J Am Oil Chem Soc (2008) 85:641–646
DOI 10.1007/s11746-008-1244-8

ORIGINAL PAPER

Sensory Thresholds of Selected Phenolic Constituents from Thyme and their Antioxidant Potential in Sunflower Oil

Alain Bitar · Tarek Ghaddar · Amal Malek · Tharwat Haddad · Imad Toufeili

Received: 17 August 2007 / Revised: 30 April 2008 / Accepted: 30 April 2008 / Published online: 18 May 2008

Abstract The odor detection thresholds of carvacrol (5-isopropyl-2-methyl-phenol), thymol (2-isopropyl-5-methyl-phenol) and *p*-cymene 2,3-diol (2,3-dihydroxy-4-isopropyl-1-methyl-benzene) in sunflower oil, determined by the three-alternative, forced-choice procedure, were 30.97, 124 and 794.33 mg kg^{-1}, respectively. Sunflower oil containing 13, 70, or 335 mg kg^{-1} of carvacrol, thymol or *p*-cymene 2,3-diol, respectively, was judged to be similar ($P < 0.01$) in taste and odor to its antioxidant-free counterpart. The rate constant of sunflower oil oxidation, measured from the increase in peroxide value during storage at 25 °C, was 9.2×10^{-9} mol kg^{-1} s^{-1} while the rate constants were 9.3×10^{-9}, 9.8×10^{-9}, and 4.3×10^{-9} mol kg^{-1} s^{-1} in the presence of 13 mg kg^{-1} carvacrol, 70 mg kg^{-1} thymol, and 335 mg kg^{-1} *p*-cymene 2,3-diol, respectively. At a level of 335 mg kg^{-1}, *p*-cymene 2,3-diol did not impart flavor taints and effected a 46.7% reduction in the rate of oxidation of sunflower oil. These findings indicate that the diphenolic *p*-cymene 2,3-diol could potentially replace synthetic antioxidants and is a valuable addition to the antioxidants used by the food industry in its quest to meet consumer demands for synthetic-additives-free and 'natural' foods.

Keywords Carvacrol · Thymol · *p*-Cymene 2,3-diol · Sensory thresholds · *Mono and diphenolic antioxidants* · Sunflower oil · *Thymus vulgaris* · Natural antioxidants

A. Bitar · A. Malek · I. Toufeili (✉)
Department of Nutrition and Food Science,
Faculty of Agricultural and Food Sciences,
American University of Beirut,
Riad El Solh 1107 2020, Beirut, Lebanon
e-mail: toufeili@aub.edu.lb

T. Ghaddar
Department of Chemistry, Faculty of Arts and Sciences,
American University of Beirut,
Riad El Solh 1107 2020, Beirut, Lebanon

T. Haddad
Department of Plant Sciences,
Faculty of Agricultural and Food Sciences,
American University of Beirut,
Riad El Solh 1107 2020, Beirut, Lebanon

Introduction

Bulk oils and fat- and oil-containing foods are susceptible to oxidation during processing and storage. Lipid oxidation negatively impacts the sensory quality of foods through production of unpleasant/offensive flavors, reduces the nutritional quality of foods, produces potentially toxic compounds, and causes significant economic losses to the food industry [1]. Synthetic phenolic antioxidants [e.g. butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ) and propyl gallate (PG)] are extensively used to retard lipid oxidation in foods [2]. However, concerns about the safety of synthetic antioxidants [3, 4] and consumers' demands for synthetic-additives-free foods triggered the search for natural compounds with antioxidative properties [5]. Herbs and spices are rich sources of phenolic antioxidants and much effort has been spent in isolating and characterizing their active compounds [6]. However, the antioxidant potential of herbs and spices is largely limited by the characteristic flavors of their phenolic constituents, which as antioxidants must not impart flavors/taints to foods [7].

