Antioxidant Activity of Some Spice Essential Oils on Linoleic Acid Oxidation in Aqueous Media

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Some spice essential oils (caraway, clove, cumin, rosemary, sage and thyme) and their major constituents were added to emulsified linoleic acid in aqueous media to examine their antioxidant activity. The methods used for measuring linoleic acid oxidation were coupled oxidation of β-carotene, conjugated diene formation and thiobarbituric acid test. The essential oils under study possess an antioxidant effect and this phenomenon was increased by increasing their concentration. Generally, the effectiveness of the various essential oils on linoleic acid oxidation was in the following descending order: caraway > sage > cumin > rosemary > thyme > clove. It appears that there was a relationship between the antioxidant effect and the chemical composition of the oils.

Consumers all over the world are becoming increasingly conscious of the nutritional value and the safety of their food and its ingredients. At the same time, there is an increased preference for natural foods and food ingredients which are generally believed to be safer, more healthy and less subject to hazards than foods containing artificial food additives. Lipid substances are easily deteriorated by oxidative rancidity from the reaction with atmospheric oxygen and hydrolytic reactions catalyzed by lipases from food or from microorganisms (1).

Antioxidants such as butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA) and propyl gallate (PG) are widely used in many foods to prevent the fat rancidity. These compounds are added at concentrations ranging from 50 to 200 ppm to fats and oils to suppress the development of peroxides during food storage (2,3). There has been some discussion recently of the undesirable use of synthetic antioxidants. For example, dietary administration of BHT to rats caused fatal hemorhages in the pleural and peritoneal cavities and in organs such as epididymis testes and pancreas (4). Also, BHT caused changes in rat thyroids, stimulation of DNA synthesis and induction of enzymes (5). BHA had toxic and carcinogenic effects (6). However, these antioxidants are approved for food use within limits. Consequently, there is an urgent need for other types of compounds to act as antioxidants. The present work was conducted to study the effects of some naturally occurring essential oils as antioxidants for linoleic acid autoxidation in an aqueous media.

MATERIALS AND METHODS

Source of essential oil plants. The flower buds, leaves or fruits of six spice plants were collected from the Pharmacy Farm, Cairo University, Giza, Egypt. The Latin names, family names and plant parts used in the present study are presented in Table 1.

Extraction of essential oils. The essential oils of sage, rosemary, caraway, clove, cumin and thyme were obtained by steam distillation.

β-carotene, BHT and linoleic acid. Crystalline *cis*-β-carotene, butylated hydroxy toluene (BHT) and linoleic acid, purest grade (99% by GLC) were obtained from Sigma Chemical Company (London Ltd., Poole, England). The purity of linoleic acid was checked by TLC and GLC and gave one spot and one peak, respectively.

Tween 20, EDTA and TBA. Tween 20 and ethylenediaminetetraacetate disodium salt (EDTA) were Merck grade. Thiobarbituric acid (TBA, 98%) was obtained from Aldrich Chemical Co. Ltd., England.

Solvents. All solvents used throughout the present work were BDH grade and were distilled before use.

Prevention of contamination by heavy metals. Scrupulous care was taken to avoid contamination by heavy metals. All experimental work was carried out in all glass equipment to minimize metal contamination. All glassware was immersed for at least 24 hr in EDTA (0.5%, w/v), rinsed several times with deionized water and dried at 150°C before use.

Preparation of linoleic acid (10^{-2} M)-β-carotene emulsion. An aliquot from β-carotene dissolved in chloroform (10 ml, 0.05%) was pipetted into a flask containing linoleic acid (ca. 1.4 g) and Tween 20 (1 ml, 0.02%). The solvent was evaporated, deionized water (500 ml) was then added and emulsification was achieved by agitation using a Julabo ultrasonic bath (40 Khz) for 15 min. This system was stable for at least 1.5 months.

TABLE 1

Scientific Names, Family Names and Plant Parts for Some Spice Essential Oils

Spice plant	Scientific name	Family name	Plant part
Sage	*Salvia officinalis*, L.	Labiatae	Leaves
Rosemary	*Rosmarinus officinalis*, L.	Labiatae	Leaves
Clove	*Eugenia* sp.	Myrtaceae	Flower buds
Caraway	*Carum corui*, L.	Umbellifereae	Fruits
Cumin	*Cumin cyminum*, L.	Umbellifereae	Fruits
Thyme	*Thymus vulgarius*, L.	Labiatae	Leaves

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Preparation of stock solutions of emulsified essential oils and their major compounds. Tween 20 (0.25 ml, 0.2%) was introduced into a volumetric flask (50 ml) then varied amounts of essential oils or their major compounds were added and the flask was filled to the mark with deionized water. Emulsification was performed by agitation using a Julabo ultrasonic bath for 15 min. The solutions of emulsified essential oils and their major compounds were stable for at least 1.5 months.

