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ORIGINAL PAPER

Volatile Profiles of Rapeseed Oil Flavored with Basil, Oregano, and Thyme as a Function of Flavoring Conditions

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Abstract The flavoring of oils with herbs gives a specific taste and aroma to the oils and may increase their antimicrobial and antioxidant activity. The volatile aroma compounds in flavored rapeseed oil were studied as a function of flavoring conditions, by means of headspace solid-phase microextraction (SPME) followed by gas chromatography-mass spectrometry (GC-MS) analysis. Rapeseed oils were flavored with dried basil, oregano, and thyme at two different concentrations (3 and 6%) and were heated at different temperatures and for various time intervals, followed by filtration. In the headspace of flavored oils, the main volatiles of the dried herbs were detected. In general, the share of monoterpenes in the headspace of flavored oil was higher than in the original dried herbs, while sesquiterpenes and phenolic compounds were detected to a considerably lower extent in the oil than in the herbs. The concentrations of the volatiles detected in the oil increased with increasing heating time and temperature.

Keywords Flavored oil · Rapeseed oil · Basil · Oregano · Thyme · Flavoring conditions · Aroma

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Introduction

Herbs are used all over the world to improve the taste and flavor of food products. In addition, they have antioxidant [1–3] and antimicrobial [4, 5] properties, and can be beneficial in the prevention of different human diseases, such as breast, colon, and lung cancer [6], and thrombus formation [7]. The most important compounds in herbs which ensure these characteristics are phenolic compounds, terpenoids, and derivatives of fatty acids and amino acids [8]. Some of the biologically active compounds in herbs are oil-soluble and, therefore, herbs can be used to flavor edible oils. The infusion of oils with herbs responds to the consumer demand for oils with added value, such as oils with improved sensorial properties or a higher oxidative stability [9]. In addition, consumers prefer food products which do not contain synthetic additives [10]. For this reason, opportunities are being sought to utilize the antioxidant properties of plants. In recent years, many studies have been performed on the characteristics of natural herbs, the possibilities for their use, and the characteristics of the different herb extracts that can be obtained. It was shown that natural herbs, in general, display a higher antioxidant activity than different solvent extracts obtained from these herbs [11]. While the presence of volatile aroma compounds and phenolic compounds in a wide variety of herbs has been well studied, little information is available on their partitioning into oils after infusion. Only a few studies report the extraction of antioxidant compounds from herbs [10]. In this context, some flavored olive oils have been prepared and different quality parameters are described in the literature [12, 13].

In this study, flavored rapeseed oils were investigated, since the interest for this vegetable oil has grown over the last several years. The number of oil manufacturers producing natural cold-pressed rapeseed oil for consumption in food is

increasing in Europe. The refined food oil market is stable with some growth, but the demand for cold-pressed rapeseed oil is growing rapidly, especially in Germany [14]. The main objective of this study was to evaluate the influence of flavoring conditions on the flavor properties of rapeseed oil flavored with basil, oregano, or thyme.

Experimental Procedures

Plant and Rapeseed Oil Material

Green basil (*Ocimum basilicum* L. cv. '*Green basil*'), Greek oregano (*Origanum heracleoicum* L. cv. '*Greek oregano*'), and thyme (*Thymus vulgare*) were grown in the experimental fields of the Latvia University of Agriculture, Faculty of Agriculture (Jelgava, Latvia). The herbs were collected in the flowering stage and were dried in a well-ventilated room at 30 ± 2 °C until a moisture content of 14% was reached (similar to the commercial dry herbs). The dry matter content of the herbs was determined by means of ISO method 6496:1999. After drying, the herbs were stored in capped vessels. Commercial basil, oregano, and thyme (Santa Maria, Estonia) were analyzed in comparison. These were purchased dry in a sealed plastic vessel.

Refined rapeseed oil was purchased from the local market (Risso, Belgium). For the preparation of the flavored oils, newly opened bottles were used, and to avoid accelerated oxidation, only oils with a peroxide value lower than 1 mmol kg^{-1} were used.

Preparation of Flavored Oils

Flavored oil was prepared by two methods:

1. A bottle containing 100 ml of oil to which chopped herbs were added (3 or 6% w/w) was placed in a water bath, which was held at temperatures of 50, 60, 70, 80, or 90 °C for a certain period of time (0–60 min). The time point 0 min was defined as the time when the flavored oil reached the required temperature, experimentally determined with oil flavored with Greek oregano. The reference time was 7 min at 50 °C, 8 min at 60 °C, 9 min at 70 °C, 10 min at 80 °C, and 12 min at 90 °C. The heating times given in the tables thus correspond to the time which the samples were kept at the required final temperature. After heating, the oil samples were cooled, stored for 48 h, filtered, and analyzed.
2. A bottle containing 100 ml of oil to which chopped herbs were added (3% w/w) was stored at 18 ± 2 °C for 1–28 days. The bottle was sealed with a plastic cap and elastic tape (Parafilm). After the storage period, the oil samples were filtered and analyzed.

All flavored oil samples were prepared in triplicate.

Determination of Volatile Aroma Compounds

Volatiles from herbs and oils were extracted using solid-phase microextraction (SPME). For this purpose, 1.2 g of herbs or 5 g of flavored oil (to obtain a comparable ratio of headspace/sample volume) were weighed in a 20-ml headspace vial (Gerstel, Mülheim a/d Ruhr, Germany) and capped with a septum. SPME extraction and injection were performed automatically by an MPS-2 autosampler (Gerstel). A divinylbenzene/Carboxen/polydimethylsiloxane (DVB/Car/PDMS) fiber (Supelco Inc., Bellefonte, PA, USA) was used for headspace SPME sampling. The SPME parameters were: incubation time 2 min, extraction temperature 30 °C, extraction duration 60 min, rotating speed 250 rpm, agitator on time 1 s, agitator off time 10 s, desorption 5 min, 250 °C. For the analysis of the SPME extracts, a Hewlett-Packard 6890 GC Plus coupled with an HP 5973 MSD (mass-selective detector, quadrupole type) equipped with a CIS-4 PTV (programmed temperature vaporization) Injector (Gerstel) and an EC5-MS capillary column (30 m × 0.25 mm i.d., coating thickness 0.25 μm) was used. The working conditions were: injector 250 °C; transfer line to MSD 260 °C; oven temperature start 35 °C, hold 5 min, programmed from 35 to 60 °C at 2 °C min^{-1} and from 60 to 250 °C at 20 °C min^{-1}, hold 5 min; carrier gas (He) 1 ml min^{-1}; splitless; ionization EI 70 eV; acquisition parameters in full-scan mode: scanned *m/z* 40–200 (0–20 min), 40–400 (>20 min).

