Influence of Thyme and Clove Essential Oils on Cottonseed Oil Oxidation

R.S. Farag[a,*], A.Z.M.A. Badei[b] and G.S.A. El Baroty[a]
[a]Biochemistry Department and [b]Food Science and Technology Department, Faculty of Agriculture, Cairo University, Giza - Egypt

Simple model systems composed of refined cottonseed oil and thyme oil or clove oil, without additives, were designed to approximate conditions in natural oils in order to study their oxidation behavior. Three methods were used to follow cottonseed oil oxidation, i.e., coupled oxidation with β-carotene, the TBA test and hydroperoxide number. The results illustrate that clove and thyme oils at various concentrations exhibit antioxidant activity and this phenomenon for clove oil is superior to that of thyme oil. Sensory evaluation tests indicate that the addition of thyme oil or clove oil to cottonseed oil at concentrations ranging from 50 to 1200 ppm do not affect the odor note of cottonseed oil. Therefore, thyme and clove essential oils are recommended for use as natural antioxidants to suppress lipid oxidation.

The oil industry is one of the most important food industries that needs great attention during processing, transport, handling and storage of products. Polyunsaturated fatty acids, especially those with a methylene interrupted diene system, are extremely susceptible to peroxidation, react readily with atmospheric oxygen and produce undesirable flavors. Heavily oxidized fats and oils are no longer suitable for nutritive purposes, because the oxidation products of the unsaturated fatty acids have toxic effects (1). In order to maintain the original structure of the polyunsaturated fatty acids in lipid materials, great care must be taken in handling and storing these materials. Consequently, more attention has been paid to discovering substances that can suppress lipid oxidation. Many synthetic substances such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate are commonly added to lipids to retard oxidation (1). Recently, these synthetic substances have been shown to cause the following symptoms: enlarge the liver size, increase liver microsomal enzyme activity, and convert some ingested materials into toxic or carcinogenic substances, especially if they are present in excessive amounts (2–4). Therefore, there is need for other compounds to act as antioxidants and to render food products safer for mankind.

Essential oils are used in medicinal drugs and in controlling harmful insects. These oils contain some phenolic compounds which can act as antioxidants. The present investigation was planned to study the antioxidant activity of some spice essential oils on cottonseed oil oxidation. Also, the acceptability to mankind of these spicy oils when mixed with cottonseed oil for processing certain foods was evaluated.

MATERIALS AND METHODS

Source of essential oil plants. The flower buds and leaves of clove *(Eugenia sp.)* and thyme (*Thumus vulgarius*, L.), respectively were obtained from the Pharmacy Farm, Cairo University, Giza-Egypt.

Extraction of essential oils. The essential oils of clove flower buds and thyme leaves were obtained by steam distillation.

β-Carotene and BHT. Crystalline *cis-β*-carotene and butylated hydroxy toluene (BHT) were obtained from Sigma Chemical Co. (London, Ltd. Poole).

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

Cottonseed oil. Refined cottonseed oil was obtained from the Cairo Company for Oil and Soap. Its peroxide number was 4.4 meq/Kg and it was nearly free of free fatty acids.

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. The glass equipment was immersed for at least 24 hr in EDTA solution (0.5%, w/v), rinsed several times with deionized water and dried at 150°C before use.

Reaction mixture. Different concentrations of thyme (200, 600 and 1200 ppm) and clove (400, 1200 and 2400 ppm) essential oils were added separately to cottonseed oil (100 g) containing β-carotene (0.5 mg). The oxidation rate of cottonseed oil was followed at room temperature for 35 days.

Measurements of cottonseed oil oxidation. A minimum of two flasks containing cottonseed oil-β-carotene and essential oils under study were run against appropriate controls. Three methods were used to follow up the cottonseed oil oxidation, i.e., coupled oxidation with β-carotene (5), peroxide number (6) and TBA test (7).

Coupled oxidation of cottonseed oil-β-carotene method. An aliquot from the reaction mixture (0.2 ml) was dissolved in hexane at intervals, vortexed for 30 sec and the absorbance recorded at 462 nm using an LKB Ultrospec II spectrophotometer.