Reaction mixture. The emulsified essential oils or their major compounds were added to emulsified linoleic acid-β-carotene at different concentrations depending on the oil type. The major compounds of the essential oils were added individually to the lipid material at concentrations similar to their presence in the neat oil. Another experiment was conducted where BHT (200 ppm) was added to emulsified linoleic acid instead of essential oils in order to compare the antioxidant efficiency of the essential oils. The oxidation components and conditions of linoleic acid are presented in Table 2.

Measurements of linoleic acid oxidation. A minimum of two flasks containing linoleic acid-β-carotene and essential oils under study or their major compounds were run against appropriate controls (flasks containing emulsified linoleic acid and emulsified linoleic acid-β-carotene). Three methods were used to follow up the oxidation of linoleic acid, i.e., coupled oxidation with β-carotene (7), conjugated diene formation (8) and TBA-test (9).

Coupled oxidation of linoleic acid-β-carotene method. An aliquot from the reaction mixture (0.2 ml) was diluted at intervals with ethanol (3 ml, 99%), vortexed for 30 sec and the absorbance was recorded at 362 nm against a blank containing emulsified linoleic acid-β-carotene using an LKB Ultrospec II spectrophotometer.

Conjugated diene formation method. An aliquot from the linoleate reaction mixture (0.1 ml) was diluted at intervals with methanol (3 ml), vortexed and the absorbance was recorded at 232 nm. The absorbance values were converted into conjugated diene concentration using a molar extinction coefficient of 26000 M^{-1} cm^{-1}.

TBA-test. An aliquot from the linoleate reaction mixture (0.1 ml) was pipetted into a test tube; then trichloroacetic acetic acid (1 ml, 35%) and TBA solution (2 ml, 0.75%) were added and vortexed. The tubes were placed in a boiling water bath for 15 min and after cooling them the absorbance was recorded at 532 nm against a blank containing all the reagents except linoleic acid. The extinction coefficient of TBA-malonaldehyde product of 1.56×10^5 was used to convert the absorbance values into concentration of the secondary reaction products.

RESULTS AND DISCUSSION

The essential oils used in the present work have shown positive and effective inhibitory effects on synthetic media containing bacteria, yeast and fungi (10,11). These microorganisms are known to be responsible for food spoilage. GLC analysis indicate that some of these oils contained compounds with phenolic nucleus which possess antioxidant properties (10). Accordingly, the essential oils under study were added to linoleic acid in an attempt to study their effect on prevention of lipid oxidation. The minimum inhibitory concentrations (MIC) required to prevent certain microorganisms from growth were 200, 400, 400, 600, 2000 and 2000 ppm for thyme, clove, cumin, caraway, rosemary and sage, respectively (10). Hence, the essential oils were added to linoleic acid oxidation systems at different concentrations, i.e., one, three and six times the MIC. The levels of essential oils added to lipid substance under study are beyond the concentration of antioxidants added in industry to food products. The major components of the essential oils were added to the model systems at concentrations similar to those in the neat oils in order to evaluate their antioxidant efficiency. An experiment was conducted using BHT at 200 ppm along with other experiments in order to compare the antioxidant potentiality of the essential oils towards linoleic acid oxidation.

Oxidation of linoleic acid in aqueous emulsion is of great importance in handling and storing of these materials