Compounds were identified by the comparison of their mass spectra with mass spectral libraries (NIST98 and Wiley 6th), and by the calculation of linear retention indexes and comparison with the literature data [15]. All analyses were performed in triplicate (residual standard deviation [RSD] < 5%). The compounds in the tables are shown in the order of their retention time. As a quantitative measure, the share in the total gas chromatography (GC) peak area for each compound is given.

Determination of Peroxide Value

To characterize the influence of heating on the oxidation of flavored rapeseed oil, accelerated oxidation tests were performed. Flavored oils were held for 48 h at 60 °C and, afterwards, the peroxide value was determined by Wheeler's method [16].

Statistical Analysis

All flavored oil samples were prepared in triplicate. Repeatability of the analytical procedure was evaluated

beforehand (RSD < 5%). The data given in the tables and graphs are the means of independent repetitions ± standard deviation (SD). The influence of different flavoring conditions was statistically evaluated, and significant differences are denoted with different letters in Tables 5, 6, and 7 and Fig. 2. Statistical tests were performed using the SPSS program (v16.0). Significance of the results was determined by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test at a significance level of 0.05.

A coefficient K (Table 5) was calculated using the least-squares method to compare the relative amounts of volatiles in herbs with flavored oil:

$$K = (A_h - A_o)^2 + (B_h - B_o)^2 + (C_h - C_o)^2 + \cdots + (X_h - X_o)^2$$

with A_h, B_h, C_h, X_h being the percentage of volatile compounds A, B, C, etc. in the headspace of herb, and A_o, B_o, C_o, X_o being the percentage of volatile compounds A, B, C, etc. in the headspace of flavored oil.

Thus, the smaller the coefficient K, the greater the similarity of the oil with the herb.

Results and Discussion

In the first instance, the headspace profiles of the herbs used are compared with the corresponding flavored oils. Afterwards, the impact of different flavoring conditions (time, temperature, concentration) on these headspace profiles are discussed.

Aroma Compounds of Dried Herbs and Flavored Oils

For each of the three herbs analyzed, basil, oregano, and thyme, commercial dried herbs were compared with a locally grown variety. Each of these herbs was used to prepare a flavored rapeseed oil (3%) by heating at 70 °C for 20 min, followed by filtration. In Tables 1, 2, and 3, the total GC peak areas are given as a measure for the total amount of volatiles extracted, while the composition of each headspace extract is displayed as well.

Altogether, 32 different compounds were identified (>0.5%) in the basil (*Ocimum basilicum*) samples analyzed in this study (Table 1). The main components of basil oil are reported to be linalool and methyl chavicol (estragole) [17], as is confirmed in this study. Also, 1,8-cineole was an important constituent of the basil flavor, as has been reported by various authors [3, 18, 19]. The content of linalool was much higher in the headspace of the green basil as compared to the commercial basil, and no methyl chavicol was detected in the green basil.

The composition of the headspace of commercial basil and of rapeseed oil flavored with this herb differed significantly ($p < 0.05$), qualitatively as well as quantitatively. The total GC peak area of the headspace of flavored oil (5 g) was considerably lower than for the corresponding herbs (1.2 g). Considering that oils were prepared with 3% herbs, the amount of herbs in 5 g oil is eight times lower than in 1.2 g of herbs analyzed. In Fig. 1, the chromatograms of the dried herbs are compared with the resulting flavored oil. In this figure, the signal of the flavored oil is multiplied by eight to obtain comparable chromatograms for equal amounts of herbs. Thus, for equal amounts of basil, the total GC peak area for the flavored oil was about ten and three times lower than for the commercial and green basil, respectively (Table 1). For the other herbs studied, this ratio falls within the same range (Fig. 1; Tables 1, 2, and 3).

Only eight different compounds were identified in the basil-flavored oil, while 12 compounds were detected in the green basil-flavored oil. Whereas methyl chavicol (43%) and linalool (19%) were the most important constituents of the headspace of the commercial basil, linalool was extracted to a relatively higher extent in the oil, and, as a result, 35% of the headspace of the flavored oil consisted of linalool as compared to 27% for methyl chavicol. In addition, the share of 1,8-cineole increased from 4.5% in the dried herbs to 24% in the flavored oil. The other main compounds identified in the headspace of commercial basil were *trans*-α-bergamotene and (*E*)-methyl cinnamate, but these were not detected in the headspace of the corresponding flavored oil. Apparently, the headspace profiles of the herbs and the corresponding flavored oils can differ considerably. In rapeseed oil flavored with green basil, no methyl chavicol was detected. Also in this case, the relative amounts of the hydrocarbon monoterpenes in the oil were, in general, higher than in the herbs, and this was especially the case for 1,8-cineole (22% in oil as compared to 9% in basil) and myrcene (7% as compared to 3%). The relative amount of linalool was proportionally lower in the oil. A comparison of the chromatograms of the commercial basil with the resulting flavored oil is shown in Fig. 1a.

In total, 31 different compounds were identified in the oregano samples analyzed in the study (Table 2). There was a large difference between both oregano varieties studied. For example, carvacrol was the most important compound in Greek oregano (64%), while it was only a minor constituent of the headspace of commercial oregano (0.48%). Different *Origanum* essential oils are known to differ especially in the content of carvacrol and thymol [17]. In the commercial and Greek oregano analyzed in this study, thymol only accounted for 4.9 and 0.8% of the headspace volatiles, respectively. Oil with a high carvacrol content typically has a spicy, herbaceous odor, reminiscent

Table 1 Volatile flavor compounds (%) as measured by solid-phase microextraction (SPME) followed by gas chromatography-mass spectrometry (GC-MS) in basil and in basil-flavored rapeseed oil

