Inhibition of lard oxidation by fractions of different essential oils

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RESUMEN

Inhibición de la oxidación de la manteca de cerdo por fracciones de diferentes aceites esenciales.

Se examinó la capacidad de los aceites esenciales de *Origanum vulgare* L. spp. *hirtum, Thymus vulgaris* L., *Thymus serpyllum* L., *Satureja montana* L. y *Satureja cuneifolia* Ten. para inhibir la oxidación de la manteca de cerdo pura. Excepto Satureja cuneifolia Ten., todos los aceites esenciales mostraron un acusado perfil fenólico caracterizado por la presencia de fenoles monoterpénicos- timol y carvacrol. El método Rancimat ha sido aplicado a manteca de cerdo sembrada con los aceites esenciales y sus fracciones. La capacidad de los aceites y sus fracciones para actuar como inhibidores de la oxidación de lípidos fue menor en comparación con la de antioxidante sintéticos (BHA y BHT), ácido ascórbico y α-tocoferol. El efecto antioxidante de las sustancias ensayadas dependió de la dosis. El periodo de inducción de la manteca de cerdo pura no se afectó por la cantidad de muestra presente en el sistema de reacción.

PALABRAS-CLAVE: Aceite esencial - Antioxidante - Hierbas medicinales - Método de Rancimat - Oxidación de lípidos.

SUMMARY

Inhibition of lard oxidation by fractions of different essential oils.

The ability to inhibit lard oxidation by the essential oils of *Origanum vulgare* L. spp. *hirtum, Thymus vulgaris* L., *Thymus serpyllum* L., *Satureja montana* L. and *Satureja cuneifolia* Ten. was examined. Except *Satureja cuneifoila* Ten. essential oil, all the essential oils studied showed a strong phenolic profile characterized by the presence of phenolic monoterpenes - thymol and carvacrol. The Rancimat method has been applied on lard spiked with essential oils and their fractions. The ability of essential oils tested and their fractions to act as inhibitors of the lipid oxidation process was lower in comparison with reference antioxidants (BHA and BHT), ascorbic acid and α-tocopherol.
The antioxidant effect of the antioxidants tested was dose-dependent. Induction time of pure lard is not effected by the quantity of the oil sample in the reacting system.

KEY-WORDS: Antioxidant - Essential oil - Lipid oxidation - Medicinal herbs - Rancimat method.

1. INTRODUCTION

The two most important types of chemical reactions responsible for quality loss in processed foods have been identified as browning and oxidation (Löliger, 1991). The process of lipid oxidation in foods is responsible for the formation of off-flavors and undesirable chemical compounds, which may be detrimental to health (Brand-Williams *et al.,* 1995).The progression of oxidation in a food system occurs from the formation of radicals through primary oxidation products (lipid hydroperoxides) and secondary oxidation products (aldehydes and ketones) to protein damage.

Antioxidants may be active at different stages of the progression of oxidation in food systems. However, the conditions for which the different antioxidative mechanisms contribute to the protection of an actual food product are not well understood. The use of real food systems for detailed studies of antioxidants is complicated by a large number of factors, which are often unknown or cannot be controlled due to the complex nature of foods. Using simplified model systems, which mimic the main features of a given food system, or antioxidant assays for quantifying the antioxidant action, can be very helpful in clarifying the action of potential antioxidants (Andersen *et al.*, 2002).

Because of market requirements, the use of synthetic antioxidants is being replaced more and more by natural antioxidants from plant sources. Many sources of antioxidants of plant origin have been studied in recent years and numerous types of antioxidants with varied activities were identified (Lagouri *et al.*, 1993; Tsimidou and Boskou, 1994, Milos *et al.*, 2000, Radonic and Milos, 2003). It has been clearly demonstrated in numerous model systems that plant phenolic compounds have antioxidative properties (Schwarz *et al.*, 2001). Previous investigations proved the strong phenolic character of essential oils from oregano (*Origanum vulgare* L.) (Lagouri *et al.,* 1993; Milos *et al.*, 2000; Vichi *et al.*, 2001), thyme (*Thymus vulgaris* L.) (Schwarz and Ernst, 1996), wild thyme (*Thymus serpyllum* L.) (Mailhebiau, 1994) and savory (*Satureja montana* L.) (Radonic and Milos, 2003). On the other hand, the *Satureja cuneifolia* essential oil (of Croatian origin) was found to contain a low percentage of phenolic compounds (Milos *et al.*, 2001).