Carvacrol (5-isopropyl-2-methyl-phenol), thymol (2-isopropyl-5-methyl-phenol), and *p*-cymene 2,3-diol (2,3-dihydroxy-4-isopropyl-1-methyl-benzene) (Fig. 1) are phenolic compounds responsible for the distinct flavor of

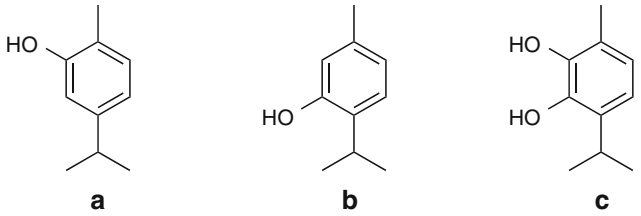

Fig. 1 Structures of carvacrol (**a**), thymol (**b**) and *p*-cymene 2,3-diol (**c**)

thyme (*Thymus vulgaris* L.) leaves. These compounds have been reported to exhibit pronounced antioxidant activity [8, 9]. The aforementioned phenolic antioxidants of thyme would be useful for controlling lipid oxidation in bulk oils and fat-containing foods if they were to possess antioxidant activities at levels below their detection thresholds. Detection thresholds are usually determined by a small group of subjects in a laboratory setting and lower values are expected for the population due to the presence of more sensitive individuals [10]. Therefore, the usage levels at which thyme antioxidants do not impart flavor needed to be determined.

The objectives of the present work were to determine the (1) odor detection thresholds of carvacrol, thymol and *p*-cymene 2,3-diol in sunflower oil, (2) maximum levels at which these antioxidants can be added to sunflower oil with the resulting antioxidant-treated oil still being judged as similar to its antioxidant-free counterpart and (3) antioxidant efficacy of these phenolics at their maximum level of usage in sunflower oil.

Experimental Procedures

Materials

Carvacrol, thymol and *p*-cymene-2,3-diol were obtained from Sigma-Aldrich Co. (Taufkirchen, Germany). Sunflower oil, free from added antioxidants, was obtained from a local refinery (Dora Oil Refinery, Beirut, Lebanon). The free fatty acids and peroxide value of sunflower oil were 0.05% (as oleic acid) and 0.2 mequiv kg^{-1}. The sample was stored at 6 °C.

Procedures

Sensory Testing

Determination of Odor Thresholds Odor detection thresholds were determined according to ASTM method E1432 [11]. Twelve panelists (eight females, four males; age 21–28) were recruited from the Faculty of Agricultural and Food Sciences at the American University of Beirut. The panelists were nonsmokers, had no self-reported problems in their sense of smell and were selected on the basis of their ability to detect high levels of the stimuli (0.1–0.3% for carvacrol and thymol; 2% for *p*-cymene 2,3-diol), their motivation and willingness to fast for one hour prior to testing and to participate in the whole study. The panelists were asked to provide odor descriptors of the antioxidants solutions at suprathreshold levels. The panelists were familiarized with the logistics of the Three-Alternative Forced-Choice (3-AFC) procedure and were not compensated for serving on the panel.

