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STUDY ON SOME ESSENTIAL OILS AS NATURAL PRESERVATIVE AGENTS IN FOOD .

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ABSTRACT

The present study was done to study the antioxidant activity of four essential oils namely thyme ,clove , cumin and caraway as preservatives agents in refined cottonseed oil in the comparison with synthetic BHA by using three different *concentrations of the oils (300,500,700 ppm) and 200 ppm* Buotylated hydroxy anisole (BHA). The physico – chemical properties of the oils were determined . Also, the chemical composition was determined by GLC Analysis . The main components were identified . The antioxidant activity of these oils revealed that all the tested essential oils had different antioxidant activity and the effect was increased by increasing oils concentration from 300 to 700 ppm . Clove oil had the highest antioxidant activity on cottonseed oil comparison with the control sample and , it retched 93.7 % of Synthetic BHA antioxidant effect , followed by thyme oil which represented 87.5 % of BHA . On the other hand , caraway essential oil was the lowest one which it represented 62.5 % of BHA effect . in addition , cumin oil had a moderate effect and represented 75 % of BHA action .

The addition of clove , thyme and cumin essential oils at the minimum inhibitory concentration 300-700 ppm to cottonseed oil did not alter the acceptability of cottonseed oil used for processing food .

INTRODUCTION

There is an argent need for another compound instead of Synthetic compound act as an antimicrobial and antioxidant and safer for human being. Essential oils are used in medical drugs and can be used as a strong antioxidant . Such oils contain some phenolic compounds which can act as antioxidant .

Guenther (1961) reported that thyme oil consisted of thymol (50 %) Geraniol , borneol , linalool , camphene , cymene , B-pinene , caryophillene and α-pinene .

EL- Hamidi and Richter (1965) fractionated the Egyptian cumin oil components by thin layer chromatography . The results showed the presence of cuminalcohol , periallialdhyde , crypt one and cuminaldhyde .

Osol *et al.*, (1967) mentioned that the chief constituent of the clove oil was eugenol and it also contained sesquiterpene , caryophillene , furfural which was probably the cause of the oil darkening on storage, methylpentyl ketone which gave the much valued fruity odour to the oil , vanillin and up to about 10 % of acetyl Eugenol .

Antioxidant activity of many spices including clove , thyme , cinnamon . tumeric , ginger and black pepper were investigated by several outhers (Bishov *et al.*, (1977)) and Al.jalay *et al.*, (1987) . Egenol of the clove

oil is 3- methoxy phenolic derivative which has been reported to posses 90 % of the antioxidant activity of BHA .

Farag *et al.*, (1989) obtained essential oils by stamdistillationt from clove flower buds and thyme leaves . Thyme (200,600,1200 ppm) And clove (400,1200,2400 ppm), oils were added separately to 1000 g cottonseed oil containing 0.5 mg B-carotene . Oxidation rate of cottonseed oil was monitored at room temperature for 35 days . by coupled oxidation with B–carotene .

Oxidation rate of cottonseed oil was mentioned at room temperature for 35 days . by coupled oxidation with B–carotene .

Peroxide number and TBA value . The results indicated that clove oil had more effect than thyme oil. Lee and Winder (1994) evaluated acommercial oleoresins from *rosmerinus officinalis , pimpinella anisum , carum carvi and Anethum graveolens* as an antioxidant for limonene oxidation , antioxidant efficiency was compared with that of food grazle antioxidant . Oleoresin from *R.officinalis* inhibited the oxidation of limonene and with more potent than 2 food – grade .

antioxidants : mixed to copherpls (50 % and to copherol mixture) and butylated hydroxy anisole . oleresins from *P . anisum* and *C.carvi* exhibited weak antioxidant properties . The oleoresins from *A.graveolens* was nearly in active against limonene oxidation .

MATERIAL AND METHODS

Source of essential oil plants

The leaves of thyme plant , flower buds of clove , fruits of cumin and caraway plants were collected from the farm of medicinal and Aromtie plants at EL Kanater EL Khairiya Barrage . Horticulture research institute during the summer season of 2001 . Family names and plant parts used as source of essential oils are listed in table (1) . All the essential oils samples were produced from crude parts by steam distillation as described by Guenther (1961).