TABLE 2

Components and Conditions of Linoleic Acid Oxidation Model Systems

Essential oil concentration (ppm)	Basic ompound concentration (ppm)	β-carotene concentration (ppm)	Reaction conditions		Emulsifier (0.02%)
			Temperature (°C)	Oscillation/min	
Thyme (200,600,1200)	Thymol (85.4,256.2,512.4)	10	50	110	1 ml
Cumin (400,1200,2400)	Cumin aldehyde (222.4,668.4,1336.8)	10	50	110	1 ml
Caraway (600,1800,3600)	Carvone (487.8,1463.4,2926.8)	10	50	110	1 ml
Clove (400,1200,2400)	Eugenol (340,1022,2044)	10	50	110	1 ml
Rosemary (2000,6000,12000)	Borneol (530,1590,3180)	10	50	110	1 ml
Sage (2000,6000,12000)	Thujone (830,2490,4980)	10	50	110	1 ml
BHT (200)	—	10	50	110	1 ml

and, in particular, in dairy products. It was intended to propose a model system as simple as possible in order to minimize variables and obtain reproducible results. A number of restrictions were imposed, including the concentration of linoleic acid, the type and concentration of emulsifier, as well as the temperature and shaking rate of reaction vessels. These together with scrupulous avoidance of contamination by extraneous metal ions and careful adherence to the routine preparation of the emulsions, have produced reproducible and consistent results.

The variables in the model systems were restricted only on the type of essential oils, their major components and no buffers were used for the preparation of the model systems since the results of Wills (12), Haase and Dunkely (13) and Allen *et al.* (8) have shown that phosphate, tris and borate buffers increased and decreased the rate of lipid oxidation, respectively.

The effect of various essential oils in all model systems of aqueous media has shown a feature of an autocatalytic chain reaction, i.e., the rate of hydroperoxide formation increased with time, and the secondary products are necessary to catalyze linoleic acid oxidation. The results of individual experiments showed considerable variation in the rates of oxidation in comparison with the control experiments. The rate of β-carotene bleaching for linoleate systems is shown in Figure 1 and the time required for the complete disappearance of β-carotene in the model systems is shown in Table 3. In this method, β-carotene was added to the model systems as a marker for linoleate oxidation. In other words, the bleaching of β-carotene is entirely dependent on the rate of hydroperoxide formation. It seems that β-carotene is bleached by linoleic acid hydroperoxides with a nonstoichiometric reaction. Consequently, one would consider that this method is a preliminary and fast test to distinguish the antioxidant activity of certain compounds. The data presented in Table 3 show that all the essential oils and their major components possessed antioxidative effect and the extent of antioxidant activity was largely dependent on the oil or major component type.

The commonly used methods for measuring lipid oxidation are conjugated diene formation and TBA-test. These methods were also used to follow up the linoleic acid oxidation in the presence of various essential oils. The first method is currently used for measuring the hydroperoxide formation, while the latter method estimates the production of secondary products such as aldehydes, ketones, etc. Therefore, these two methods will indeed give an accurate data on the course of lipid oxidation, since no further compounds such as β-carotene were used as a marker for lipid oxidation.

In order to compare the antioxidative behavior of the essential oils under study in an aqueous media, values of 1.5 mM and 7 mM for the formation of conjugated dienes and secondary products were chosen respectively, as some of these model systems differ greatly in the rate of linoleic acid oxidation. The catalytic effects of various essential oils and their major components on the stability of linoleic acid in aqueous media are shown in Figures 2, 3 and Tables 3, 4. The effectiveness of the various essential oils on linoleic acid oxidation was in the following descending order: caraway > sage > cumin > rosemary > thyme > clove. It has been reported that rosemary, sage, thyme and clove exhibited antioxidant activity (14-21). The antioxidant efficiency of various essential oils was basically depend on their concentrations. An increase of the concentration from one to three- and sixfold the MIC caused an increase in the oxidation activity of the essential oils (Figs. 2, 3). However, this phenomenon was not found with carvone, thujone, cumin aldehyde and borneol, the