In this study we report the investigation of the ability of the previously mentioned herbs to inhibit the process of lard oxidation with essential oils in relation to their chemical composition. The Rancimat method was applied for these investigations because it mimics the process of lipid oxidation in a simple,

reproducible and fast manner. The antioxidant effect of different essential oils and their fractions was evaluated in comparison with, the frequently used antioxidants (BHT, BHA, ascorbic acid and α-tocopherol) and pure constituents thymol and carvacrol.

2. EXPERIMENTAL

2.1. Materials

The plant materials of oregano (*Origanum vulgare* L. spp. *hirtum*), thyme (*Thymus vulgaris* L.), wild thyme (*Thymus serpyllum* L.) and two savory species (*Satureja montana* L. and *Satureja cuneifolia* Ten.) were collected in Central Dalmatia (Croatia). Plant material (flower tops and stalks), after air-drying, was used for the isolation of the essential oils. A hundred grams of each dried plant material was subjected to a 3 h hydrodystillation using a Clevenger-type apparatus. The obtained essential oils were dried over anhydrous sodium sulphate and stored under nitrogen in a sealed vial at – 20 ^{o}C until needed . The voucher specimens of plant material as well as their essential oils are stored in the Department of Biochemistry and Food Chemistry, Faculty of Chemical Technology, Split, Croatia.

The essential oils of oregano, thyme, wild thyme and two savory species (0.5 g) were fractionated using a silica gel (30-60 μm, Mallinckrodt Baker B.V., Deventer, The Netherlands) column (length 20 cm; i.d. 2 cm). Pentane (50 mL) was used to obtain a fraction, which contained only non-polar hydrocarbons (CH fraction), and diethyl ether (50 mL) was used to obtain a fraction of polar (oxygen containing, CHO fraction) compounds. These fractions were concentrated to 0.5 mL and subjected to thin layer chromatography (TLC) on silica gel plates in order to check results of the column chromatography separation. Different solvents were used as mobile phase: *n*-hexane for CH fraction and *n*-hexane:ethyl acetate 85:15 (v/v) for CHO fraction. Two percent vanillin-sulphuric acid was used as a detection reagent. The fractions obtained by column chromatography were also subjected to GC/MS analysis and good separation results were confirmed.

In order to obtain a fraction of phenolic compounds, 0.5 g of the essential oils from *Origanum vulgare* L. spp. *hirtum*, *Thymus vulgaris* L. and *Satureja montana* L. was dissolved in 5 mL pentane and extracted with sodium hydroxide solution (20%) in water. In this manner, phenolic compounds were removed from the pentane layer. The aqueous phase, containing dissolved phenolic compound sodium salts, was neutralized with hydrochloric acid solution (10%) in water. Finally, isolation of the phenolic compounds was performed by extraction with pentane (5 x 5mL). The effectiveness of this separation method was tested by TLC on silica gel plates (mobile phase: *n*-hexane:ethyl-acetate 85:15 v/v). Purity of the phenolic compounds fraction was confirmed by GC/MS analysis.

The lard applied in the Rancimat method was home made (free of added antioxidants or preservatives).

2.2. GC-MS analysis

The analyses of the volatile compounds were run on a Hewlett-Packard GC-MS system (GC 5890 series II; MSD 5971A, Hewlett Packard). The fused-silica HP-20 M polyethylene glycol column (50 m x 0.2 mm, 0.2 μm thickness, Hewlett-Packard) was directly coupled to the mass spectrometer. The carrier gas was helium (1 mL/min). The program used was 4 min isothermal at 70 ^{o}C, then 4 ^{o}C/min to 180 ^{o}C and 10 min isothermal. The injection port temperature was 250°C and the detector temperature was 280°C. Ionization of the sample components was performed in the EI mode (70 eV).

The linear retention indexes for all the compounds were determined by co-injection of the sample with a solution containing the homologous series of C_8-C_{22} *n*-alkanes (Van den Dool and Kratz, 1963). The individual constituents were identified by their retention indexes referring to the compounds known from literature data (Adams, 1995); and also by comparing their mass spectra with spectra of either the known compounds or with those stored in the Wiley mass spectral database (Hewlett-Packard).