Solutions for testing were made by preparing stock solutions of antioxidants in sunflower oil and diluting to provide samples differing in concentration by a factor of 2. Levels of the antioxidants were chosen, on the basis of preliminary screening experiments, and were varied by a factor of 2 to provide a reasonable bracketing of threshold. Seven solutions were prepared with levels ranging between 0.0004 and 0.0256, 0.003 and 0.192, and 0.03 and 1.92 g per 100 mL sunflower oil for carvacrol, thymol and *p*-cymene 2,3-diol, respectively. Concentrations of antioxidants were converted to mg kg^{-1} of oil by multiplying with density of sunflower oil (925 kg m^{-3}). Panelists who were able to detect the lowest concentrations (i.e. the 0.0004, 0.003 and 0.03 g per 100 mL levels of carvacrol, thymol and *p*-cymene 2,3-diol, respectively) 100% of the time were tested at two lower concentrations. Samples of antioxidants in oil (20 mL) were placed in transparent glass bottles (30 mL capacity) coded with 3-digit numbers. The 3-AFC sets, each set consisting of three bottles with two bottles containing sunflower oil with no added antioxidants and the third bottle a solution of antioxidant in sunflower oil, were presented to the panelists at room temperature. Each session consisted of seven sets with the antioxidant level of the odd sample in each set having successively higher values. The panelists were asked to proceed from the set with the lowest value to the set with the highest concentration, with order being specified from left to right, open the bottles and sniff the solutions, and indicate the odd sample. The assessors were not told that the antioxidant concentrations were increasing in the specified direction of testing to eliminate the expectation error [10]. Samples were arranged in a balanced random order, within each triad, in order for each sample to appear in a given position an equal number for times to eliminate positional bias [10]. Testing was replicated 6 times with two sessions, 1 h apart, being conducted in a day. Testing was conducted daily, and for some panelists every other day, with the 42 presentations of the 3-AFC sets evaluated by each panelist, for each antioxidant, being completed in a week. Testing was conducted in partitioned booths equipped with daylight. The individual thresholds were determined by fitting data to the logistic model:

$$P = \frac{\frac{1}{3} + e^{B(T-\mathrm{Log}\,X)}}{1 + e^{B(T-\mathrm{Log}\,X)}} \quad (1)$$

where

P = Proportion of correct identifications at each concentration in the six replicate determinations,

B = Slope of logistic curve,

X = Concentration of stimulus (mg kg^{-1}) in oil, and

T = Threshold value in Log (mg kg^{-1}).

The individual threshold value (T) corresponds to the concentration at which the predicted correct identification is 50% in the normalized curve.

The group detection threshold was determined by computing the geometric mean of individual thresholds.

Similarity Testing: Odor One hundred and eight subjects (65 females, 43 males; age 17–68 years), recruited from the American University of Beirut campus, served on panels aimed at establishing odor similarity between antioxidant-containing oils and their antioxidant-free counterparts. The concentrations of carvacrol, thymol, and *p*-cymene 2,3-diol corresponding to the respective group detection thresholds (i.e. 30.97 mg kg^{-1} for carvacrol, 124 mg kg^{-1} for thymol and 794.33 mg kg^{-1} for *p*-cymene 2,3-diol) were lowered in steps differing by 25% and added to sunflower oil. More specifically, carvacrol was tested at concentrations of 23.23, 17.42, 13.07 mg kg^{-1}, etc.; thymol was tested at 93, 69.75, 52.31 mg kg^{-1}, etc.; and *p*-cymene 2,3-diol was tested at 595.75, 446.81, 335.1 mg kg^{-1}, etc. The resulting antioxidant-containing oils were tested for similarity to regular sunflower oil. The triangle test was used and subjects were instructed to identify the sample that was most different from the other two. Half of the triads contained two antioxidant-treated oils and one regular oil while the other half of triads contained two regular and one antioxidant-treated oil samples. Samples were presented in a balanced random order with sample size and testing logistics (sample coding, sample handling, testing area) being as described in the previous section.

Similarity Testing: Taste Sunflower oil was dosed with concentrations of carvacrol, thymol, and *p*-cymene 2,3-diol, determined not to impart odor to the oil (i.e. 13 mg kg^{-1} for carvacrol, 70 mg kg^{-1} for thymol and 335 mg kg^{-1} for *p*-cymene 2,3-diol), and judged for similarity in taste to regular sunflower oil using the triangle test. Sixty four panelists (41 females, 23 males; age 18–35 years), recruited from the Faculty of Agricultural and Food Sciences, participated in this part of the study. Subjects were asked to place samples of oil (5 mL) in the mouth, swish them thoroughly throughout the oral cavity, and indicate the odd sample. The panelists were asked to rinse their palates before and after testing of each sample with bottled water (Nestlé PureLife, Falougha, Lebanon). Apart from the difference in evaluating samples (taste, sniff), the logistics of tests (sample coding, composition of triads, testing area) were the same as those in odor testing sessions.