Peroxide number. A known weight from the oxidation mixture (0.5 g) was dissolved in a mixture consisting of $CHCl_3$:acetic acid (30 ml, 3:2, v/v) then freshly prepared saturated potassium iodide solution was added (1 ml) and vortexed for exactly one min. Distilled water (30 ml) and starch solution (0.5 ml, 1%) were added and the liberated iodine was titrated with sodium thiosulfate (0.001 N).

TBA test. A known weight from the reaction mixture (0.5 g) was thoroughly mixed with TBA reagent (2 ml) and benzene (5 ml). After shaking the mixture, the aqueous phase was separated and heated in a boiling water bath for 30 min. The absorbance of the resultant color was recorded at 532 nm.

Sensory evaluation. Sensory evaluation tests were performed to illustrate the effect of mixing thyme and clove essential oils with cottonseed oil (8). Two techniques were

*To whom correspondence should be addressed.

used in the sensory tests, as follows, a sensitivity (threshold) test and a ranking test.

Sensitivity (threshold) test. Two sets of beakers (7 each) containing cottonseed oil were mixed with different concentrations of thyme oil (50, 100, 200, 600, 1200, 2400 and 3600 ppm) and clove oil (50, 200, 400, 1200, 2400, 3000 and 3600 ppm). Panelists (10) were chosen from personnel within the Faculty of Agriculture, Cairo University, and asked to sniff each sample, characterize the odors and rate the odor intensity on a scale of 0 (no odor) to 3 (nonacceptable odor).

Ranking test. The acceptability of cottonseed oil mixed with essential oils (clove and thyme) was evaluated using heated (frying potato chips) and unheated (cooked horse beans) foods. The panelists (25) ranked all food samples with code numbers (first = 9; second, 6; third, 3, and fourth, 0) according to the intensity of the characteristic flavor and their preference. The results were subjected to analysis of variance and least significant difference (9).

RESULTS AND DISCUSSION

Much work has been carried out by many researchers dealing with the oxidation of lipid materials using model systems in aqueous media (10–13). The model systems consisted of linoleic acid catalyzed by ascorbic acid, Cu^{+2}, amino acids, and bases, respectively. Few attempts have been undertaken to elucidate the course of lipid oxidation in anhydrous sources. For instance, the oxidation of linoleic acid, methyl linoleate and trilinolein was performed in model systems based on various solid supports and at varying relative humidity (14). Also, the antioxidant efficacy of some essential oils as natural preservatives for butter was evaluated (15).

In the present study, simple model systems composed of refined cottonseed oil and thyme oil or clove oil, without recorse to any additives such as emulsifiers, were designed to approximate conditions in natural oils in order to study their oxidation behavior. The addition of thyme and clove essential oils at various concentrations did not affect the color or the appearance of cottonseed oil at all. An experiment was conducted where cottonseed oil was catalyzed by BHT (200 ppm) in order to compare the antioxidant efficiency of the essential oils under study with the most commonly used synthetic antioxidant material. The results relevant to linoleic acid oxidation catalyzed by various essential oils in aqueous media show that clove and thyme oils possess high antioxidant activity compared with other essential oils (16). Consequently, clove and thyme oils were used in this set of experiments only at various concentrations (one, three and six times the minimum inhibitory concentration required for antimicrobial activity) (17). Determination of peroxide number, coupled oxidation with β-carotene and TBA test of the model systems at intervals were the methods of choice to follow up the course of cottonseed oil oxidation at room temperature. The samples were stored and tested for a period of 35 days. Results of this work illustrate that

