, 0.09% of beta-myrcene, and 0.07% of alpha-pinene.

At the end of the study period, the yolks of 48 eggs, including 12 eggs (three eggs × four replicates) from each group, were analysed according to the TS EN ISO 12966: 2 (2017) method (Anonymous, 2017).

During the last five days of the study, eggs were collected for use in analysis. From each group, 48 eggs were stored (12×4 = 48 eggs) for each of the tested storage periods. For the five different storage periods tested (1, 14, 28, 42, and 56 days), in total 48×5 = 240 eggs were stored.

The eggs were stored in the Kırşehir province, located in the Central Anatolian region of Turkey, during the months of July and August, at 40-42% humidity and a mean temperature range of 22-34 °C, under room conditions in covered carton boxes.

Before being placed in the carton boxes for storage, the eggs were weighed on a precision balance (to an accuracy of 0.01 g), and the weights were recorded. At the end of each storage period, the eggs were weighed for a second time to calculate the absolute (g) and relative (%) egg weight losses.

At the end of each storage period, yolk MDA analysis was performed as described by Buege and Aust (1978).

The data obtained in the study was analysed using the Statistical Package for Social Sciences (SPSS, Version 21.0) software (Inc., Chicago, IL, USA, 2012) by one-way analysis of variance (ANOVA). The significances of the mean differences among the groups were compared with Duncan's test.

The statistical model used to test the effect of treatment was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

in which  $Y_{ij}$  = represents the j-th observation on the i-th treatment,  $\mu$  = overall mean,  $T_i$  = the main effect of the i-th treatment, and  $e_{ij}$  = random error present in the j-th observation on the i-th treatment.

### 3. Results

When compared with the eggs of the control group, no difference was observed for egg weight loss in the eggs laid by the groups that received an antibiotic, cinnamaldehyde, and 1,8-cineole in feed ( $P > 0.05$ ; Table 1). However, egg weight loss increased with prolonged storage (Figure 1).

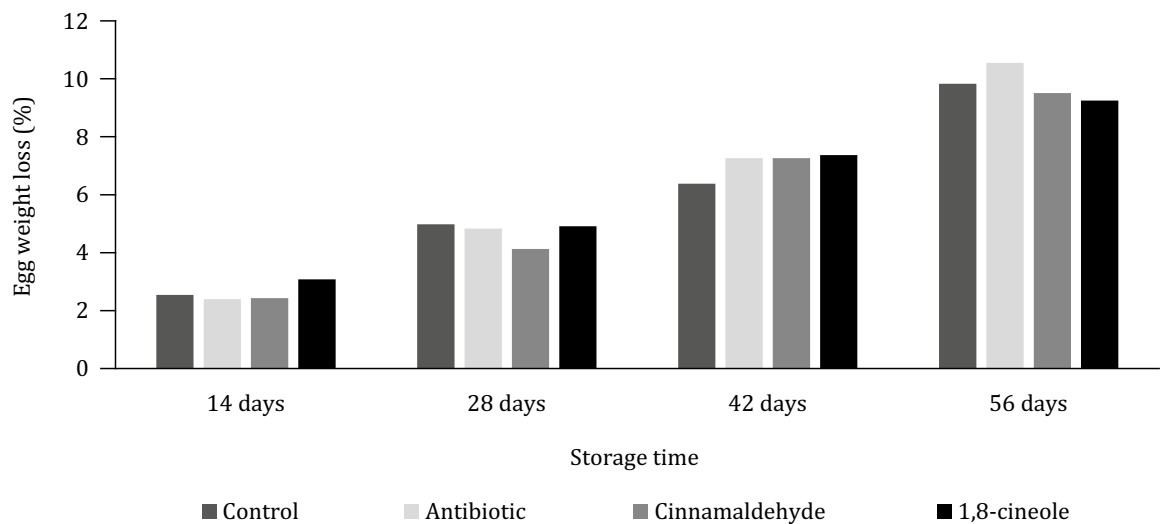
**Table 1** - Absolute and relative egg weight losses detected in the eggs of the study groups for the different egg storage periods

Group	Storage time (days)							
	14		28		42		56	
	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)
Control	2.54	3.92	4.98	7.43	6.35	10.22	9.83	15.43
Antibiotic	2.39	3.91	4.82	7.86	7.25	11.60	10.55	16.60
Cinnamaldehyde	2.43	3.76	4.13	6.40	7.26	11.23	9.51	14.47
1,8-cineole	3.08	4.71	4.96	7.83	7.37	11.18	9.25	14.57
P	0.371	0.381	0.358	0.273	0.441	0.510	0.461	0.465
SEM	0.15	0.21	0.19	0.29	0.25	0.38	0.30	0.53

g - absolute weight loss; % - relative weight loss; P-value - significance level; SEM - standard error of the mean.