Table (1) Latin names , family names and botanical parts as sources of essential oils for same spice plants

Plant	Latin names	Familynames	Plant part
Clove	*Eugenia caryophyllus*	Myrtaceae	Flower buds
Thyme	*Thymus vulgarius , L*	Labiatae	Leaves
Cumin	*Cuminum cyminum,L*	Umbelliferaere	Fruits
Caraway	*Carum carvi, L*	Umbelliferaere	Fruits

Source of main chemical compounds of essential oils .

The main components of essential oils were obtained by Alderch Company .

Antioxidant activity test :

Fresh refined cottonseed oil obtained from Cairo oil and soap company .EL-Ayat Factory , Giza .

Determination of physical and chemical properties of essential oils

Specific gravity, refractive index , optical rotation , solubility , acid value , ester number and estter number after acetylation were determined according to the method described by Guenther (1961)

Identification and determination of essential oil composition .

The G.L.C. analysis was carried out in the central laboratory of Fac.Agric.,Cairo univ .The condition used are described in :

PRO-GCPye Unicom

Colume :PEGA 10 %

Tem .programming :

Initial temp	70°C
Rate	4°C/ min
Final temp	190°C
Final time	20 min
Detector temp	300°C
Injection temp	250°C
Char speed	2 min / cm
Fow rate of gasses	
N_2	**30 ml/min**
H_2	**33 ml/min**
Air	**330 ml/min**

Antioxidant activity :

Antioxidant activity divided into 5 tests :

a- specific gravity and refractive index were determined by the method described by Guenther (1961).

b-Oven test :

The antioxidant activity of the essential oils were measured in refiend cottonseed oil modified oven stability procedure of Holley and Hammons (1968), as reported by Brown et al ., (1974).

C- Peroxide number :

It was determined according to the method described in A.O.A.C (1984).

D- TBA – Test:

It was carried out according to the method described by Ottolenghi (1959)

Sensory evaluation

Ranking test : The acceptability of cottonseed oil mixed with essential oils evaluated using heated (friend fish) and unheated (cooked hars beans foods). The panalists (10 persons) ranked all food samples with code numbers (first 10 , second 8 , third 5 and fourth 5) according to the intensity of the characteristic flavour and their preference.The results were subjected to analysis of variance and least significant difference according to Larmonrd (1970) .

RESULTS AND DISCUSSION

The most important physical and chemical properties of clove .

Thyme , cumin and caraway essential oils are determined and the results are shown in table (2) . Most of the values were found to be within the range mentioned by Guenther (1961).

I)physical and chemical properties of the essential oils :

Table (2) : the physico – chemical properties of the tested essential Oils .

Property	Clove oil	Thyme oil	Cumin oil	Caraway oil
Specific gravity at 20 °c	1.1292	0.9621	0.9213	0.9861
Optical rotation at 20 °c	-33°	+ 6° 22	+ 7° 32	+ 68°
Refractive index at 20°c	1.5324	1.4835	1.4970	1.5238
Acid value	1.59	1.7	2.6	1.2
Ester number	4.8	3.2	-	-

B) Chemical composition of the tested essential oils

Gas liquid chromatography was used to determined the chemical composition of four essential oils (clove, thyme, cumin and caraway). The chemical composition of these oils is listed in table (3) and their chromatograms in figs. (1-4).