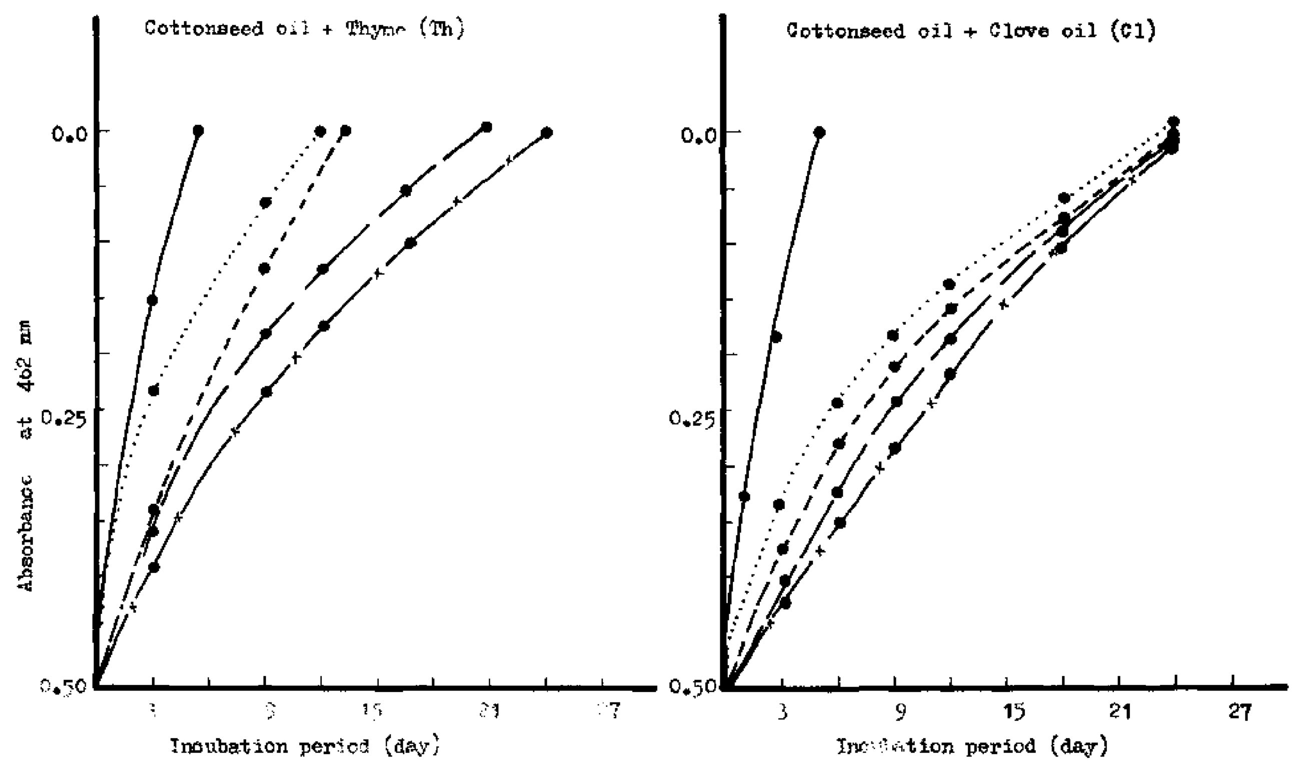

FIG. 1. Antioxidant activity of different concentrations of thyme and clove essential oils on cottonseed oil oxidation. ●—●, Control (cottonseed oil); ●······●, cottonseed oil + Th (200 ppm):cottonseed oil + Cl (400 ppm); ●– – –●, cottonseed oil + Th (600 ppm):cottonseed oil + Cl (1200 ppm); ●—–●, cottonseed oil + Th (1200 ppm):cottonseed oil + Cl (2400 ppm); ●–X–●, cottonseed oil + BHT (200 ppm).