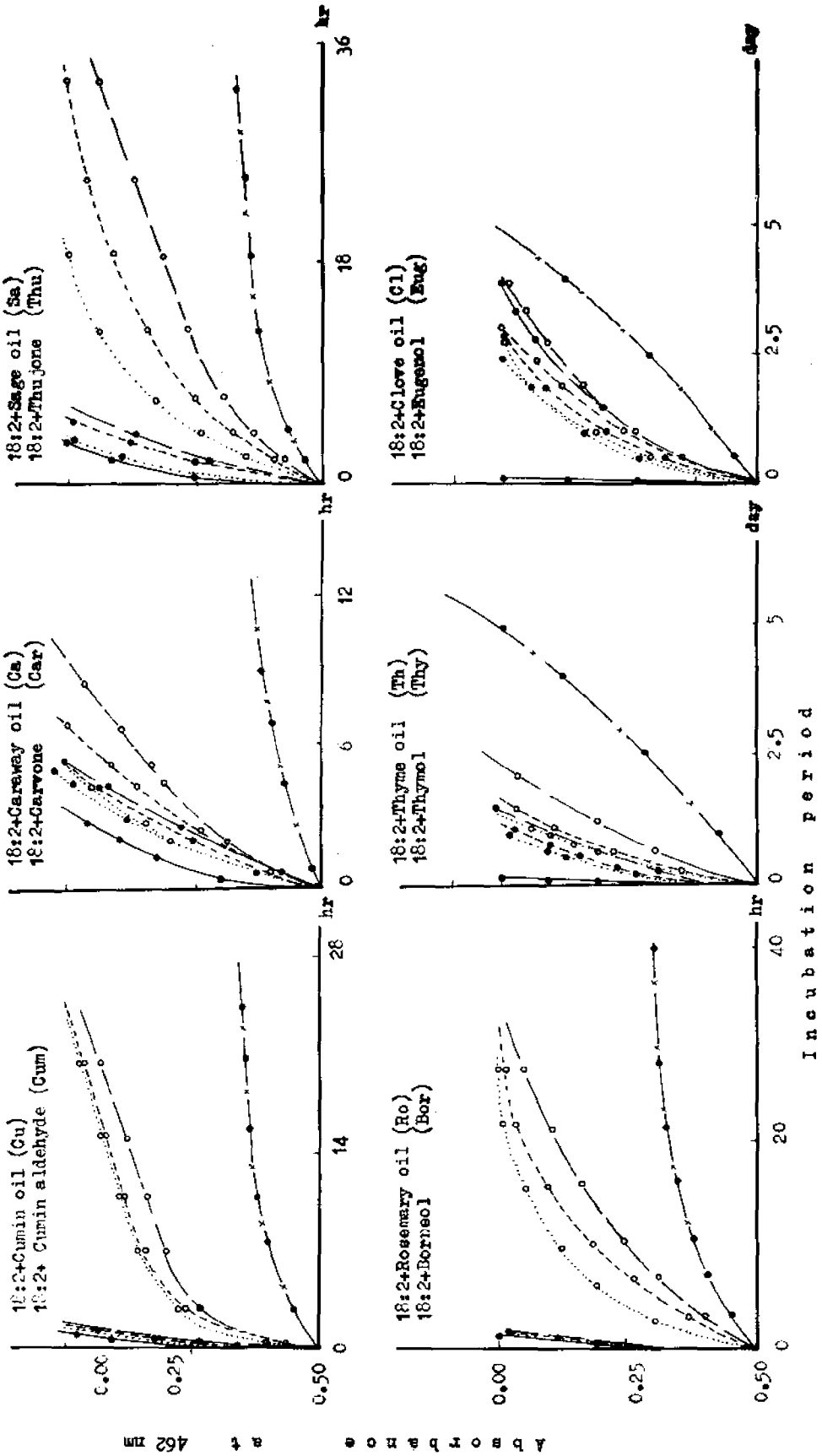
TABLE 3

Coupled Oxidation of β-carotene and Linoleic acid[a]

Oil	Concentration (ppm)	Antioxidant index[b]	Basic compound	Concentration (ppm)	Antioxidant index[b]
Caraway	600	4.4	Carvone	487.8	5
	1800	6.6		1463.4	5
	3600	9.0		2926.8	5
Cumin	400	6.6	Cuminaldehyde	222.4	1.8
	1200	26.5		668.4	1.8
	2400	28.5		1336.8	1.8
Sage	2000	18.0	Thujone	530	4.0
	6000	31.1		1590	5.0
	12000	35.0		3180	6.0
Rosemary	2000	33.5	Borneol	530	1.6
	6000	35.5		1590	1.6
	12000	42.0		3180	1.6
Thyme	200	31.2	Thymol	85.4	24
	600	38.4		256.2	28.8
	1200	57.6		512.4	32.4
Clove	400	67.2	Eugenol	340	60.0
	1200	74.4		1022	72.0
	2400	96.0		2044	93.6
Control	—	1.5	BHT	200	120

[a]Catalyzed by some essential oils and their basic compounds in aqueous media.

[b]Antioxidant index refers to the time (hr) required for the complete disappearance of β-carotene.