Testing was done at an α-level of 0.2 (probability of concluding that a perceptible difference exists when one does not), a β-level of 0.01 (probability of concluding that no perceptible difference exists when one does); P_d (true proportion of population able to detect a difference between samples) was set at 25 and 30% in odor and taste tests, respectively. The significance of the test was ascertained by reference to tables of correct responses required to establish significance in the triangle test [10]. The confidence limits on the proportion of the population that can distinguish between samples were determined by calculating $1.5 \times c/n - 0.5 - z_\alpha(1.5)\sqrt{(c/n)(1-c/n)/n}$ and $1.5 \times c/n - 0.5 + z_\beta(1.5)\sqrt{(c/n)(1-c/n)/n}$, where c is the number of correct responses in the triangle test, n total number of assessors and Z_α and Z_β are critical values of the standard normal distribution [10].

Determination of Antioxidant Activity

The efficacy of carvacrol, thymol, and *p*-cymene-2,3-diol in inhibiting oxidation of sunflower oil was determined by the method described by Yanishlieva et al. [12]. Concentrations of the antioxidants, at levels at which sunflower oil was judged to be similar in odor and taste to its antioxidant-free counterpart, were added to sunflower oil. Solutions of the antioxidants-in-oil were placed in Petri dishes (diameter = 8 cm) to provide layers that were 1 mm thick and stored in the dark at 25 ± 1 °C. Aliquots were withdrawn, at 4-day intervals, and their peroxide values (PV) determined, in triplicate, by iodometric titration [13]. Under these conditions of high oxygen tension, the rate of oxidation is not affected by oxygen diffusion and is, therefore, equal to the rate of peroxide accumulation [14]. The rate of hydroperoxide formation was expressed in mol kg^{-1} s^{-1} using the relationship 1 mequiv kg^{-1} h^{-1} = 0.14 μmol L^{-1} s^{-1} [14].

Statistical Analysis

The parameters B (slope of logistic curve) and T (threshold value) were estimated by fitting the proportion of correct identifications, in the six replications of the ascending forced choice tests, to Eq. (1) using the sequential quadratic programming option, with a maximum of 12 iterations, of the Nonlinear Regression module of SPSS software [15]. The logistic curves of individual panelists for the three antioxidants, corresponding to Eq. (1), were plotted using

Origin software [16]. PV were fitted to days of storage by linear regression using Microsoft Excel®.

Results and Discussion

Representative logistic curves fitted to an individual panelist's data and individual and group detection thresholds of carvacrol, thymol and *p*-cymene 2,3-diol are presented in Fig. 2 and Table 1.

The individual detection thresholds of the antioxidants in sunflower oil ranged over 8–106 orders of magnitude with differences being smallest for thymol and largest for *p*-cymene 2,3-diol (Table 1). Differences in olfactory sensitivity to chemical stimuli have been attributed to physiological differences and random fluctuations in alertness, attention, fatigue and adaptation with a decrease in sensitivity being observed after prolonged exposure [10]. The group detection thresholds of thymol and *p*-cymene 2,3-diol were 4 and 25.5 times higher than that of carvacrol, respectively (Table 1). Odor detection thresholds are shaped by the molecular architecture of chemical stimuli and marked differences in thresholds' magnitudes have been reported for isomers and homologous series in different classes of compound [17, 18]. Consumers are intolerant to the presence of flavor taints in edible oils and a major objective of the refining process is removal of foreign flavors. The bland flavor of edible oils would be jeopardized when treated with carvacrol, thymol or *p*-cymene 2,3-diol especially as these antioxidants evoked sensations described as herbal, sweet, warm, penetrating, and tar-like at suprathreshold concentrations.