Compounds	LRI exp[a]	LRI lit[b]	Commercial basil		Green basil	
			Dried herb	Flavored oil	Dried herb	Flavored oil
α-Thujene	925	931	0.12 ± 0.01[c]	n.d.	n.d.	n.d.
α-Pinene	932	939	0.40 ± 0.01	2.39 ± 0.18	0.54 ± 0.03	n.d
Benzaldehyde	960	961	0.63 ± 0.02	n.d.	n.d.	n.d
Sabinene	971	976	0.44 ± 0.02	1.55 ± 0.08	0.58 ± 0.03	1.91 ± 0.12
β-Pinene	975	980	0.64 ± 0.03	3.16 ± 0.05	0.9 ± 0.04	2.76 ± 0.13
Myrcene	989	991	0.66 ± 0.02	n.d.	2.95 ± 0.18	7.24 ± 0.28
p-Cymene	1,024	1,026	1.80 ± 0.05	2.97 ± 0.19	0.55 ± 0.04	n.d.
Limonene	1,028	1,031	1.63 ± 0.05	3.8 ± 0.24	0.91 ± 0.06	2.16 ± 0.1
1,8-Cineole	1,031	1,033	4.50 ± 0.21	23.95 ± 1.24	9.17 ± 0.52	21.9 ± 1.29
(*Z*)-β-Ocimene	1,047	1,040	n.d.	n.d.	0.66 ± 0.04	0.93 ± 0.05
cis-Sabinene hydrate	1,069	1,068	0.35 ± 0.01	n.d.	0.5 ± 0.03	0.74 ± 0.04
trans-Linalool oxide	1,086	1,088	0.50 ± 0.03	n.d.	0.48 ± 0.04	n.d
Linalool	1,102	1,098	18.93 ± 0.67	35.35 ± 1.76	63.72 ± 4.04	53.38 ± 2.79
Camphor	1,146	1,143	0.37 ± 0.01	n.d.	0.76 ± 0.05	n.d
Menthone	1,156	1,154	n.d.	n.d.	2.67 ± 0.11	3.35 ± 0.16
Borneol	1,170	1,165	0.25 ± 0.01	n.d.	0.58 ± 0.03	n.d
Menthol	1,177	1,173	n.d.	n.d.	1.77 ± 0.13	2.37 ± 0.14
Terpin-4-ol	1,180	1,177	0.99 ± 0.03	n.d.	n.d.	n.d
α-Terpineol	1,194	1,189	0.67 ± 0.02	n.d.	0.71 ± 0.05	n.d
Methyl chavicol	1,202	1,195	42.93 ± 1.62	26.85 ± 1.56	n.d.	n.d
Bornyl acetate	1,285	1,285	0.50 ± 0.01	n.d.	1.04 ± 0.06	n.d
(*E*)-Anethole	1,287	1,283	1.96 ± 0.06	n.d.	n.d.	n.d
Thymol	1,290	1,290	0.58 ± 0.02	n.d.	n.d.	n.d
(*Z*)-Methyl cinnamate	1,303	1,301	1.90 ± 0.06	n.d.	n.d.	n.d
Eugenol	1,354	1,356	1.92 ± 0.06	n.d.	1.34 ± 0.03	n.d
(*E*)-Methyl cinnamate	1,386	1,379	4.49 ± 0.19	n.d.	n.d.	n.d
β-Elemene	1,393	1,390	0.50 ± 0.01	n.d.	0.92 ± 0.06	1.66 ± 0.07
(*E*)-Caryophyllene	1,423	1,418	0.64 ± 0.02	n.d.	n.d.	n.d
trans-α-Bergamotene	1,436	1,436	5.21 ± 0.24	n.d.	5.18 ± 0.28	n.d
α-Guaiene	1,439	1,439	0.36 ± 0.01	n.d.	0.78 ± 0.02	n.d
Germacrene D	1,484	1,480	n.d.	n.d.	1.14 ± 0.11	n.d
γ-Cadinene	1,517	1,513	1.38 ± 0.05	n.d.	1.29 ± 0.09	n.d
Total GC peak area			$(1.24 \pm 0.05) \times 10^9$	$(1.49 \pm 0.05) \times 10^7$	$(6.34 \pm 0.32) \times 10^8$	$(2.88 \pm 0.12) \times 10^7$

n.d. not detected

[a] Experimental linear retention index (LRI) determined on EC5-MS stationary phase

[b] LRI from the literature [15]

[c] Values are expressed as means of the percentage of each compound in the total gas chromatography (GC) peak area ± standard deviation (SD) ($n = 3$). Only compounds with a relative peak area >5% are shown

of thyme [17]. Other constituents, detected in significant amounts especially in commercial oregano, were *p*-cymene, *trans*-sabinene hydrate, linalyl acetate, and terpin-4-ol. High levels of carvacrol and *p*-cymene were found in oregano grown in the south of Greece [20], Italy [21], and in a German greenhouse [22]. In the studies of Juliani and Simon [23], carvacrol and γ-terpinene were reported as the main constituents of oregano.

p-Cymene was quantitatively the most important constituent of the oregano-flavored rapeseed oils. Oils flavored with commercial oregano were rich in *trans*-sabinene hydrate, γ-terpinene, and sabinene, whereas Greek oregano-flavored oils were characterized by a high carvacrol, γ-terpinene, and myrcene content. While carvacrol was a dominant constituent in the herb headspace extract (64%), this was much less the case in the headspace of the

Table 2 Volatile flavor compounds (%) as measured by SPME followed by GC-MS in oregano and in oregano-flavored rapeseed oil