Table (3) chemical composition of the tested essential oils

Component %	Clove	Thyme	Cumin	Caraway
α- pinene	-	1.1	0.30	-
B- pinene	-	0.3	20.6	-
Comphene	-	-	0.6	-
Limonene	-	0.3	5.1	-
δ- terpinene	-	0.1	0.3	0.4
Phellandrene	-	1.5	0.2	-
Terpinolen	-	-	12.5	-
Myrcene	-	-	4.0	15.8
p- cyemene	-	36.2	0.4	0.8
Caryophillene	6.1	-		-
Eugenol	85.8		-	-
Thymol	-	42.7	-	81.3
Carrene	-	-	-	-
Thjone	-	-	-	-
Cuminaldhyde	-	-	55.8	-
Linalool	-	-	-	-
Geraniol	-	-	-	-
α- Terpinol	2.8	-	-	-
Cineole	-	-	-	-
Borneol	-	0.7	-	-
Linalyl acetate	7.5	1.0	-	-
Eugenol acetate	-	-	-	-

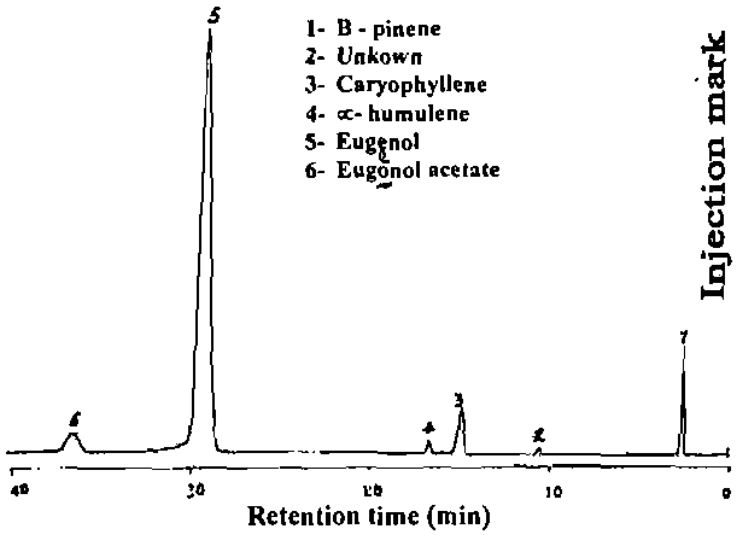

Fig . (1) : GLC chromatogram of clove essential oil

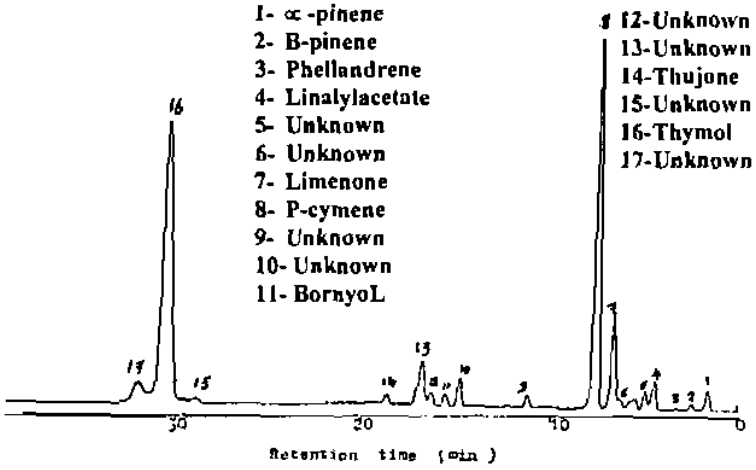

Fig . (2) : GLC chromatogram of thyme essential oil

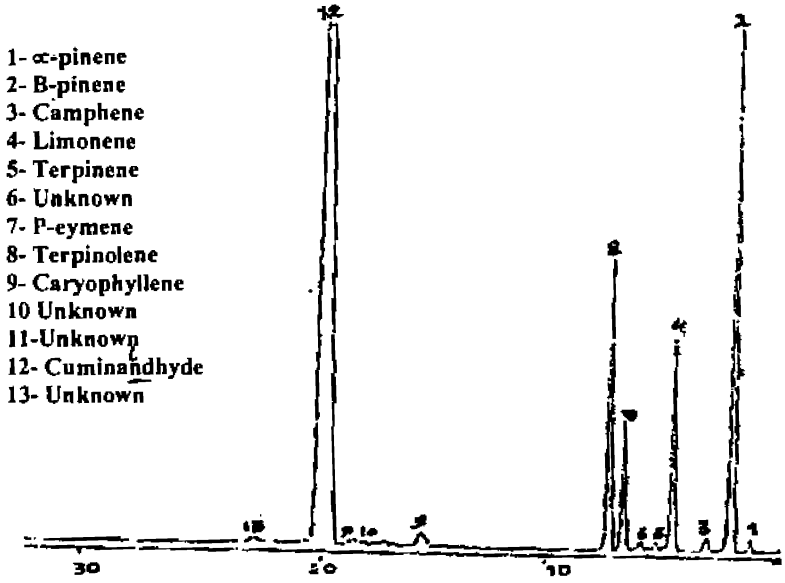

Fig . (3) : GLC chromatogram of eumin essential oil

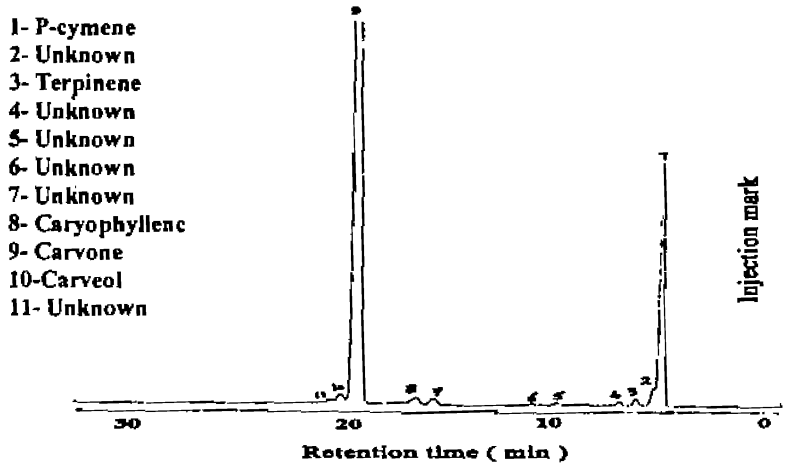

Fig . (4) : GLC chromatogram of caraway essential oil

GLC analysis of the fresh essential oils showed that clove oil was characterized by the presence of eugenol as a major compound (85.8%). Caryophylene and eugenolacetate were present as a minor compounds.

Thyme oil had the highest concentrations of thymol and P-cymene (42.65 % and 36.2% respectively) α-pinene and phellandrene were present as minor compounds while B-pinene , limonene and linalyl acetate occurred as trace compounds .

The major substances for cumin was cuminaldhyde (55.8 %) B-pinene (20.6 %) and terpinolene (12.5 %). Limonene and P-cymene were present in amounts less than 10 % .

Caraway oil was characterized by the presence of carvone (81.3 % and P-cymene (15.8 %) as major materials.

C- Antioxidant effect

The antioxidant properties of clove, thyme, cumin and caraway essential oils were studied with various concentration (300,500 and 700 ppm) on cottonseed oil . BHA was used as a standard synthetic antioxidant (at 200 ppm) to evaluate and compare the antioxidant effect of these oils.

The effect of these oils on refined cottonseed oil oxidation is graphically shown in figs. (5-24) by tests of oven test, peroxide number, specific gravity, refractive index and TBA value.

The data in all figs. (5-24) of these tested oils showed that all oils at various concentration had considerable antioxidant effect on cottonseed oil in comparison with the control.

The results illustrated in figs. (5 – 8) of stability in oven of cottonseed oil during storage (50 days) of the four oils indicated that the stability of cottonseed oil increased gradually by increasing the concentration addition of any of the four oils from (300-700 ppm).

Caraway essential oil led to an increase in the induction period of the cottonseed oil from 10 days in the control sample oil to 2.6 time more than the control sample. while, the addition of 200 ppm of BHA to cottonseed oil led to increase in the induction period of 4 times more than control sample . further more , caraway essential oil represented 62.5 % of BHA .