The yolk MDA levels measured at the end of the different storage periods tested (14, 28, 42, and 56 days) in the eggs laid by the hens that received cinnamaldehyde and 1,8-cineole in the feed, were significantly lower than the levels measured in the eggs laid by the control group and antibiotic-treated group ( $P < 0.01$ ;  $P < 0.001$ ; Table 2).

The assessment of the differences in the yolk fatty acid profile of the eggs demonstrated that, when compared with the control group and cinnamaldehyde-treated group, feed supplementation with 1,8-cineole significantly increased the yolk myristic acid (C14:0) level ( $P < 0.05$ ; Table 3). The level of palmitoleic acid (C16:1) was higher in the eggs of the antibiotic-treated group, compared with those of the control, cinnamaldehyde-, and 1,8-cineole-treated groups ( $P < 0.001$ ; Table 3). While yolk myristic acid (C14:0) levels were significantly lower in the eggs of the group that received cinnamaldehyde in feed and control group, when compared with the eggs of the groups that were given an antibiotic and 1,8-cineole in feed ( $P < 0.05$ ), yolk oleic acid (C18:1n9c) levels were determined to be significantly higher than those of all the remaining groups ( $P < 0.01$ ; Table 3). No significant difference was detected between the groups for saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) ( $P > 0.05$ ). The highest MUFA level (50.31%) and the lowest PUFA level (9.45%) were detected in the eggs laid by the group given cinnamaldehyde-supplemented feed.



**Figure 1** - Relative weight losses in the eggs of the study groups for the different egg storage periods (%).

**Table 2** - Yolk malondialdehyde levels for the different egg storage periods (nmol/mg)

Group	Storage time (days)				
	1	14	28	42	56
Control	0.202	0.249a	0.337a	0.385a	0.434a
Antibiotic	0.190	0.235ab	0.322a	0.365a	0.401a
Cinnamaldehyde	0.181	0.216b	0.273b	0.305b	0.323b
1,8-cineole	0.187	0.220b	0.278b	0.311b	0.332b
P	0.316	0.003	0.010	0.002	0.000
SEM	0.004	0.003	0.007	0.008	0.008

P-value - significance level; SEM - standard error of the mean.

a, b - Means in the same column with different letters differ significantly ( $P < 0.05$ ).

**Table 3** - Egg yolk fatty acid profiles of the study groups (%)

Fatty acid	Group				P	SEM
	Control	Antibiotic	Cinnamaldehyde	1,8-cineole		
C14:0	0.357bc	0.431ab	0.326c	0.478a	0.020	0.02
C16:0	26.88	27.41	26.98	27.22	0.677	0.16
C18:0	13.01	12.28	12.92	12.77	0.542	0.18
C16:1	2.73b	3.35a	2.31b	2.67b	0.002	0.07
C18:1n9c	44.83b	43.79b	46.28a	44.98b	0.008	0.20
C18:1n7c	1.86	1.99	1.72	1.76	0.170	0.04
C18:2n6	10.32	10.73	9.45	10.12	0.308	0.14
SFA	40.26	40.12	40.23	40.48	0.976	0.28
MUFA	49.42	49.14	50.31	49.40	0.197	0.19
PUFA	10.32	10.73	9.45	10.12	0.308	0.14

a,b - Significant difference among the averages having different letters in the same row ( $P < 0.05$ ).

Myristic acid (C14:0); palmitic acid (C16:0); stearic acid (C18:0); palmitoleic acid (C16:1); oleic acid (C18:1n9c); vaccinic acid (C18:1n7c); linoleic acid (C18:2n6); SFA - saturated fatty acids (C14:0 + C16:0 + C18:0); MUFA - monounsaturated fatty acids (C16:1 + C18:1n9c + C18:1n7); PUFA - polyunsaturated fatty acids (C18:2n6); P-value - significance level; SEM - standard error of the mean.

## 4. Discussion

The results of the present study demonstrated that the addition of an antibiotic, cinnamaldehyde, and 1,8-cineole to laying hen feed did not affect egg weight loss. The weight losses detected in the eggs were determined to be associated with the length of the storage period (14, 28, 42, and 56 days), such that longer storage periods significantly increased egg weight losses (Table 1; Figure 1). On the other hand, egg weight losses, which exceeded the normal level and increased rapidly as of the 14th day of storage, were attributed to high storage temperatures, for the mean temperature of the storage room (22-34 °C) was rather high due to the season being summer, and the egg albumen is considered to have lost water more rapidly by gas exchange through the eggshell pores at high storage temperature (34 °C).