Compounds	LRI exp[a]	LRI lit[b]	Commercial oregano		Greek oregano	
			Dried herb	Flavored oil	Dried herb	Flavored oil
α-Thujene	925	931	1.29 ± 0.05[c]	4.43 ± 0.24	2.72 ± 0.15	9.37 ± 0.41
α-Pinene	932	939	0.4 ± 0.02	1.04 ± 0.05	1.72 ± 0.09	4.31 ± 0.16
Sabinene	971	976	3.08 ± 0.11	6.85 ± 0.32	0.12 ± 0.01	n.d.
β-Pinene	975	980	0.33 ± 0.01	0.52 ± 0.03	0.39 ± 0.02	n.d.
1-Octen-3-ol	979	978	0.15 ± 0.01	n.d.	n.d.	n.d.
Myrcene	989	991	2.53 ± 0.1	1.61 ± 0.09	3.72 ± 0.17	14.15 ± 0.86
α-Phellandrene	1,003	1,005	0.45 ± 0.01	1.07 ± 0.05	0.58 ± 0.03	1.67 ± 0.08
Δ-3-Carene	1,008	1,011	0.51 ± 0.02	0.59 ± 0.03	0.26 ± 0.01	n.d.
α-Terpinene	1,016	1,018	1.95 ± 0.07	4.79 ± 0.19	1.92 ± 0.11	6.08 ± 0.22
p-Cymene	1,024	1,026	18.45 ± 0.52	32.29 ± 1.26	6.73 ± 0.39	21.35 ± 1.19
Limonene	1,028	1,031	3.08 ± 0.12	3.28 ± 0.15	0.41 ± 0.02	0.97 ± 0.06
β-Phellandrene	1,028	1,031	n.d.	n.d.	0.81 ± 0.04	1.23 ± 0.07
1,8-Cineole	1,031	1,033	1.93 ± 0.06	4.43 ± 0.24	n.d.	n.d.
(*Z*)-β-Ocimene	1,047	1,040	0.72 ± 0.03	n.d.	n.d.	n.d.
γ-Terpinene	1,058	1,062	4.25 ± 0.15	8.38 ± 0.38	5.37 ± 0.33	18.48 ± 1.01
cis-Sabinene hydrate	1,069	1,068	3.4 ± 0.17	4.09 ± 0.21	1.14 ± 0.06	2.28 ± 0.11
Terpinolene	1,083	1,088	0.73 ± 0.03	1.07 ± 0.04	0.23 ± 0.01	n.d.
trans-Sabinene hydrate	1,101	1,098	12.84 ± 0.42	17.99 ± 0.93	n.d.	n.d.
Linalool	1,102	1,098	n.d.	n.d.	0.79 ± 0.04	1.37 ± 0.04
trans-p-Menth-2-en-1-ol	1,124	1,121	0.87 ± 0.03	n.d.	n.d.	n.d.
cis-p-Menth-2-en-1-ol	1,142	1,140	0.65 ± 0.02	n.d.	n.d.	n.d.
Terpin-4-ol	1,180	1,177	7.65 ± 0.29	4.58 ± 0.23	0.61 ± 0.04	n.d.
α-Terpineol	1,194	1,189	1.83 ± 0.06	0.52 ± 0.03	n.d.	n.d.
Methyl chavicol	1,202	1,195	0.75 ± 0.02	n.d.	n.d.	n.d.
Thymol methyl ether	1,235	1,235	4.38 ± 0.17	0.55 ± 0.03	0.31 ± 0.01	n.d.
Carvacrol methyl ether	1,241	1,244	0.63 ± 0.02	1.03 ± 0.05	n.d.	n.d.
Linalyl acetate	1,251	1,257	10.01 ± 0.4	0.90 ± 0.04	n.d.	n.d.
Thymol	1,290	1,290	4.94 ± 0.17	n.d.	0.75 ± 0.04	n.d.
Carvacrol	1,299	1,298	0.48 ± 0.02	n.d.	63.92 ± 2.53	17.89 ± 0.85
(*E*)-Caryophyllene	1,423	1,418	2.13 ± 0.07	n.d.	5.42 ± 0.31	0.84 ± 0.04
β-Bisabolene	1,505	1,509	n.d.	n.d.	1.2 ± 0.08	n.d.
Total GC peak area			$(1.81 \pm 0.06) \times 10^9$	$(3.17 \pm 0.16) \times 10^7$	$(2.06 \pm 0.08) \times 10^9$	$(6.41 \pm 0.28) \times 10^7$

n.d. not detected

[a] Experimental LRI determined on HP5-MS stationary phase

[b] LRI from the literature [15]

[c] Values are expressed as means of the percentage of each compound in the total GC peak area ± SD ($n = 3$). Only compounds with a relative peak area >5% are shown

corresponding infused oil (18%). In contrast, the relative share of γ-terpinene and *p*-cymene was more than twofold higher in the oil as compared to the dried herbs (Fig. 1b). Also in this case, the aroma composition of oregano and oregano-flavored oil differed significantly ($p < 0.05$).

In the thyme samples analyzed in this study, 26 different compounds were identified (>0.5%) (Table 3). Among those, 16 were found in both herb samples studied. *p*-Cymene was the most dominant compound in the headspace of commercial thyme (37%), followed by thymol (21%). In thyme grown in Latvia, on the other hand, *p*-cymene made up 7% of the volatiles, while thymol and carvacrol were only minor constituents (2 and 6%, respectively). This herb was characterized by a high content of linalyl acetate (46%). Thyme oils are generally characterized by a high thymol and carvacrol content (up to 70%), but specific chemotypes with a varying composition are known [17]. For example, the essential oil of the

Table 3 Volatile flavor compounds (%) as measured by SPME followed by GC-MS in thyme and in thyme-flavored rapeseed oil

Compounds	LRI exp[a]	LRI lit[b]	Commercial thyme		Thyme	
			Dried herb	Flavored oil	Dried herb	Flavored oil
α-Thujene	925	931	0.61 ± 0.02	0.99 ± 0.05[c]	0.95 ± 0.05	2.71 ± 0.11
α-Pinene	932	939	0.78 ± 0.03	1.17 ± 0.04	0.46 ± 0.02	1.35 ± 0.07
Camphene	947	953	0.47 ± 0.02	1.02 ± 0.06	n.d.	4.94 ± 0.24
Sabinene	971	976	0.22 ± 0.01	n.d.	1.67 ± 0.08	n.d.
1-Octen-3-ol	979	978	0.99 ± 0.03	n.d.	n.d.	n.d.
3-Octanone	985	986	0.28 ± 0.01	n.d.	1.51 ± 0.08	1.93 ± 0.08
Myrcene	989	991	2.88 ± 0.13	3.11 ± 0.14	5.33 ± 0.28	15.32 ± 0.6
3-Octanol	996	993	n.d.	n.d.	1.57 ± 0.09	3.73 ± 0.17
α-Terpinene	1,016	1,018	1.48 ± 0.07	2.99 ± 0.13	0.77 ± 0.04	2.02 ± 0.13
p-Cymene	1,024	1,026	37.25 ± 1.24	66.06 ± 3.85	7.2 ± 0.34	8.39 ± 0.38
Limonene	1,029	1,031	3.05 ± 0.08	1.68 ± 0.09	0.99 ± 0.05	1.88 ± 0.07
1,8-Cineole	1,031	1,033	0.94 ± 0.03	1.75 ± 0.09	n.d.	n.d.
γ-Terpinene	1,058	1,062	6.78 ± 0.22	14.47 ± 0.74	5.96 ± 0.31	6.37 ± 0.35
cis-Sabinene hydrate	1,070	1,068	0.89 ± 0.03	2.72 ± 0.1	7.28 ± 0.37	20.6 ± 1.31
Linalool	1,099	1,098	5.08 ± 0.16	n.d.	2.09 ± 0.13	3.6 ± 0.13
Camphor	1,146	1,143	0.53 ± 0.02	n.d.	n.d.	n.d.
Borneol	1,170	1,165	0.85 ± 0.03	n.d.	n.d.	n.d.
Terpin-4-ol	1,180	1,177	1.3 ± 0.03	n.d.	n.d.	n.d.
Methyl chavicol	1,198	1,195	0.78 ± 0.03	n.d.	n.d.	n.d.
Thymol methyl ether	1,231	1,235	3.18 ± 0.13	n.d.	1.05 ± 0.05	n.d.
Carvacrol methyl ether	1,240	1,244	2.96 ± 0.09	n.d.	4.59 ± 0.29	0.86 ± 0.05
Linalyl acetate	1,254	1,257	n.d.	n.d.	45.87 ± 2.32	26.3 ± 1.21
Thymol	1,290	1,290	21.06 ± 0.83	2.56 ± 0.14	1.58 ± 0.08	n.d.
Carvacrol	1,300	1,298	1.46 ± 0.06	n.d.	6.21 ± 0.32	n.d.
(*E*)-Caryophyllene	1,423	1,418	2.55 ± 0.12	n.d.	3.15 ± 0.16	n.d.
β-Bisabolene	1,510	1,510	n.d.	n.d.	1.35 ± 0.08	n.d.
Total GC peak area			$(9.42 \pm 0.50) \times 10^8$	$(3.47 \pm 0.13) \times 10^7$	$(9.31 \pm 0.44) \times 10^8$	$(4.67 \pm 0.22) \times 10^7$

n.d. not detected

[a] Experimental LRI determined on HP5-MS stationary phase

[b] LRI from the literature [15]