From the same data, the addition of 300-700 ppm of cumin oil to cottonseed oil led to an increase of the induction period of it 3.0 times more than the control sample. At the same time, cumin oil led to an increase in the induction period 75 % of BHA.

As for thyme oil, the addition of it from 300-700 ppm lead to an increase in the induction period 3.3 times than the control sample .

The addition of 300-700 ppm of clove oil to cottonseed oil led to an increase to the induction period of it 3.5 times than the control sample. So, it represent 87.5 % of BHA effect.

The data in figs . (9-12) of peroxide number of caraway , cumin , thyme and clove essential oils showed that there was a sharp increase of peroxide number of the control sample of cottonseed oil with comparison to the other samples of cottonseed oil with various concentrations of the four essential oils under study .

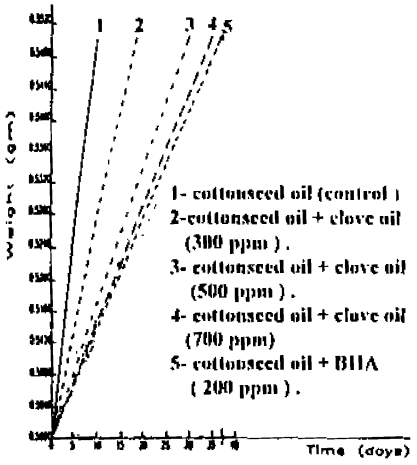

Fig.(5) effect of different concentrations of clove essential oil added to the cottonseed oil on its stability as checked by the oven test .

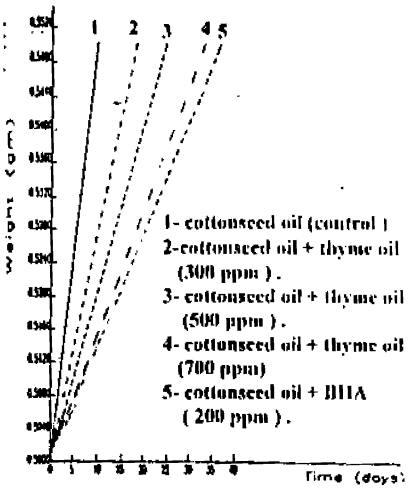

Fig.(6) effect of different concentrations of thyme essential oil added to the cottonseed oil on its stability as checked by the oven test

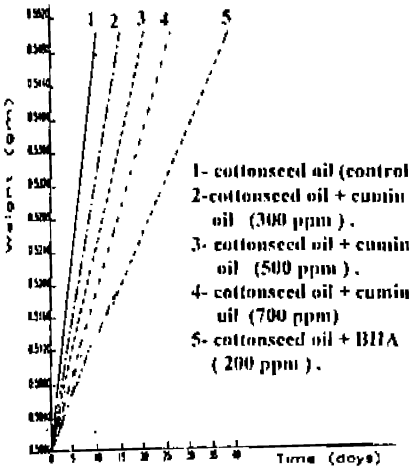

Fig.(7) effect of different concentrations of cumin essential oil added to the cottonseed oil on its stability as checked by the oven test .

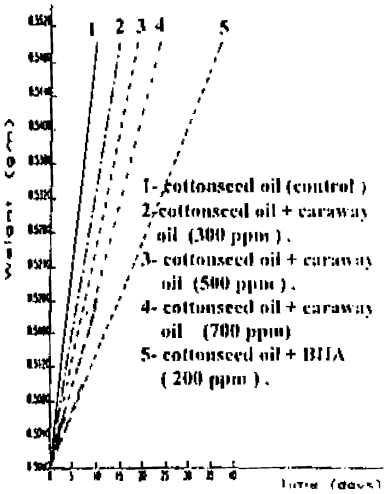

Fig.(8) effect of different concentrations of caraway essential oil added to the cottonseed oil on its stability as checked by the oven test .