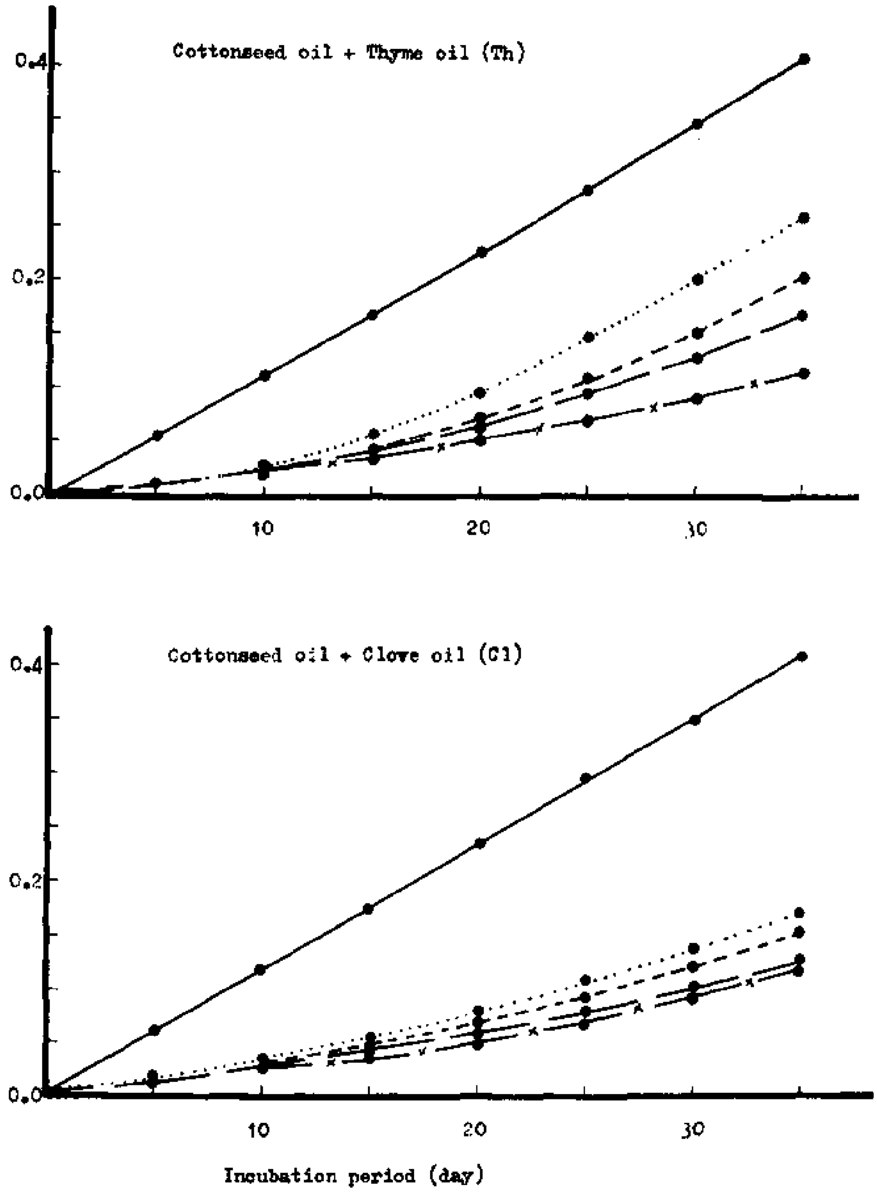

FIG. 2. Antioxidant activity of different concentrations of thyme and clove essential oils on cottonseed oil oxidation. ●—●, Control (cottonseed oil); ●······●, cottonseed oil + Th (200 ppm):cottonseed oil + Cl (400 ppm); ●– – –●, cottonseed oil + Th (600 ppm):cottonseed oil + Cl (1200 ppm); ●——●, cottonseed oil + Th (1200 ppm):cottonseed oil + Cl (2400 ppm); ●—X—●, cottonseed oil + BHT (200 ppm).

clove and thyme oils at various concentrations exhibited antioxidant activity in comparison with the control experiments, and showed features of an autocatalytic chain reaction in all cases (Figs. 1–3).

In order to compare the antioxidant behavior of the two essential oils at different concentrations, a value of 18 mM hydroperoxides was chosen because all the model systems showed different oxidation patterns during the incubation period. Figure 3 shows the oxidation curves for refined cottonseed oil catalyzed by thyme and clove oils at various concentrations. Table 1 shows the time required by the model systems to produce 18 mM hydroperoxides. These data indicate that the antioxidant efficiency of the clove oil was superior to that of thyme oil. This result also was found with linoleic acid oxidation catalyzed by these oils in an aqueous system (16). However, the antioxidant phenomenon of these two oils were more pronounced in a nonaqueous system than in an aqueous one. Clove and thyme oils at 2400 and 1200 ppm, respectively, produced an antioxidant power similar to that produced by BHT at 200 ppm. The levels of thyme and clove oils were 12 and 6 times that of BHT. However, natural antioxidants are preferred over synthetic antioxidants, to minimize adverse effects on mankind.