[c] Values are expressed as means of the percentage of each compound in the total GC peak area ± SD ($n = 3$). Only compounds with a relative peak area >5% are shown

so-called '*p*-cymene' chemotype is mainly composed of *p*-cymene (about 32%), thymol (21%), γ-terpinene (9.5%), and linalool (2.8%) [24].

Eleven of the 23 compounds found in commercial thyme were also identified in the corresponding flavored oil, in percentages significantly different from the thyme itself ($p < 0.05$). The share of thymol, thymol methyl ether, and carvacrol methyl ether decreased considerably from the herb to the oil, as was also found for carvacrol in oregano (Fig. 1c). Rapeseed oil flavored with thyme grown in Latvia showed a completely different flavor profile composed mainly of linalyl acetate, *cis*-sabinene hydrate, and myrcene.

In general, the percentages of hydrocarbon monoterpenes, such as myrcene and γ-terpinene, and oxygenated monoterpenes, especially 1,8-cineole, in the headspace of flavored oil were higher than in the original dried herbs, while sesquiterpenes and phenolic compounds were detected to a considerably lower extent in the oil than in the herbs. These last compounds generally have a lower vapor pressure and, thus, volatility as compared to the other compounds. Therefore, these effects may also be due, to some extent, to the analytical conditions, as a low vapor pressure will minimize the volatilization of these compounds from the oil to the headspace and, thus, SPME extraction.

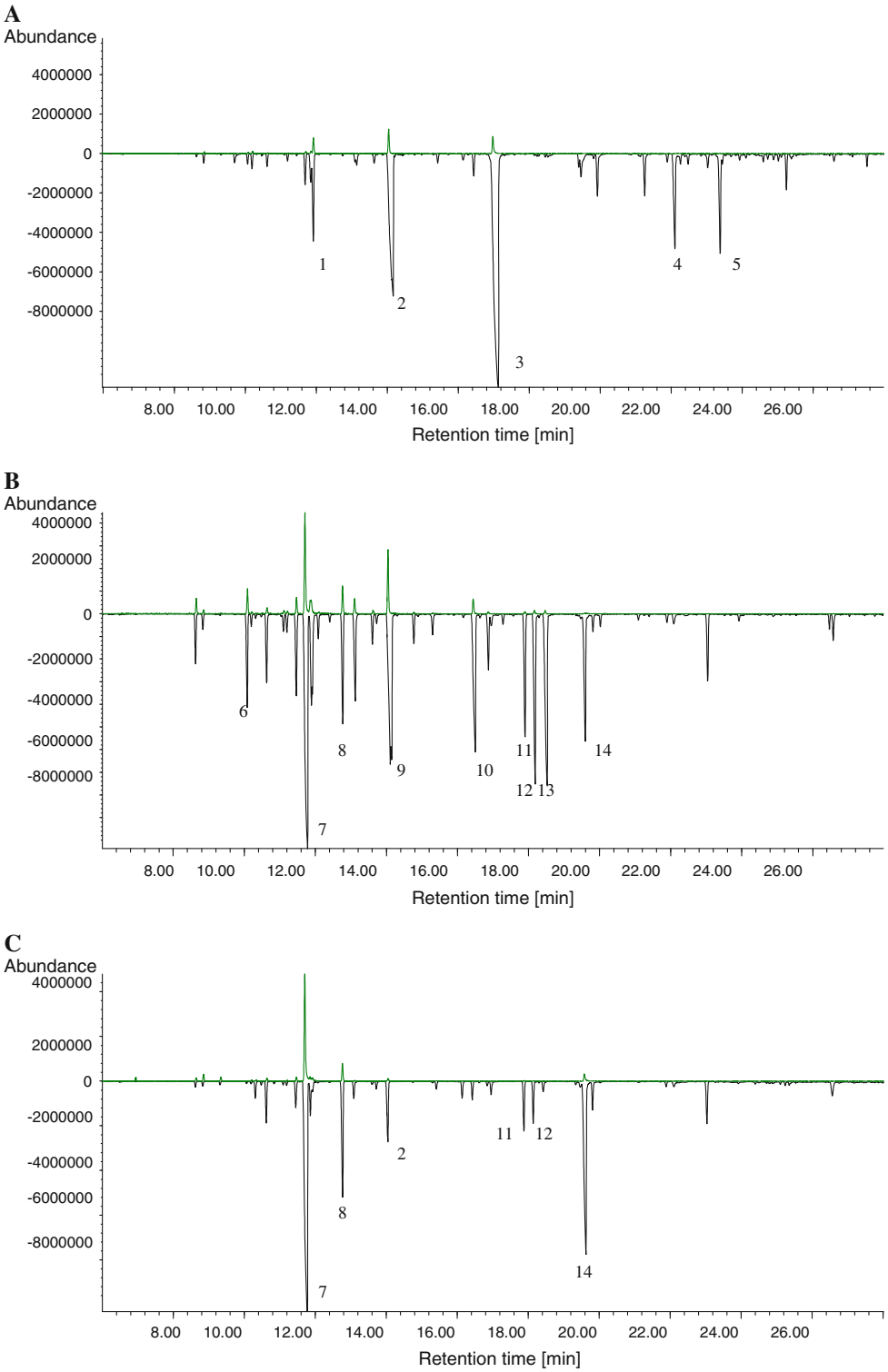

Fig. 1 Headspace solid-phase microextraction (SPME) gas chromatography-mass spectrometry (GC-MS) chromatograms of dried herbs (*downwards*) and corresponding flavored rapeseed oil (*upwards*), representing equal amounts of herbs (signal of the flavored oil × 8), for basil (**a**), oregano (**b**), and thyme (**c**). Indicated compounds: *1* 1,8-cineole; *2* linalool; *3* methyl chavicol; *4* (*E*)-methyl cinnamate; *5* *trans*-α-bergamotene; *6* sabinene; *7* *p*-cymene; *8* γ-terpinene; *9* *trans*-sabinene hydrate; *10* terpinen-4-ol; *11* thymol methyl ether; *12* carvacrol methyl ether; *13* linalyl acetate; *14* thymol

Influence of Extraction Conditions on the Flavored Oil Characteristics

The influence of different extraction conditions, contact time, time and temperature of heating, and herb concentration on the headspace profile of the flavored rapeseed oils was studied.