●—● = 18:2 (Control)
●····● = 18:2 + Cum (222.4 ppm); 18:2 + Car (487.8 ppm); 18:2 + Thu (830 ppm)
●– –● = 18:2 + Cum (668.4 ppm); 18:2 + Car (1463.4 ppm); 18:2 + Thu (2490 ppm)
●– –● = 18:2 + Cum (1336.8 ppm); 18:2 + Car (2926.8 ppm); 18:2 + Thu (4980 ppm)
○····○ = 18:2 + Cu (400 ppm); 18:2 + Ca (600 ppm); 18:2 + Sa (2000 ppm)
○– –○ = 18:2 + Cu (1200 ppm); 18:2 + Ca (1800 ppm); 18:2 + Sa (6000 ppm)
○– –○ = 18:2 + Cu (2400 ppm); 18:2 + Ca (3600 ppm); 18:2 + Sa (12000 ppm)

●····● = 18:2 + Bor (530 ppm); 18:2 + Thy (85.4 ppm); 18:2 + Eug (340 ppm)
●– –● = 18:2 + Bor (1590 ppm); 18:2 + Thy (256.2 ppm); 18:2 + Eug (1022 ppm)
●– –● = 18:2 + Bor (3180 ppm); 18:2 + Thy (512.4 ppm); 18:2 + Eug (2044 ppm)
○····○ = 18:2 + Ro (2000 ppm); 18:2 + Th (200 ppm); 18:2 + Cl (400 ppm)
○– –○ = 18:2 + Ro (6000 ppm); 18:2 + Th (600 ppm); 18:2 + Cl (1200 ppm)
○– –○ = 18:2 + Ro (12000 ppm); 18:2 + Th (1200 ppm); 18:2 + Cl (2400 ppm)
●–x–● = 18:2 + BHT (200 ppm)

FIG. 1. Oxidation of emulsified linoleic acid (18:2) — β-carotene catalyzed by various essential oils and their major compounds.