2.3. Induction period of lard oxidation (Rancimat method)

The induction period of lard with and without the addition of antioxidants was determined with the Rancimat model 743 (Metrohm, Switzerland) at 100 ^{o}C and the airflow of 20 L/h. The ethanolic solutions of different concentrations of antioxidant (100 μL) were added to the lard (2.5 g) giving a final concentration of 0.16%, 0.12%, 0.08%, 0.04% and 0.016% (w/w) of the antioxidant in the reacting system.

The antioxidant activity index (AI) is calculated from the measured induction times, according to the following formula by Forster *et al*. (2001).

AI = Induction time of lard with antioxidant / Induction time of pure lard

Although this technique has been questioned (Frankel, 1993), it is a procedure commonly used in the food industry and governmental analytical laboratories (Parejo *et al*., 2003).

3. RESULTS AND DISCUSSION

3.1. Chemical composition of essential oils

The chemical composition of total and fractionated essential oils from oregano, thyme and wild thyme is shown in Table I, while Table II show the chemical composition of the total and fractionated essential oil from two savory species, *Satureja montana* L. and *Satureja cuneifolia* Ten. Except *Satureja cuneifolia* Ten., all the essential oils showed qualitative similarities and a strong phenolic character.

In the essential oil of oregano eleven compounds were identified (97.9% of total oil) with phenolic monoterpenes thymol (35.0%) and carvacrol (32.0%) as the major compounds. Monoterpenic hydrocarbons γ-terpinene (10.5%), *p*-cymene (9.1%) and α-terpinene (3.6%) represented other important constituents of oregano essential oil (Kulisic *et al*., 2004). Similar results were already reported by Vokou *et al*. (1993).

Table I
**The composition (area %) of *Origanum vulgare* L. spp. *hirtum, Thymus vulgaris* L. and
Thymus serpyllum L. essential oils**

No	Compound	RI[1]	Peak area (%)					
			In total oil			In fraction		
			oregano	thyme	wild thyme	oregano	thyme	wild thyme
Hydrocarbons CH fraction								
1	α-Thujene	1031	1.4	-	-	5.2	0.7	-
2	α-Pinene	1038	-	-	0.6	-	-	1.2
3	β-Pinene	1102	-	-	-	0.7	-	-
4	δ-3-Carene	1131	-	-	-	-	0.3	-
5	β-Myrcene	1148	-	-	0.4	6.1	-	1.0
6	α-Terpinene	1161	3.6	-	0.7	10.4	0.8	2.3
7	γ-Terpinene	1231	10.5	5.5	5.3	31.0	41.1	29.5
8	*p*-Cymene	1247	9.1	11.6	5.2	22.1	26.5	26.1
9	Terpinolene	1262	-	-	-	0.9	0.1	-
10	Alloocimene[2]	1351	-	-	-	0.3	-	-
11	α-Copaene	1466	-	-	-	0.4	-	-
12	β-Burbonene	1496	-	-	-	0.3	-	-
13	*trans-*Caryophyllene	1578	2.4	-	3.5	9.1	23.9	29.3
14	Aromadendrene	1583	-	-	-	0.4	-	-
15	α-Humulene	1638	-	-	-	1.5	0.5	0.6
16	Ledene	1644	-	-	-	0.3	-	-
17	β-Bisabolene	1694	1.4	-	-	2.0	-	-
18	β-Cubebene	1694	-	-	-	-	0.7	-
19	Germacrene D	1680	-	-	-	-	1.5	-
20	δ-Cadinene	1729	0.5	-	-	3.8	2.8	-
21	α-Murolene	1735	-	-	-	0.2	-	-
22	uknown compound		-	-	-	-	-	6.1
					Total	95.8	98.8	96.3
Oxygen containing compounds fraction (CHO)								
23	1-Octen-3-ol	1411	1.0	-	-	0.8	-	-
24	Linalool	1507	-	-	-	-	1.3	-
25	Borneol	1653	1.0	-	-	1.0	-	-
26	Benzyl alcohol	1919	-	-	-	-	-	0.2
27	Thymol	2115	35.0	80.4	30.0	47.3	91.8	35.4
28	Carvacrol	2140	32.0	2.1	49.4	46.4	5.8	62.7
			Total					
			97.9	98.9	98.3			
Phenolic fraction								
29	Thymol	2115				58.9	91.0	/[3]
30	Carvacrol	2140				41.1	8.9	/

[1]Retention indices relative to C8-C22 alkanes on polar HP-20M column
[2]Correct isomer is not identified
[3]Phenolic fraction was not isolated

Table II
The composition (area %) of *Satureja montana* L. and *Satureja cuneifolia* Ten. essential oils