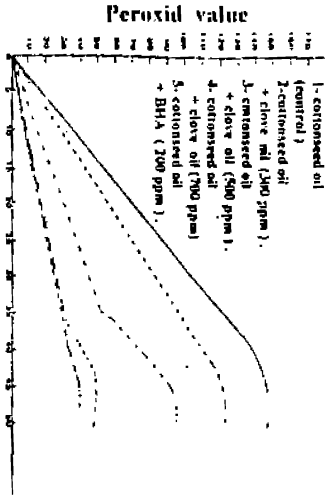

Fig. (9) Effect of difrent concentration of clove essential oil on peroxide value of cottonseed oil during oxidation .

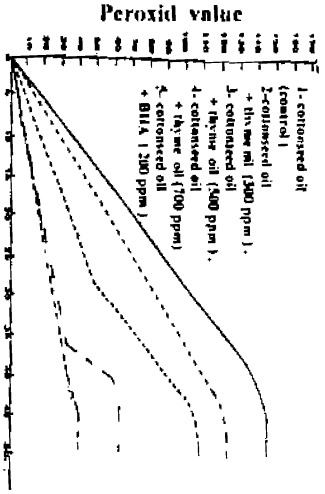

Fig. (10) Effect of difrent concentration of thyme essential oil on peroxide value of cottonseed oil during oxidation .

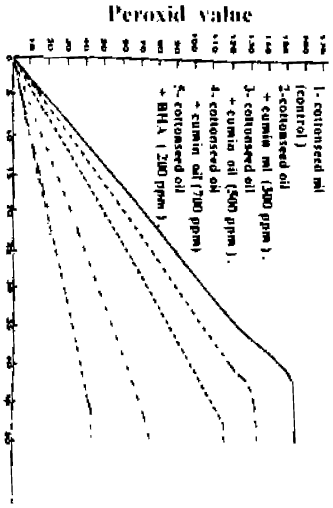

Fig. (11) Effect of different concentration of cumin essential oil on peroxide value of cottonseed oil during oxidation .

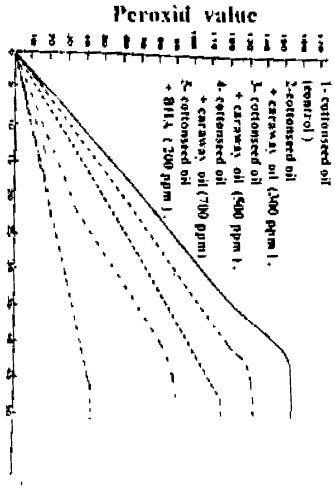

Fig. (12) Effect of different concentration of caraway essential oil on peroxide value of cottonseed oil during oxidation .

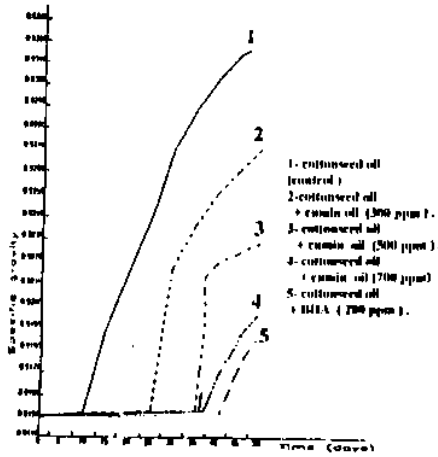

Fig.(13) effect of different concentrations of clove essential oil on specific gravity of cottonseed oil during oxidation .

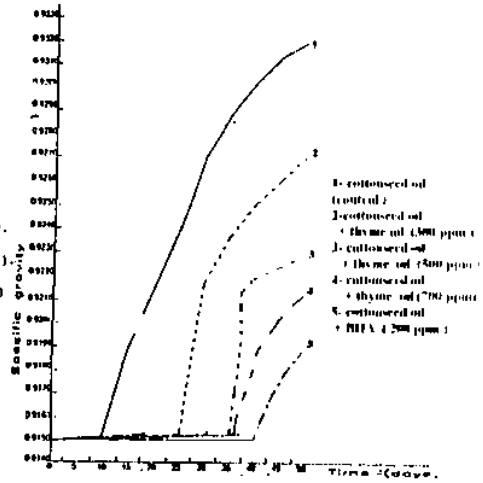

Fig.(14) effect of different concentrations of thyme essential oil on specific gravity of cottonseed oil during oxidation .