Triangle tests indicated that antioxidant-treated sunflower oil samples were similar ($P < 0.01$), in odor, to antioxidant-free sunflower oil when dosed at levels lower by two 25% intervals for thymol and three 25% intervals for carvacrol and *p*-cymene 2,3-diol than the corresponding group detection thresholds. More specifically, sunflower oil was judged to have a similar odor to its antioxidant-free counterpart when treated with 13, 70, and 335 mg kg^{-1} of carvacrol, thymol and *p*-cymene 2,3-diol, respectively. At these levels the 99% confidence intervals (CI) of the true proportion of the population that can detect differences in odor between antioxidant-treated and antioxidant-free sunflower oil ranged between 0 and 8%, 0 and 19%, and 0 and 22% for carvacrol, thymol and *p*-cymene 2,3-diol, respectively.

At the aforementioned levels of the antioxidants, sunflower oil was judged similar ($P < 0.01$) in taste to its antioxidant-free counterpart with the 99% CI of the true proportion of the population that can detect differences in taste ranging between 0 and 20%, 0 and 17%, and 0 and 22% for carvacrol, thymol and *p*-cymene 2,3-diol, respectively. These findings indicate that sunflower oil, and possibly other bulk oils, can be treated with *p*-cymene 2,3-diol at substantially higher levels than carvacrol and thymol without undue effects on its taste and odor.

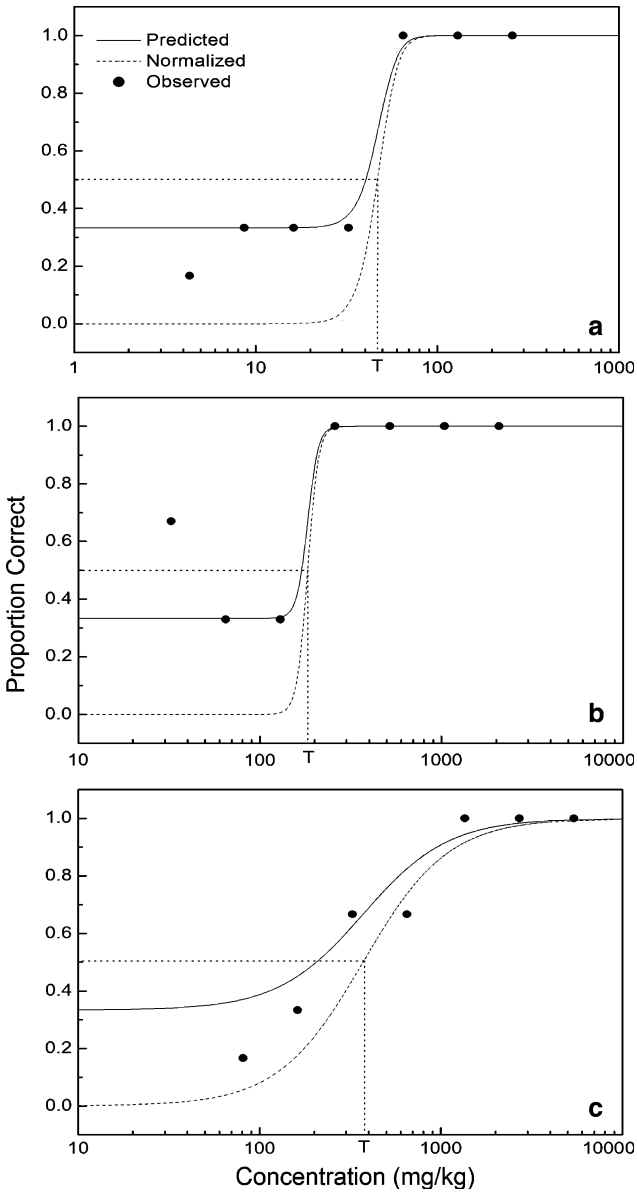

Fig. 2 Proportion of correct judgments by assessor # 9 at different concentrations of: carvacrol (**a**), thymol (**b**) and *p*-cymene 2,3-diol (**c**). Each point (*filled circle*) is the proportion correct of six judgments in 3-Alternative Forced Choice (AFC) tests with one sample containing the indicated concentration of the antioxidant and the other two samples having no added antioxidant. The parameters *B* and *T* (in Log units) of the curves as determined by logistic regression of Eq. (1) were −22.4, 1.673 for carvacrol (**a**), −35.24, 2.263 for thymol (**b**) and −4.27, 2.570 for *p*-cymene 2,3-diol (**c**). The threshold value (*T*) is the concentration that corresponds to 50% correct identifications on the normalized curves