The influence of the concentration of herbs in the oil (3 and 6% w/w) during extraction on the headspace profile of the resulting flavored oils was studied (Table 4). The GC

Table 4 Peak area ratio of volatile flavor compounds in oil flavored with 60 g herbs/kg towards oil flavored with 30 g herbs/kg, as measured by SPME GC-MS

Compounds	Peak area ratio (oil with 6% herbs/oil with 3% herbs)					
	Green basil	Commercial basil	Greek oregano	Commercial oregano	Thyme	Commercial thyme
α-Thujene	–	–	1.7	1.59	1.69	–
α-Pinene	–	2.10	1.8	–	1.9	–
Camphene	–	–	–	–	1.83	–
Sabinene	–	–	–	1.53	–	–
β-Pinene	1.97	2.00	–	–	–	–
Myrcene	1.95	–	1.87	–	1.65	1.56
3-Octanol	–	–	–	–	1.85	–
α-Terpinene	–	–	1.8	1.50	1.63	1.50
p-Cymene	–	2.16	1.92	1.55	1.7	1.55
Limonene	1.92	1.95	–	1.74	–	–
1,8-Cineole	1.89	1.88	–	2.43	–	–
γ-Terpinene	–	–	1.65	1.45	1.78	1.45
cis-Sabinene hydrate	–	–	1.94	1.74	1.87	1.74
trans-Sabinene hydrate	–	–	–	1.67	–	–
Linalool	2.01	2.05	–	–	1.67	–
Menthone	1.97	–	–	–	–	–
Menthol	1.99	–	–	–	–	–
Terpin-4-ol	–	–	1.89	–	–	–
Methyl chavicol	–	2.26	–	–	–	–
Linalyl acetate	–	–	–	–	1.84	–
Carvacrol	–	–	1.89	–	–	1.95

–, indicates not detected

peak areas of selected compounds in oil flavored with 6% of herbs were about two times higher than for the corresponding oils flavored with 3% of herbs. This suggests that the migration of the volatiles to the oil is directly proportional to the concentration of herbs used to infuse the oils. Depending on the application, more or less intensely flavored oils can, thus, be prepared. As the same trends were found from both sets of oils, the following experiments were all performed with oils containing 3% of herbs (as lower concentrations usually give better chromatograms).

Oils were prepared by applying a certain time and temperature of heating, after which the oils were cooled, stored for 48 h, and filtered. This storage time between cooling and filtration of the oils was optimized as well. Comparing storage times between 24 and 172 h showed that the amount of volatiles extracted increased from 24 to 48 h, but did not increase significantly afterwards (data not shown). Therefore, 48 h was selected as the most appropriate time between the cooling and filtration of the samples.

In the first instance, basil-flavored oil samples were prepared at room temperature applying different contact times (Table 5). The extraction of volatile compounds in the unheated oil samples occurred very slowly. There was a significant positive linear correlation ($R = 0.97$) between the holding time and the total GC peak area of volatiles in the oil. For the range of contact times studied, an equilibrium in the headspace concentration for the volatile compounds in the oil was not reached within 28 days, as the content of volatile compounds still increased.

Heating the oils with basil for a specific time (10, 20, 40, and 60 min) and at a specific temperature (60, 70, and 80 °C) significantly ($p < 0.05$) affected the quantity of volatile compounds in the oil (as measured by the SPME GC-MS peak area) (Table 5). For all of the heated flavored oil samples, the total GC peak area was higher than for unheated oil after 24 h. Thus, heating increased the concentration of volatile compounds in the headspace of the oil and creates, as such, the possibility to obtain flavored oils in a shorter time period.

For the same heating time, applying a higher temperature resulted in a significantly higher extraction of volatiles. For the same heating temperature, a longer heating time generally increased the total GC peak area obtained, and, thus, the amount of volatiles extracted. At 70 and 80 °C, however, a maximum was reached after heating for

Table 5 Volatile flavor compound peak area as measured by SPME GC-MS in basil-flavored oils depending on the preparation conditions (T, temperature; t, time) and calculated coefficient K (sum of squared deviations between basil and basil-flavored oil)

T (°C)	t (min)[A]	Peak area ($\times 10^6$)[B]				K
		Total	Methyl chavicol	Linalool	1,8-Cineole	
18 (rt)	1 day	3.24^{a}	1.04^{a}	1.19^{a}	0.67^{a}	1,411
	7 days	5.63^{b}	1.70^{c}	2.50^{b}	1.27^{b}	1,961
	14 days	14.06^{f}	3.43^{f}	6.05^{f}	3.24^{d}	1,947
	21 days	14.02^{f}	3.93^{g}	6.15^{f}	3.13^{d}	1,863
	28 days	19.89^{g}	6.14^{i}	7.88^{g}	3.56^{e}	1,558
60	10	5.42^{b}	1.49^{b}	2.88^{c}	1.05^{b}	2,329
	20	6.06^{b}	1.33^{b}	3.16^{cd}	1.58^{bc}	2,707
	40	7.34^{c}	1.48^{b}	3.75^{d}	2.11^{c}	2,847
	60	12.11^{de}	2.25^{de}	5.49^{e}	3.64^{d}	2,664
70	10	10.64^{d}	2.11^{d}	4.64^{e}	3.21^{d}	2,520
	20	14.89^{ef}	2.34^{de}	6.30^{f}	4.94^{f}	2,838
	40	19.95^{g}	3.59^{f}	8.55^{g}	5.72^{g}	2,503
	60	18.04^{fg}	2.60^{e}	7.54^{g}	5.68^{g}	2,789
80	10	17.61^{fg}	2.60^{e}	7.12^{fg}	5.52^{fg}	2,690
	20	25.40^{h}	3.74^{f}	10.30^{h}	8.29^{h}	2,795
	40	39.13^{j}	5.11^{h}	14.57^{j}	13.18^{i}	2,805
	60	29.92^{i}	2.50^{de}	11.77^{i}	6.15^{g}	2,900

[A] Results obtained at 18 °C (room temperature) are presented in days

[B] Values in the table are the averages of three measurements. The different superscripts within each column represent significant differences ($p < 0.05$)

40 min, while heating for 60 min did not significantly change, or even decreased, the total GC peak area as compared to 40 min.