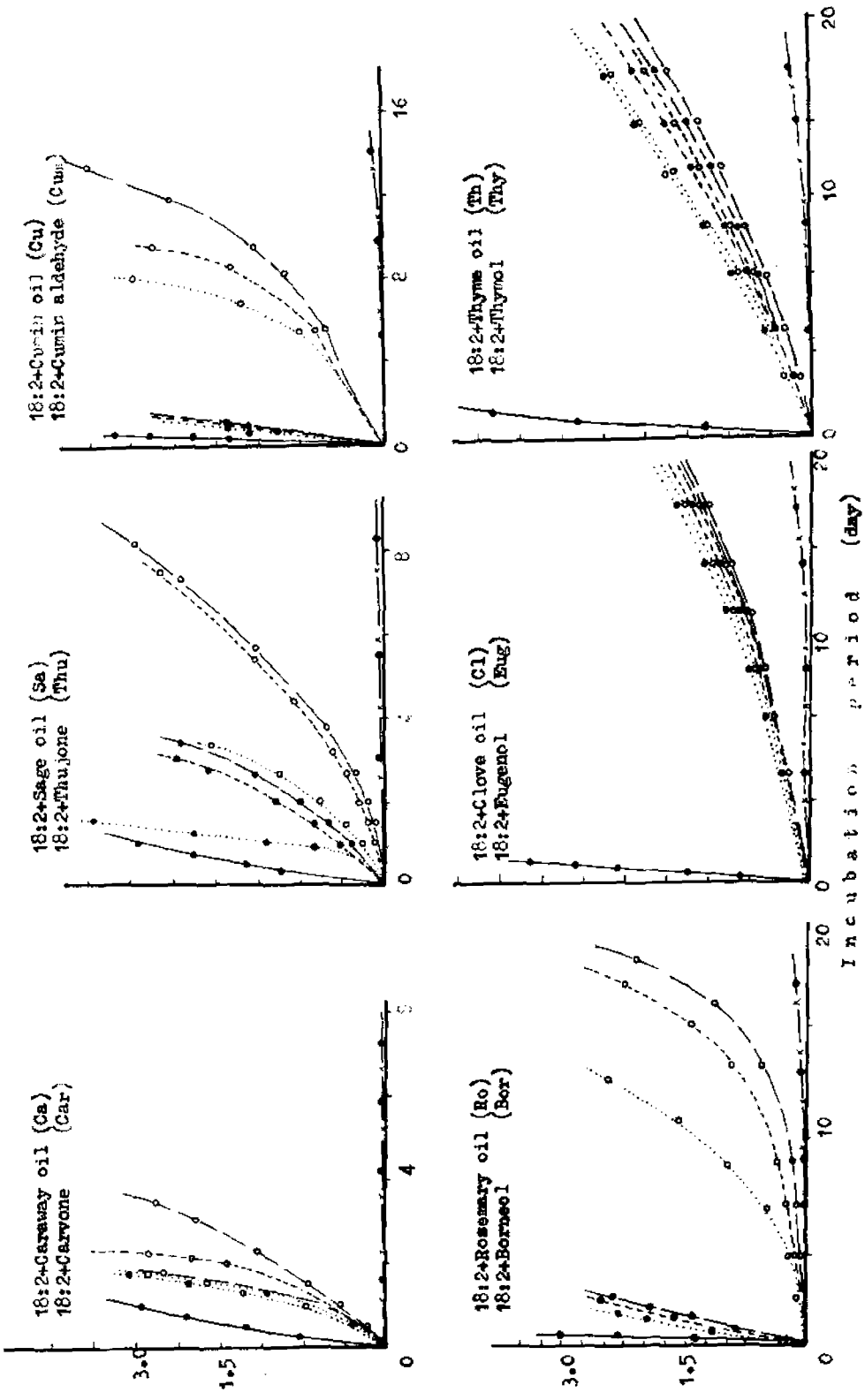


FIG. 2. Conjugated diene hydroperoxide formation by emulsified linoleic acid (18:2) catalyzed by various essential oils and their major components.