A set of experiments was conducted to detect the acceptability of cottonseed oil mixed with thyme or clove

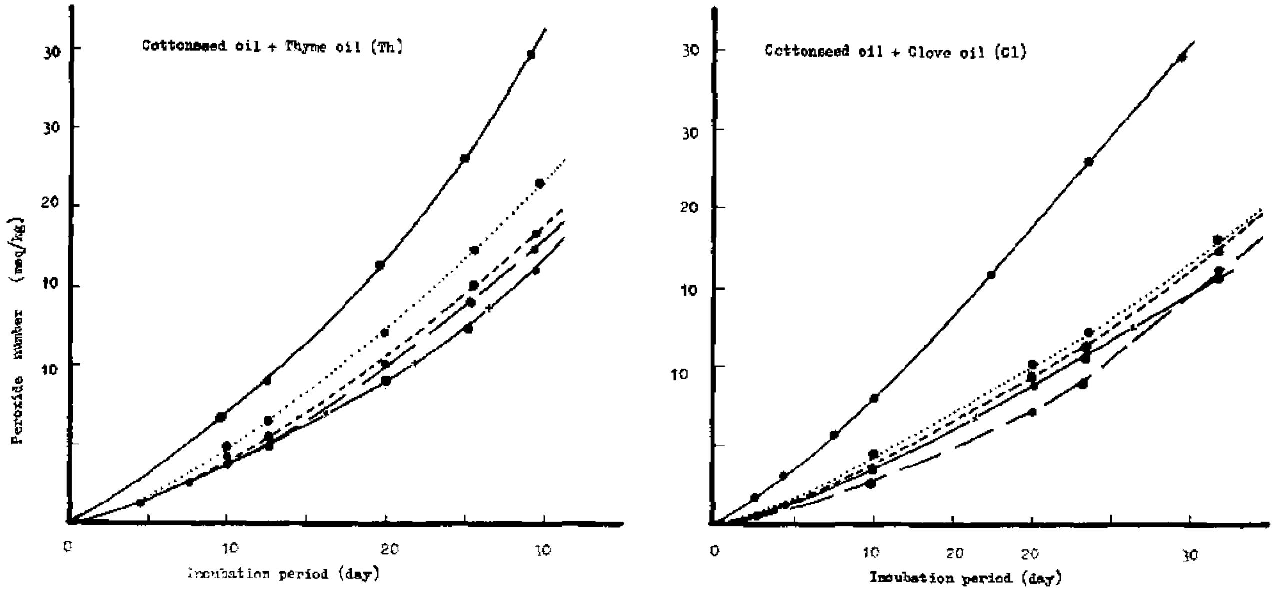

FIG. 3. Antioxidant activity of butylated hydroxytoluene (BHT), thyme oil and clove oil on cottonseed oil oxidation. ●—●, Control (cottonseed oil); ●······●, cottonseed oil + Th (200 ppm):cottonseed oil + Cl (400 ppm); ●– – –●, cottonseed oil + Th (600 ppm):cottonseed oil + Cl (1200 ppm); ●——●, cottonseed oil + Th (1200 ppm):cottonseed oil + Cl (2400 ppm); ●—X—●, cottonseed oil + BHT (200 ppm).

TABLE 1

Incubation Periods (Days) for Cottonseed Oil Catalyzed by Thyme, Clove and BHT at a Value of 18 mM Peroxide Content

System	Incubation period (days)	System	Incubation period (days)
Cottonseed oil (control)	23	Cottonseed oil (control)	19.5
Cottonseed oil + thyme oil (200 ppm)	28	Cottonseed oil + clove oil (400 ppm)	33.5
Cottonseed oil + thyme oil (600 ppm)	31	Cottonseed oil + clove oil (1200 ppm)	35
Cottonseed oil + thyme oil (1200 ppm)	32	Cottonseed oil + clove oil (2400 ppm)	37
Cottonseed oil + BHT (200 ppm)	34.5	Cottonseed oil + BHT (200 ppm)	37

TABLE 2

Mean Threshold Values and Standard Errors for Thyme and Clove Essential Oils Added to Cottonseed Oil