Table 1 Individual and group detection thresholds for carvacrol, thymol and *p*-cymene 2,3-diol in sunflower oil

Panelist number	Individual threshold (mg kg^{-1})[a]		
	Carvacrol	Thymol	*p*-Cymene-2,3-diol
1	38.02	367.28	3845.92
2	11.91	71.29	1352.07
3	76.38	365.59	2296.15
4	47.32	219.28	743.02
5	64.71	174.98	2094.11
6	37.84	85.31	2697.74
7	71.29	232.27	114.55
8	64.86	85.31	7311.39
9	47.10	183.23	371.54
10	1.07	45.92	125.89
11	15.07	46.77	69.02
12	55.46	55.21	473.15
Group mean threshold (mg kg^{-1})	30.97	124	794.33
Standard log deviation	0.526	0.334	0.658

[a] Determined by logistic regression of percentage correct identifications in six replicate presentations of seven stimulant-containing solutions as per the ASTM method E 1432

The behavior of hydroperoxide accumulation was apparently pseudo-zero-order with the rate being independent of oxygen concentration:

$$C = C_0 + kt \quad (2)$$

where C_0 and C are the concentrations of hydroperoxides at time (0) and after time (t), respectively, and k the rate constant of the reaction (Fig. 3). The PV increased linearly with time and the system did not show distinct induction period and propagation phase, under the conditions of measurement, typical of lipids undergoing oxidation. The coefficients of determination (R^2) of the linear regression equations of PV vs. days of storage were $\geq$0.997 with the CI of regression coefficients being $\leq$0.06 at $P < 0.05$. The rate constant of sunflower oil oxidation was 9.2×10^{-9} mol kg^{-1} s^{-1}, with the rate constants being 9.3×10^{-9}, 9.8×10^{-9}, and 4.3×10^{-9} mol kg^{-1} s^{-1} in the presence of 13 mg kg^{-1} carvacrol, 70 mg kg^{-1} thymol, and 335 mg kg^{-1} *p*-cymene 2,3-diol, respectively. The apparent lack of antioxidative effect for carvacrol and thymol is presumably due to their low concentrations especially as these monophenols have been reported to exhibit marked antioxidant activity, in sunflower oil, at concentrations higher than those used in the present work [12]. The lower value of k observed for oxidation of sunflower oil in the presence of *p*-cymene 2,3-diol can be attributed to its higher concentration, as compared to those of carvacrol and thymol, in the oxidizing substrate. Furthermore, the presence of two

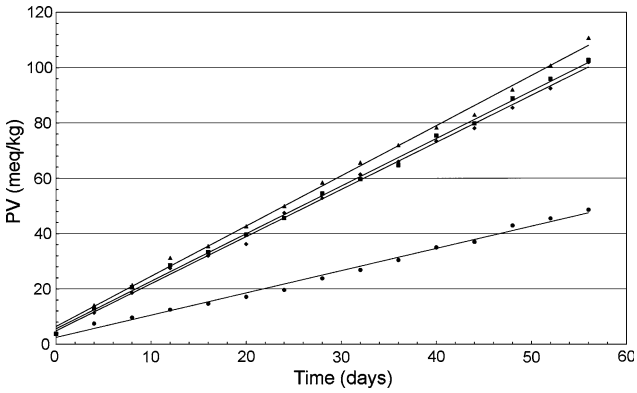

Fig. 3 Changes in peroxide values (PV) during oxidation of sunflower oil at 25 °C in the absence (*filled diamonds*) and presence of 13 mg kg^{-1} carvacrol (*filled squares*), 70 mg kg^{-1} thymol (*filled triangles*), or 335 mg kg^{-1} *p*-cymene 2,3-diol (*filled circles*)

phenolic hydroxyl groups in the molecule in contrast with the monophenolic nature of the two other antioxidants contributes to the improved antioxidant activity (Fig. 1). The antioxidant activity is known to increase with increasing numbers of hydroxyl groups in phenolic antioxidants [19, 20]. This work indicated that *p*-cymene 2,3-diol could be a potential replacement for synthetic antioxidants, provided that this compound be extracted from natural sources.