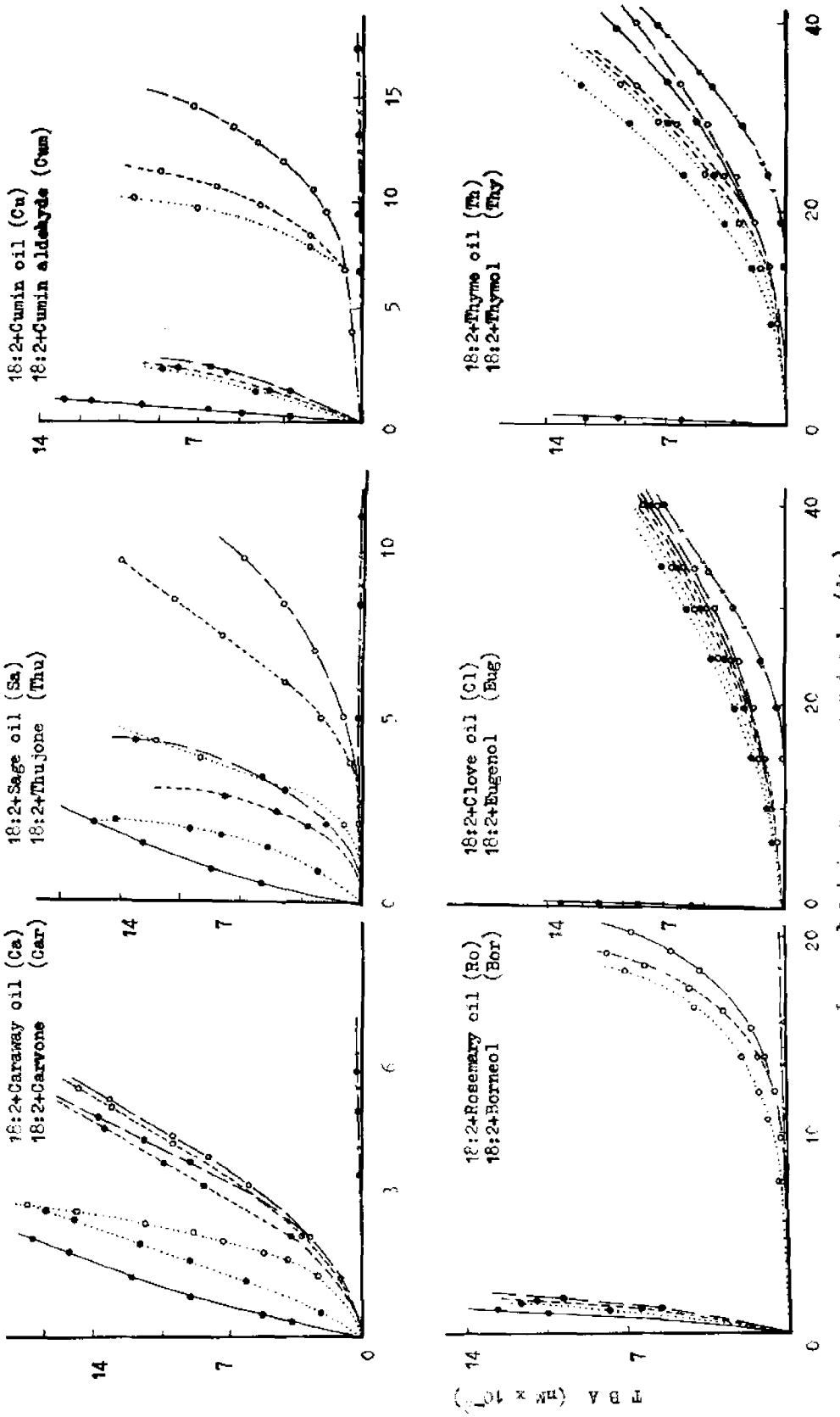


FIG. 3. The effect of various essential oils and their major components on the secondary oxidation products of emulsified linoleic acid (18:2).

TABLE 4

Incubation periods (Day) for Linoleic acid[a]

Oil	Concentration (ppm)	ROOH[b]	TBA[c]	Basic compound	ROOH[b]	TBA[c]
Caraway	600	1.15	1.6	Carvone	5	1.25
	1800	1.70	4.1		5	1.25
	3600	2.40	4.3		5	1.30
Sage	2000	2.75	3.50	Thujone	1.0	1.5
	6000	5.25	6.70		2.1	2.7
	12000	6.00	9.0		2.5	3.0
Cumin	400	6.3	8.7	Cuminaldehyde	0.9	1.95
	1200	8.1	11.4		0.9	2.0
	2400	9.5	15.0		0.9	2.5
Rosemary	2000	10.4	17.6	Borneol	0.8	0.9
	6000	15.5	18.2		1.0	1.1
	12000	17.0	19.6		1.2	1.3
Thyme	200	14.5	31	Thymol	14	28
	600	17.5	33		17	32
	1200	19.2	38		19	36
Clove	400	20.0	37	Eugenol	19.7	36.0
	1200	21.1	40.4		20.6	38.0
	2400	22	41.8		21.9	40.4
BHT	200	30.0	43.00	Control	0.50	0.98

[a]Catalyzed by some essential oils and their basic compounds in aqueous media.

[b]Indicates number of days required to reach 1.5 mM hydroperoxides by a model system.

[c]Indicates number of days required to reach 7 mM TBA products as malonaldehyde by a model system.

major components of caraway, sage, cumin and rosemary, respectively. On the contrary, thymol and eugenol were exempted from this rule, since the antioxidant efficiency of these compounds increased with increasing the oil concentration. The relatively high TBA values for thujone and cumin aldehyde might stem from the presence of carbonyl groups which react readily with TBA reagent. It is worth mentioning that the antioxidant activity of thymol and eugenol at 1200 ppm were nearly 0.6 and 0.7 times the effectiveness of BHT at 200 ppm, respectively. Despite the fact that the levels of thymol and eugenol were 6 times that of BHT, natural antioxidants are preferred over synthetic antioxidant from a food safety view point.

It appears that there is a relationship between the antioxidant efficiency and the chemical composition of the oils. Comparison of the tested essential oils and their major components showed that the structural feature required for antioxidant activity was a phenolic ring containing an electron repelling group in the *ortho*-position to the phenolic group such as isopropyl or methoxy group. These structural requirements were supported by the powerful antioxidant activity of the well-known BHT or BHA. Borneol, thujone and carvone had little antioxidant activity compared with thymol or eugenol due to absence of aromaticity. Thymol and eugenol had the higher antioxidant action due to the presence of phenolic OH groups. One would relate the antioxidant activity of thyme and clove oils and their major substances to the inhibition of the hydroperoxide formation. The first step in lipid oxidation is the abstraction of hydrogen atom from a fatty acid and oxygen involvement gives a peroxy radical. Generally, the antioxidants suppress the hydrogen atom abstraction from the fatty acid which leads to the decrease of hydroperoxide formation (22). It is well known that phenolic compounds act as hydrogen donors to the reaction mixture and therefore, the formation of hydroperoxides is decreased. The slow formation of conjugated dienes and consequently the secondary products by thyme and clove oils and their major compounds indicated that these materials acted as hydrogen donors to the peroxy radicals. Thus, retarding the autoxidation of linoleic acid by chain radical termination.

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