Comparing the total GC peak areas for the flavored oil samples heated at different temperatures showed that there was no significant difference between, for example, oil heated at 60 °C for 60 min and at 70 °C for 10 min. There was also no significant difference between the peak areas of volatile compounds in unheated oil held for 7 days and oils heated for 10 and 20 min at 60 °C ($p > 0.05$). Thus, it is possible to achieve equivalent levels of volatile compounds in flavored oils at lower temperatures for a longer period or at higher temperatures for a shorter period.

The qualitative composition of heated and unheated basil-flavored oils, however, differed significantly. The three most important compounds in heated and unheated oil samples, linalool, 1,8-cineole, and methyl chavicol, were studied separately. These three compounds together make up more than 65% of the total headspace extract (Table 5).

At room temperature, the extraction of linalool and methyl chavicol occurred gradually, and there was a positive linear correlation between the increase in peak area and the flavor contact time ($R = 0.96$ for both compounds). On the other hand, 1,8-cineole reached a maximum peak area after 14 days, and did not change significantly afterwards ($p > 0.05$). Thus, unheated oil held for a longer time contained a relatively higher percentage of linalool and methyl chavicol, but a relatively lower percentage of 1,8-cineole.

Methyl chavicol levels in basil-flavored oil heated at 60 °C did not change significantly with time between 10 and 40 min. Only after heating for 60 min did the methyl chavicol level increase significantly as compared to shorter heating times. At 70 and 80 °C, the peak areas for methyl chavicol increased gradually with time until 40 min, and decreased afterwards. Methyl chavicol is quite heat-sensitive and is extracted more efficiently at room temperature (for a longer extraction time). The content of linalool and 1,8-cineole in the oil, on the contrary, were efficiently increased by heating. The level of volatiles obtained after keeping the herbs in the oil for 28 days at room temperature was reached after heating at 70 °C for 40 min for linalool and already after heating at 60 °C for 60 min for 1,8-cineole. For both compounds, the extracted amounts generally increased with longer heating times, except for heating at 70 and 80 °C, where the extracted levels decreased when the heating time was increased from 40 to 60 min.

The total GC peak area of volatile compounds as well as the peak areas of the most important constituents in basil-flavored oil, linalool, 1,8-cineole, and methyl chavicol, reached a maximum when the oil was extracted at 80 °C for 40 min. Thus, these extraction conditions may be considered as optimal for oils flavored with green basil, if the goal is to extract maximal amounts of flavor compounds. Methyl chavicol was quantitatively the most important compound in the headspace of commercial basil (43%), but its relative content was significantly lower in the corresponding flavored oils. In unheated oil, its content was 31% as compared to only 13% in oil heated at 80 °C for 40 min. An opposite trend was noted for 1,8-cineole. Its level was only 4.5% in the headspace of dried herbs, but after 28 days in unheated oil and 40 min in oil at 80 °C, it was 19 and 34%, respectively.

As the volatile composition of flavored oil is significantly affected by the extraction conditions, a comparison was made between the headspace profiles of basil with basil-flavored oil. A coefficient K (sum of squared deviations) was calculated to compare the relative amounts of volatiles in basil with basil-flavored rapeseed oil (Table 5). Thus, the smaller the coefficient, the greater the similarity of the flavored oil with the original dried herbs. These results showed that the volatile composition of herbs is more similar to the volatile composition of the unheated flavored oil than to oil samples flavored by heating.

Similar results were found for oils flavored with oregano at different contact times (0–60 min) and temperatures (50–90 °C) (Fig. 2; Table 6). In general, the concentration of volatiles in the flavored oil (measured as the total GC peak area; Fig. 2) increased with temperature for the same heating time, except for the strongest heating conditions applied (90 °C for 40 and 60 min). The increase of total GC peak area with heating time was not very pronounced. A general increasing trend was noted, but at 80 and 90 °C, the extraction of volatiles reached a maximum value at around 20 min of heating, after which a decrease was observed. Thus, the maximum volatile compound concentrations were obtained at the following conditions, with no significant difference ($p > 0.05$): 80 °C for 20 min, 90 °C for 10 min, and 90 °C for 20 min.

Concerning the qualitative composition of the heated oils flavored with oregano, the most important constituents (>3%) in the headspace extracts of the flavored oils are listed in Table 6. It concerns *p*-cymene (19–27%), carvacrol (14–21%), γ-terpinene (11–20%), α-thujene (12–19%), and myrcene (4–12%). The qualitative composition of the oils did not change much for the different heating conditions applied. Considering that the maximum amount of volatiles (measured as the GC peak area) was extracted after heating the oil at 80 °C for 20 min, Table 6 shows that the qualitative composition of the oregano-flavored oil at these heating conditions did not differ much from the other conditions. Only the relative content of myrcene was higher when stronger heating conditions were applied.

In order to investigate the oxidation of rapeseed oil at the heating conditions applied for flavoring of the oil, the peroxide value of rapeseed oil flavored with oregano was determined at different times (0–60 min) and temperatures (50–90 °C) of heating (Table 7). In oil samples heated at 50, 60, and 70 °C, the peroxide values were not significantly different for the different heating times. Significant increases of peroxide values (primary oxidation products) were observed only in oil samples which were heated at 80 and 90 °C for 20 min and longer. Considering these data together with the extraction of oregano volatiles shown in Table 6, heating at 80 °C for 10 min can be considered as the optimum conditions for extracting oregano aroma volatiles into rapeseed oil with minimum oxidation.

Conclusion

The volatile constituents of rapeseed oil flavored with basil, oregano, or thyme were analyzed (after filtration) by means of headspace solid-phase microextraction (SPME) followed by gas chromatography-mass spectrometry (GC-MS) analysis. The headspace profiles of the flavored oils were different from the corresponding profiles of the original dried herbs. In general, the percentages of hydrocarbon monoterpenes, such as myrcene and γ-terpinene, and oxygenated monoterpenes, especially 1,8-cineole, in the headspace of flavored oil were higher than in the original dried herbs, while sesquiterpenes and phenolic compounds represented a considerably lower headspace fraction in the oil than in the herbs.