References

1. Choe E, Min DB (2006) Mechanisms and factors for edible oil oxidation. Compr Rev Food Sci Food Safety 5:169–186
2. Frankel EN (1998) Lipid oxidation. The Oily Press, Dundee, pp 115–160
3. Miková K (2001) The regulation of antioxidants in food. In: Pokorný J, Yanishlieva N, Gordon M (eds) Antioxidants in food. Woodhead Publishing, Cambridge, pp 267–284
4. Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K, Taniguchi K, Tsuda S (2002) The comet assay with 8 mouse organs: results with 39 currently used food additives. Mutat Res-Genet Toxicol Environ Mutagenesis 519:103–119
5. Pszczola DE (2001) Antioxidants: from preserving food quality to quality of life. Food Technol 55:51–59
6. Dimitrios B (2006) Sources of natural phenolic antioxidants. Trends Food Sci Technol 17:505–512
7. Shahidi F, Naczk M (2004) Phenolics in food and nutraceuticals. CRC Press, Boca Raton, pp 443–481
8. Dapkevicius A, van Beek TA, Lelyveld GP, van Veldhuizen A, de Groot A, Linsen JPH, Venskutonis R (2002) Isolation and structural elucidation of radical scavengers from *Thymus vulgaris* leaves. J Nat Prod 65:892–896
9. Schwartz K, Ernst H Ternes W (1996) Evaluation of antioxidative constituents from thyme. J Sci Food Agric 70:217–223
10. Meilgaard M, Civille GV, Carr BT (1999) Sensory evaluation techniques, 3rd edn. CRC Press, Boca Raton
11. ASTM (2000) Standard practice for defining and calculating individual and group sensory thresholds from forced-choice data sets of intermediate size. In: Annual Book of ASTM Standards, ASTM Press, Pennsylvania, Method E 1432–91

12. Yanishlieva NV, Marinova EM, Gordon MH, Raneva VG (1999) Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. Food Chem 64:59–66
13. AOCS (1998) Peroxide value: acetic acid-chloroform method. In: Official methods and recommended practices of the American Oil Chemists Society, 5th edn. American Oil Chemists Society, Illinois, Method Cd, pp 8–53
14. Marinova EM, Yanishlieva NV (1992) Effect of temperature on the antioxidative action of inhibitors in lipid autoxidation. J Sci Food Agric 60:313–318
15. SPSS (2005) User's guide: statistics, Version 14.0. SPSS Inc., Chicago
16. Origin (2000) User's guide, Version 6.0. Microcal Software Inc., Massachusetts
17. Buttery RG, Ling LC (1997) 2-Ethyl-3,5-dimethylpyrazine and 2-ethyl-3,6-dimethylpyrazine: odor thresholds in water solution. Lebensm–Wiss u–Technol 30:109–110
18. Cometto-Muniz JE, Cain WS, Abraham MH, Kumarsingh R (1998) Trigeminal and olfactory chemosensory impact of selected terpenes. Pharmacol Biochem Behav 60:765–770
19. Heijnen CGM, Haenen GRMM, Oostveen RM, Stalpers EM, Bast A (2002) Protection of flavonoids against lipid peroxidation: the structure activity relationship revisited. Free Radic Res 36: 575–581
20. Siquet C, Paiva-Martins F, Lima JLFC, Reis S, Borges F (2006) Antioxidant profile of dihydroxy- and trihydroxyphenolic acids—a structure-activity relationship study. Free Radic Res 40: 433–442