No	Compound	RI[1]	Peak area (%)			
			In total oil		In fraction	
			S. montana	*S. cuneifolia*	*S. montana*	*S. cuneifolia*
Hydrocarbons CH fraction						
1	α-Thujene	1031	1.0	-	2.7	-
2	α-Pinene	1038	1.0	8.5	2.0	13.6
3	β-Pinene	1102	-	-	2.1	-
4	Myrcene	1149	-	1.6	-	3.2
5	α-Terpinene	1161	3.5	-	7.5	-
6	Limonene	1179	-	2.7	-	4.3
7	*cis*-β-Ocimene	1218	-	6.5	-	18.9
8	γ-Terpinene	1231	5.9	-	19.8	-
9	*trans*-β-Ocimene	1235	-	4.7	-	14.1
10	*p*-Cymene	1247	6.4	1.3	23.1	2.3
11	Terpinolene	1262	-	-	0.6	-
12	Alloocimene[2]	1351	0.6	2.3	1.8	3.8
13	α-Copaene	1466	-	-	0.5	-
14	β-Burbonene	1496	-	-	0.7	-
15	β-Cubebene[4]	1524	-	-	8.9	-
16	*trans*-Caryophyllene	1578	2.3	1.1	18.2	6.9
17	α-Humulene	1638	0.3	-	1.3	-
18	Zingiberene	1659	-	-	0.6	-
19	γ-Cadinene	1677	-	-	0.4	-
20	Germacrene D	1680	-	10.3	-	20.3
21	α-Elemene	1687	0.3	-	0.7	-
22	β-Bisabolene	1694	1.1	-	3.1	-
23	δ-Cadinene	1729	-	1.7	2.3	4.7
24	α-Murolene	1735	-	-	0.2	-
			Total		96.5	92.1
Oxygen containing compounds fraction (CHO)						
25	1-Octen-3-ol	1411	0.7	-	0.8	-
26	*trans*-Sabinene hydrate	1423	0.2	-	0.3	-
27	Linalool	1507	0.6	29.3	0.7	62.0
28	Thymol methyl ether	1563	5.1	2.7	4.4	-
29	Carvacrol methyl ether	1576	5.8	2.3	6.6	3.2
30	Neral	1641	-	-	0.2	-
31	Borneol	1653	3.9	3.9	4.7	7.4
32	Geranial	1680	-	--	0.5	-
33	Geranyl acetate	1729	2.1	-	2.2	-
34	Nerol	1752	1.1	-	1.2	-
35	Geraniol	1796	5.0	-	6.2	-
36	3-Phenylpropanol[4]	1947	-	-	0.2	-
37	Thymol	2115	45.2	8.9	61.6	9.4
38	Carvacrol	2140	5.3	4.0	7.1	4.8
			Total	97.4	91.8	96.7	86.8
Phenolic fraction						
39	Thymol	2115			85.5	/[3]
40	Carvacrol	2140			14.5	/
				Total	100.0	

[1,2,3] as in Table I. [4]Tentatively identification on basis of the mass spectra (MS) only

Only four compounds were identified in thyme essential oil without fractionation: γ-terpinene (5.5%), *p*-cymene (11.6%), thymol (80.4%) and carvacrol (2.1%). In the essential oil from wild thyme eight compounds were identified with *p*-cymene (5.2%), γ-terpinene (5.3%), thymol (30.0%) and carvacrol (49.4%) as main components.

In the oregano essential oil seventeen compounds were identified in hydrocarbon (CH) fraction with γ-terpinene (31.0%), *p*-cymene (22.1%), α-terpinene (10.4%) and *trans*-caryophyllene (9.1%) as main components. The fraction with oxygen-containing compounds (CHO), was represented by four compounds, with thymol (47.3%) and carvacrol (46.4%) as the major ones (Kulisic *et al.*, 2004).

After fractionation, eleven compounds were identified in thyme hydrocarbon fraction where γ-terpinene (41.1%), *p*-cymene (26.5%) and *trans*-caryophyllene (23.9%) were main components. The analysis of the wild thyme hydrocarbon fraction showed that it consists of eight compounds with γ-terpinene (29.5%), *p*-cymene (26.1%) and *trans*-caryophyllene (29.3%) as main components. The fractions with oxygen-containing compounds in thyme and wild thyme essential oils, were represented by two phenolic compounds – thymol (91.8% in thyme, 35.4% in wild thyme) and carvacrol (5.8% in thyme, 62.7% in wild thyme) as the major compounds.