The results of several previous studies are in support of this opinion. Indeed, Yenilmez et al. (2017) reported that egg weight loss occurred at higher rates during the summer season and under the storage conditions of wholesalers (33 °C), which were determined to quicken the spoilage of eggs. These researchers also reported that while storage at +4 °C during both summer and winter extended the shelf life of eggs, the egg quality, which they graded based on the Haugh unit score, varied greatly with the storage season, length of storage period, and wholesaler and consumer storage conditions.

Jin et al. (2011) determined that increased storage temperatures and prolonged storage periods significantly increased egg weight loss. Chung and Lee (2014) affirmed that while increased storage temperature accelerated egg weight loss, prolonged storage increased egg weight loss, and both increased temperature and prolonged storage reduced egg quality. Batista et al. (2017) reported that while the internal quality traits of eggs stored at a controlled storage temperature (25.0 °C) and refrigeration temperature (5.0 °C) were adversely affected by prolonged storage (15-30 days) and high storage temperature, these adverse effects could be reduced by supplementing the feed of laying hens with organic minerals and 200 mg/kg of rosemary oil. Cimrin et al. (2019) reported that the inclusion of natural and synthetic antioxidants in laying hen feed did not show any effect on egg weight, but indicated that the two different storage temperatures (room temperature: 22-34 °C, refrigeration temperature: 4 °C) and five different storage periods (1, 14, 28, 42, and 56 days) they investigated significantly affected egg weight, such that prolonged storage and increased storage temperature augmented egg weight loss.

In the present study, the MDA levels measured in the eggs collected at the end of the study after being stored for one day in a storeroom did not show any significant difference between the study

groups. However, measurements performed at 14-day-intervals between days 14 and 56 of storage showed that the yolk MDA levels of the eggs laid by the groups that received cinnamaldehyde and 1,8-cineole in feed were significantly lower than the levels measured in the eggs of the control and antibiotic-treated groups.

These results suggest that, when consumed in feed, cinnamaldehyde and 1,8-cineole are transferred to the eggs laid by the hens, which receive these supplements, and slow down peroxidation in the yolk, rich in unsaturated fatty acids, of eggs stored under room conditions in summer. Furthermore, it was observed that in the control and antibiotic-treated groups, which did not receive any antioxidant supplement, the level of free radicals increased with higher storage temperature and prolonged storage, and eventually led to increased levels of MDA, as an indicator of peroxidation having occurred. Karabulut and Gülay (2016) indicated that unfavourable environmental conditions increase the generation of free radicals and cause oxidation, and suggested that antioxidant supplementation is required under such circumstances as the available level of antioxidants falls short.

Several other literature reports have been published, all of which support the results of this study. Gouda et al. (2017) determined that three different concentrations of trans-cinnamaldehyde, thymol, menthol, and vanillin significantly increased the antioxidant activity, and thereby, significantly altered the physical characterization of egg yolk. Ahmad et al. (2015) reported that antioxidant compounds were transferred from feed into eggs, and thereby, inhibited lipid peroxidation and extended the shelf life of eggs. Torki et al. (2018) suggested that the supplementation of feed with some plant extracts improved antioxidant enzyme activity in laying hens exposed to heat stress.

Batista et al. (2017) determined that the addition of 200 mg/kg of rosemary oil and organic minerals to feed improved the lipid oxidation stability of eggs stored at 25 °C. In another study conducted by Bayoumi and Helmy (2015), sage (in powder and oil form), containing 1,8-cineole as the main active ingredient, significantly decreased the yolk MDA level, which is used as a lipid peroxidation marker. In their research on the antioxidant activity of 1,8-cineole (eucalyptol), Juergens et al. (2018) determined that this monoterpene inhibited the superoxide radical and hydrogen peroxide and showed a strong antioxidant effect against reactive oxygen species (superoxide radical, hydrogen peroxide, hydroxyl radical). Researchers have reported that in the event of antioxidant shortage, hydrogen peroxide, in the presence of the superoxide radical, generates the most reactive and damaging free oxygen radical, the hydroxyl radical (Myers et al., 1985; Maza and Frishman, 1987).

The hydroxyl radical has been reported to peroxidise the unsaturated fatty acids in biomembranes, and thus, to cause tissue damage (Cross et al., 1987). Cinnamaldehyde and 1,8-cineole decrease yolk MDA levels by exerting a strong antioxidant effect against reactive oxygen radicals. However, when incorporated in feed, the impact of antioxidants on the oxidative stability of eggs depends on the type of the antioxidant used, the amount of the antioxidant added to feed, and the length of the storage period of eggs. Cimrin and Demirel (2016a) reported that while rosemary essential oil containing 45.04% of 1,8-cineole showed an antioxidant effect and reduced yolk MDA levels when added at a low level (100 mg/kg) into feed, no such antioxidant effect was observed when it was supplemented at high levels (200 and 300 mg/kg). Thus, phytogetic antioxidants provide protection against reactive oxygen species in animals that consume feed supplemented with these substances, as well as in the food products obtained from these animals, and humans, who consume these food products, only when incorporated in feed at an optimum level. Indeed, Fascina et al. (2017) reported significantly decreased blood and intestinal MDA levels in laying hens fed a ration supplemented with a phytogetic antioxidant (20% cinnamon).