Thyme oil			Clove oil		
Concentration (ppm)	Detection of difference	Odor score[b]	Concentration (ppm)	Detection of difference	Odor score[b]
50	None	0.0 ± 0.0	50	None	0.0 ± 0.0
100	None	0.0 ± 0.0	100	None	0.0 ± 0.0
200	None	0.0 ± 0.0	200	None	0.0 ± 0.0
600	None	0.1 ± 0.10	600	None	0.0 ± 0.10
1200	Weak	0.9 ± 0.10	1200	Weak	0.9 ± 0.10
2400	Medium[a]	2.0 ± 0.15	2400	Medium[a]	2.1 ± 0.10
3000	Medium	2.3 ± 0.15	3000	Strong	3.0 ± 0.15
3600	Strong	3.0 ± 0.15	3600	Very strong	3.9 ± 0.10

[a]Threshold value refers to the minimum concentration at which a stimulus is easily characterized.

[b]The intensity of odor was described according to the following scale: 0, None (odor of control); 1, weak (odor different from control); 2, medium (threshold, very weak odor identifiable); 3, strong; 4, very strong; 5, extremely strong.

TABLE 3

Mean Sensory Scores and Standard Errors for Bean Meal Prepared by Mixing Cottonseed Oil with Thyme Oil or Clove Oil

Concentration (ppm)	Thyme oil	Clove oil
Control	9.3 ± 0.16	8.6 ± 0.16
100	9.3 ± 0.16	8.6 ± 0.16
600	8.8 ± 0.13	8.5 ± 0.16
1200	8.5 ± 0.166	8.4 ± 0.16
2400	8.5 ± 0.166	7.9 ± 0.17

The intensity of acceptability was described according to the following scale: 10, None (acceptable as control); 8, weak (odor different from control); 5, medium (acceptable odor from control); 0, strong (unacceptable from control).

TABLE 4

Mean Sensory Scores and Standard Errors for Potato Chips Fried in Cottonseed Oil Mixed with Thyme Oil or Clove Oil

Concentration (ppm)	Thyme oil	Clove oil
Control	9.3 ± 0.16	8.6 ± 0.16
100	9.3 ± 0.15	8.6 ± 0.16
600	8.9 ± 0.10	8.6 ± 0.52
1200	8.1 ± 0.14	8.3 ± 0.48
2400	8.6 ± 0.16	7.8 ± 0.63

The intensity of acceptability was described according to the following scale: 10, None (acceptable as control); 8, weak (odor different from control); 5, medium (acceptable odor from control); 0, strong (unacceptable from control).

essential oils. The threshold values for these systems are shown in Table 2. These values show that cottonseed oil mixed with thyme or clove oils was the same at the range of 50 to 1200 ppm. Statistical analysis demonstrated that the addition of thyme oil or clove oil to cottonseed oil at concentrations ranging between 50 and 1200 ppm did not at all affect the odor note of cottonseed oil and consequently the mixed cottonseed oil is completely acceptable for human consumption.

The addition of thyme oil to cottonseed oil at 2400, 3000 and 3600 ppm possessed medium, medium and strong odor, respectively. Clove oil mixed with cottonseed oil at 2400, 3000 and 3600 ppm caused medium, strong and very strong aroma, respectively. Hence, thyme oil in the range of 2400 to 3600 ppm was more preferable than clove oil.

The minimum effective concentrations for thyme and clove oils as antioxidants as well as antimicrobial agents were 200 and 400 ppm, respectively. The data show that the addition of these essential oils to cottonseed oil at 6-fold the minimum antimicrobial concentration possessed no distinctive odor or sense to cottonseed oil. Further experiments were performed to assess the acceptability of cottonseed oil mixed with thyme oil or clove oil for fried and cold prepared foods. Potato chips and cold bean dishes, common meals in Egypt, were examples for hot and cold treatments, respectively. Analysis of variance for the overall acceptability data (Tables 3 and 4) revealed no significant differences between the food prepared with cottonseed oil alone and that mixed with thyme oil or clove oil. Here again, the addition of thyme oil or clove oil to cottonseed oil, as an antioxidant or antimicrobial agent, did not alter the acceptability of cottonseed oil used for processing food. It is worth mentioning that thyme oil was preferable to clove oil from the consumer point of view. The consumer preference was in reverse proportion to the amount of added essential oil.

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