As shown in Table II the phenolic monoterpene thymol (45.2%) was also found to be the main constituent of the essential oil of *Satureja montana* L. The presence of monoterpenic hydrocarbons *p*-cymene (6.4%) and γ-terpinene (5.9%) and oxygen-containing compounds carvacrol methyl ether (5.8%), thymol methyl ether (5.1%), carvacrol (5.3%), geraniol (5.0%) and borneol (3.9%) represented the other constituents of this essential oil (Radonic and Milos, 2003). On contrary, the essential oil of *Satureja cuneifolia* Ten. contained a low percentage of thymol (8.9%) and carvacrol (4.0%). Linalool (29.3%) and germacrene D (10.3%) were the main components of this essential oil.

After fractionation, in the essential oil of *Satureja montana* L. nineteen compounds were identified in hydrocarbon fraction with *p*-cymene (23.1%), γ-terpinene (19.8%), *trans*-caryophyllene (18.2%) and β-cubebene (8.9%) as main components. The fraction with oxygen-containing compounds, which consisted of fourteen compounds, was dominated by thymol (61.6%) and the other important components were carvacrol (7.1%), carvacrol methyl ether (6.6%), geraniol (6.2%), borneol (4.7%) and thymol methyl ether (4.4%) (Radonic and Milos, 2003). Quite different results were obtained from the fractionation of *Satureja cuneifolia* Ten. essential oil. Hydrocarbon fraction consisted of ten compounds with germacrene D (20.3%), *cis*-β-ocymene (18.9%),

trans-β-ocymene (14.1%) and α-pinene (13.6%) as main components. Among five compounds in the oxygen-containing fraction, linalool (62.0%) was the highly dominating compound.

3.2. Inhibition of lard oxidation (Rancimat method)

The autoxidation of lipids can be inhibited or retarded by adding different antioxidants. They function either by scavenging chain-carrying peroxyl radicals or by diminishing the formation of initiating lipid radicals (Yamamoto and Niki, 1990). The mechanism of the antioxidant action of phenolic antioxidants in lipids has not been completely explained so far (Yanishlieva *et al.*, 1999).

Table III shows the induction times of lard in the presence of the total essential oils and their fractions from oregano, thyme, wild thyme and two savory species in comparison with reference antioxidants (BHT, BHA, α-tocopherol and ascorbic acid) and pure compounds thymol and carvacrol. The concentration of tested ethanolic solutions of antioxidants, which were added to the lard, was 0.16% (w/w). The inhibitory effect of the samples is expressed as the antioxidant activity index (AI).

The higher induction period of the lard with antioxidant added, compared to the control (pure lard) means improved antioxidant activity of that compound (von Gadow *et al.*, 1997).

Reference compounds, BHA and α-tocopherol, were the most potent inhibitors of the autoxidation of lipids with antioxidant activity indexes of 7.2 and 6.3. Synthetic antioxidant BHA has already been approved to control lipid oxidation in foods (Imaida *et al.*, 1983; Okada *et al.*, 1990; Ayar *et al.*, 2001), while α-tocopherol is known as the main protection factor in a system like LDL, low-density lipoproteins (Jia *et al.*, 1998; Zhu *et al.*, 1999; Andersen *et al.*, 2002). Ascorbic acid also showed a potent inhibitory effect against the autoxidation of lipid (AI = 4.3). It is known that it is used in patented protection systems for lard and marine oils (Löliger, 1991). Among the reference compounds, BHT showed the lowest antioxidant activity index (3.6), which can be explained by its volatility at the high temperature of the test (100 °C) so BHT could rapidly sweep from the lipid (von Gadow *et al.*, 1997).

In comparison with reference antioxidants, the total essential oils tested and their fractions showed a poor inhibitory effect against the lard autoxidation process under the conditions of this method, but very similar to those obtained for pure thymol and carvacrol. Among the essential oils tested the longest induction time for lard was achieved by the addition of oregano essential oil (AI = 2.0). Results for the other tested essential oils decreased in the order *Thymus vulgaris* L.>*Thymus serpyllum* L.>

Table III
**Induction times of lard and the antioxidant activity index (AI) of various antioxidants determined
by the Rancimat method**