Following the optimization of different flavoring conditions, the following set of conditions were selected: rapeseed oil was flavored with 3% of dried herbs, heated at 80 °C for 10 min (or for 20 min, depending on the herb used), and kept at room temperature for 48 h prior to filtration. At these flavoring conditions, maximum amounts

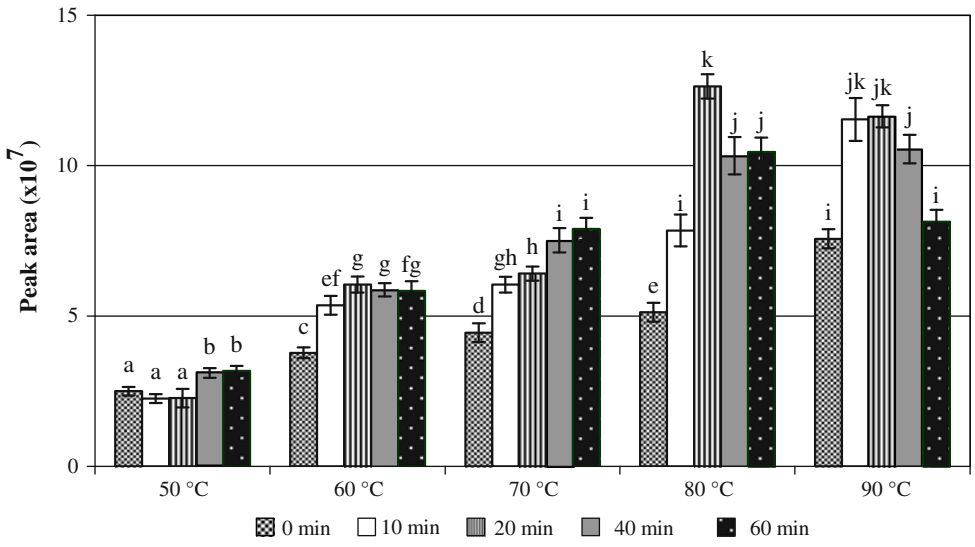

Fig. 2 Total GC peak area of identified compounds in heated flavored oils with Greek oregano

Table 6 Volatile flavor compound peak area as measured by SPME GC-MS in oils flavored with Greek oregano (*T*, temperature; *t*, time)

T (°C)	*t* (min)	Content (%)[B]					
		α-Thujene	*p*-Cymene	γ-Terpinene	Carvacrol	Myrcene	Others
50	0[A]	14.42^{def}	27.24^{de}	17.65^{ef}	18.36^{efg}	8.54^{gh}	25.56^{f}
	10	14.18^{cde}	26.50^{de}	15.98^{de}	19.33^{gh}	5.23^{b}	28.62^{g}
	20	13.33^{bcd}	26.84^{de}	16.36^{def}	21.13^{h}	4.48^{a}	28.23^{g}
	40	14.78^{def}	25.19^{cde}	17.78^{ef}	17.82^{def}	5.71^{c}	30.29^{gh}
	60	14.48^{def}	24.08^{bc}	16.72^{def}	20.35^{h}	6.27^{d}	28.81^{gh}
60	0	17.89^{h}	23.13^{b}	16.20^{de}	13.52^{a}	7.26^{e}	21.99^{de}
	10	18.50^{h}	24.70^{bcd}	15.91^{de}	13.64^{a}	9.43^{ij}	22.30^{e}
	20	15.65^{fg}	23.72^{bc}	16.53^{def}	13.39^{a}	8.16^{fg}	22.54^{e}
	40	15.81^{fg}	24.89^{bcd}	17.17^{ef}	13.17^{a}	8.98^{hi}	19.98^{cd}
	60	15.01^{ef}	24.41^{bcd}	19.66^{g}	13.21^{a}	8.59^{gh}	19.13^{bc}
70	0	15.43^{fg}	25.70^{cde}	16.81^{def}	13.47^{a}	9.01^{hi}	19.59^{c}
	10	15.35^{fg}	24.25^{bcd}	16.33^{de}	15.53^{b}	9.41^{ij}	19.14^{bc}
	20	13.90^{cd}	25.34^{cde}	16.72^{def}	15.49^{b}	9.71^{ij}	18.84^{bc}
	40	12.79^{ab}	19.40^{a}	12.60^{bc}	15.85^{bc}	7.78^{ef}	31.58^{h}
	60	16.14^{g}	24.52^{bcd}	16.03^{de}	13.37^{a}	10.07^{jkl}	19.87^{c}
80	0	15.74^{fg}	23.10^{b}	15.00^{d}	19.23^{fgh}	7.85^{f}	19.10^{bc}
	10	12.86^{bc}	25.61^{cde}	16.56^{def}	16.98^{cde}	9.72^{ijk}	18.27^{bc}
	20	12.78^{ab}	26.81^{de}	10.52^{a}	18.17^{ef}	12.22^{m}	19.50^{c}
	40	11.92^{a}	25.36^{cde}	15.32^{d}	20.06^{h}	10.06^{jkl}	17.29^{ab}
	60	12.25^{ab}	26.16^{cde}	11.99^{b}	20.53^{h}	10.44^{kl}	18.62^{bc}
90	0	11.99^{ab}	26.50^{de}	15.73^{d}	13.84^{a}	10.48^{kl}	21.46^{de}
	10	13.14^{bc}	27.33^{e}	17.79^{f}	14.01^{a}	11.75 ^{m}	15.98^{a}
	20	12.87^{bc}	25.89^{cde}	15.26^{d}	19.28^{gh}	10.73^{l}	15.97^{a}
	40	13.19^{bcd}	26.24^{de}	16.55^{def}	16.80^{cd}	11.15^{lm}	16.07^{a}
	60	15.55^{fg}	24.91^{bcd}	13.37^{c}	18.91^{fgh}	9.33^{i}	17.94^{b}

[A] The time point 0 min was defined as the time when the oil with the herbs reached the required temperature. The heating times given in the tables thus correspond to the time where the samples were kept at the required final temperature

[B] Values in the table are the averages of three measurements. The different letters within each column represent significant differences ($p < 0.05$)

Table 7 Peroxide value of flavored oils with Greek oregano prepared using different temperatures for various times

Heating temperature (°C)	Time (min)				
	0[A]	10	20	40	60
50	2.67$^{a, B}$	2.63^{a}	2.64^{a}	2.66^{a}	2.66^{a}
60	2.84^{ab}	2.97^{bc}	2.93^{bc}	2.94^{bc}	2.99^{bc}
70	2.90^{ab}	2.87^{ab}	2.72^{ab}	2.74^{ab}	2.83^{ab}
80	2.69^{a}	2.91^{ab}	3.05^{bc}	3.48^{de}	3.60^{fg}
90	2.90^{ab}	3.01^{bc}	3.22^{cd}	3.65^{e}	3.86^{e}

[A] The time point 0 min was defined as the time when the oil with the herbs reached the required temperature. The heating times given in the tables thus correspond to the time where the samples were kept at the required final temperature

[B] Values in the table are the averages of three measurements. The different letters within each column represent significant differences ($p < 0.05$)

of flavor constituents were detected in the resulting flavored oil. In addition, measurement of the peroxide value showed no significant oil oxidation at these heating conditions.

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