In the present study, the assessment made for the yolk fatty acid profile of the study groups demonstrated that dietary 1,8-cineole supplementation significantly increased myristic acid levels, in comparison with the control and cinnamaldehyde-treated groups ( $P < 0.05$ ). Cimrin and Demirel (2016b) reported that, while dietary supplementation with 100, 200, and 300 mg/kg of rosemary essential oil had no effect on yolk myristic acid level, oleic acid level significantly increased with the addition of 100 mg/kg of rosemary essential oil to feed and decreased with no antioxidant effect at

300 mg/kg. Rietjens et al. (2002) suggested that, when administered at high doses, some natural antioxidants could show an opposite effect by accelerating oxidation and increasing the degeneration of unsaturated fatty acids. Indeed, in the present study, it was determined that the active substance 1,8-cineole, used at a purity level of 98.63%, adversely affected yolk fatty acid levels by a mechanism that was not able to be explained. Therefore, it is considered that certain natural antioxidants show antioxidant effect when administered at a favourable dose, and the constituents of an essential oil may show a synergistic or antagonistic effect with each other.

In the present study, of the herbal essential oil constituents investigated, the terpenes 1,8-cineole and cinnamaldehyde were observed to show a protective effect against the lipid oxidation of egg yolk, but did not show a positive effect on the yolk fatty acid profile. The yolk level of palmitoleic acid, a monounsaturated fatty acid, was determined to be higher in the antibiotic-treated group, when compared with the control, cinnamaldehyde-treated, and 1,8-cineole-treated groups ( $P < 0.001$ ). However, given its low level in egg yolk, palmitoleic acid may not be as significant as oleic acid with respect to its percentile share in unsaturated fatty acids.

Previous research has shown that oleic acid, which is the major component of olive oil (70-85%) (Waterman and Lockwood, 2007), reduces reactive oxygen species levels (Gonçalves-de-Albuquerque et al., 2016), balances body weight (Bensinger and Tontonoz, 2008), and reduces the risk of cardiovascular disease and cancer (Perdomo et al., 2015; Medeiros-de-Moraes et al., 2018). The results of the present study demonstrated that the level of the primary yolk fatty acid, oleic acid, significantly increased in the group that received cinnamaldehyde in feed, compared with the other groups ( $P < 0.01$ ; Table 3). Furthermore, the highest MUFA and lowest PUFA levels were detected in the yolk of the eggs belonging to the cinnamaldehyde-treated group. On the other hand, Ding et al. (2017) reported that while feed supplementation with an essential oil mixture containing the active substances thymol and cinnamaldehyde showed no significant effect on the yolk fatty acid profile, SFA and MUFA levels decreased and PUFA levels increased with increased supplement levels. Abdulla et al. (2015) reported that the fatty acid profile of broiler chicken breast meat reflected the fatty acid composition of the feed provided to broiler chickens and suggested that the body fatty acid profile could change with the fatty acid composition of feed. The differences between the results of different studies could be due to a variety of reasons. However, these divergent results are mainly attributed to differences in the laying hen feed provided to the animals and differences in the constituents of the essential oils added to the feed.

## 5. Conclusions

The present study demonstrated that the two phytochemical antioxidants investigated could be used as alternative feed supplements to increase the oxidative stability of eggs. While cinnamaldehyde has a positive impact on the level of oleic acid in eggs, the active substance 1,8-cineole shows a negative impact on the same parameter. This result is significant in that not only is 1,8-cineole the major component of many herbs such as cinnamon, eucalyptus, rosemary, sage, and daphne, which have common use across the world, but also because alterations in the fatty acid composition of food of animal origin are directly associated with the development of obesity and cardiovascular disease in humans.

## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

Conceptualization: T. Cimrin and M.D. Avsaroglu. Data curation: T. Cimrin and R. Ivgin Tunca. Formal analysis: T. Cimrin and R. Ivgin Tunca. Funding acquisition: T. Cimrin. Investigation: T. Cimrin. Methodology: T. Cimrin. Project administration: T. Cimrin. Resources: T. Cimrin and M.D. Avsaroglu.

Software: T. Cimrin. Supervision: T. Cimrin and T. Ayasan. Validation: T. Cimrin. Visualization: T. Cimrin. Writing-original draft: T. Cimrin and T. Ayasan. Writing-review & editing: T. Cimrin, T. Ayasan and S. Küçükersan.

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