Antioxidant[1]	Induction time (h)	AI
Total essential oils		
Origanum vulgare L. spp. *hirtum*	10.2	2.0
Thymus vulgaris L.	9.0	1.7
Thymus serpyllum L.	7.9	1.5
Satureja montana L.	6.8	1.3
Satureja cuneifolia Ten.	4.4	0.8
Hydrocarbons CH fraction		
Origanum vulgare L. spp. *hirtum*	5.3	1.0
Thymus vulgaris L.	4.4	0.8
Thymus serpyllum L.	4.0	0.7
Satureja montana L.	5.5	1.0
Satureja cuneifolia Ten.	0.6	0.1
Oxygen-containing compounds		
fraction (CHO)		
Origanum vulgare L. spp. *hirtum*	8.0	1.5
Thymus vulgaris L.	10.2	2.0
Thymus serpyllum L.	7.2	1.4
Satureja montana L.	6.9	1.3
Satureja cuneifolia Ten.	6.3	1.2
Phenolic fraction		
Origanum vulgare L. spp. *hirtum*	6.9	1.3
Thymus vulgaris L.	8.3	1.6
Satureja montana L.	7.3	1.4
BHT	19.8	3.6
BHA	37.8	7.2
α-Tocopherol	35.4	6.3
Ascorbic acid	22.5	4.3
Thymol	7.7	1.5
Carvacrol	7.2	1.4

[1]Concentration of tested antioxidant, which was added to the lard was 0.16% (w/w) in reacting system

The induction time of pure lard was 5.2 h.

Satureja montana L.>*Satureja cuneifolia* Ten. Thymol and carvacrol, which were the main components of essential oils of *Origanum vulgare* L. spp. *hirtum*, *Thymus vulgaris* L., *Thymus serpyllum* L. and *Satureja montana* L., can be effective in the inhibition of lard autoxidation at 35 $^{\circ}$C at a concentration of 0.1% (Lagouri *et al.*, 1993). Lagouri and Boskou (1996) concluded that the inhibition of oxidation by the essential oils from plants of the oregano species was highly dependent on the content of carvacrol and thymol. However, results presented in this study proved the opinion established by Yanishlieva *et al.* (1999) concerning the volatilities of thymol and carvacrol at 100 $^{\circ}$C, which is the main reason for their relatively low inhibitory effect observed by this method. The essential oil from *Satureja cuneifolia*

Table IV
Induction times of the lard with the addition of increasing amounts of various antioxidants determined by the Rancimat method

Total essential oil	Concentration (%)			
	0.016	0.04	0.08	0.12
	Induction time (h)			
Origanum vulgare L. spp. *hirtum*	5.8	7.0	7.6	10.6
Thymus vulgaris L.	6.6	7.1	8.5	9.7
Thymus serpyllum L.	5.1	6.6	7.7	8.5
Satureja montana L.	5.1	5.4	5.9	6.1

The induction time of pure lard was 5.2 h.

Ten. showed the poorest inhibitory effect probably because of the non-phenolic character of this oil.

The inhibitory effect of oxygen-containing compound fractions (CHO) was very similar to those for essential oils. Oxygen-containing compound fraction from *Thymus vulgaris* L. showed the longest induction time (AI = 2.0). Almost all CH fractions showed a prooxidative effect (AI < 1), except *Origanum vulgare* L. spp. *hirtum* and *Satureja montana* L. CH fractions with AI = 1. The strongest prooxidative effect showed CH fraction from *Satureja cuneifolia* Ten. (AI = 0.1), which could be the reason for the lower AI value of the total oil in comparison to the AI value of the CHO fraction of this oil. Phenolic fractions from *Origanum vulgare* L. spp. *hirtum*, *Thymus vulgaris* L. and *Satureja montana* L., consisted of thymol and carvacrol (Table I and II), and exhibited lower inhibitory effect in comparison to the effect of oxygen-containing compound fractions, which continues the speculations about the sinergy among minor oxygen containing compounds (Janssen *et al.*, 1988, Kulisic *et al.*, 2004).

The results presented in Table IV prove that the antioxidant effect of tested essential oils was dose-dependent. The induction times of lard were not affected by the different quantity of lard (5.2 h was induction time for 2.0 g, 2.5 g and 3.0 g of lard).

Since the conditions of this test were extreme (temperature - 100 $^{\circ}$C and air flow - 20 L/h), the volatile nature of tested antioxidants reduces their inhibitory effect in the lard autoxidation process. However, due to their strong phenolic character, the essential oils of the herbs tested could find their use in food protection, mostly in food processing at lower temperatures or in the process of food storage.

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