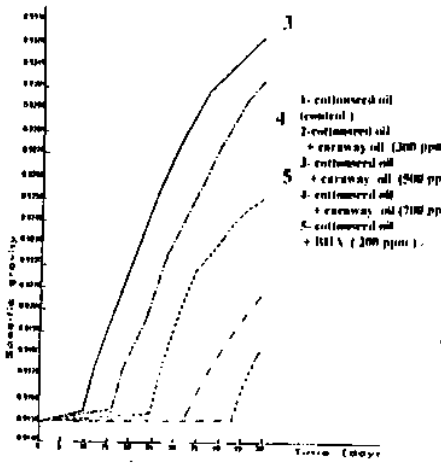

Fig.(15) effect of different concentrations of cumin essential oil on specific gravity of cottonseed oil during oxidation .

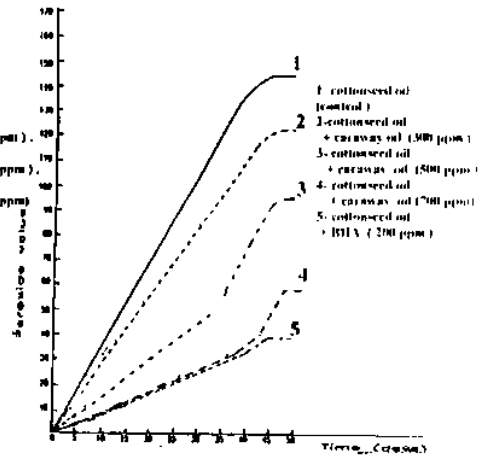

Fig.(16) effect of different concentrations of caraway essential oil on specific gravity of cottonseed oil during oxidation .

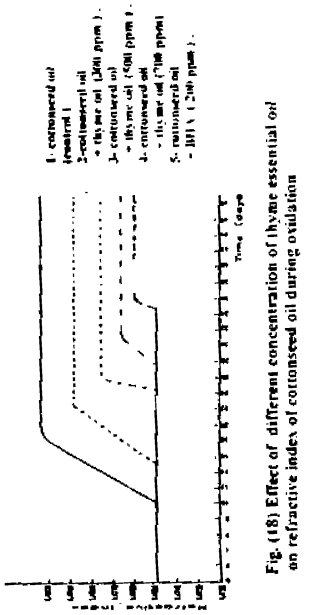

Fig. (18) Effect of different concentration of thyme essential oil on refractive index of cottonseed oil during oxidation

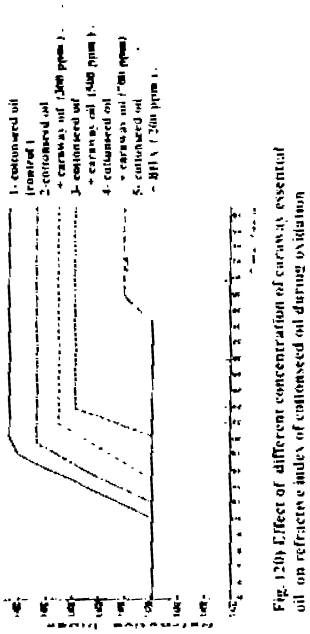

Fig. (20) Effect of different concentration of caraway essential oil on refractive index of cottonseed oil during oxidation

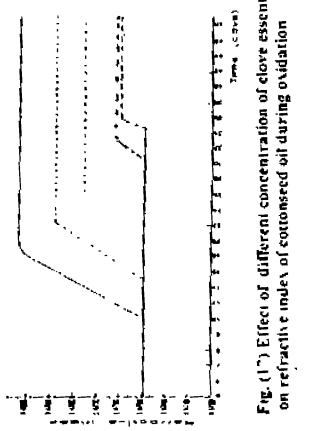

Fig. (17) Effect of different concentration of clove essential oil on refractive index of cottonseed oil during oxidation

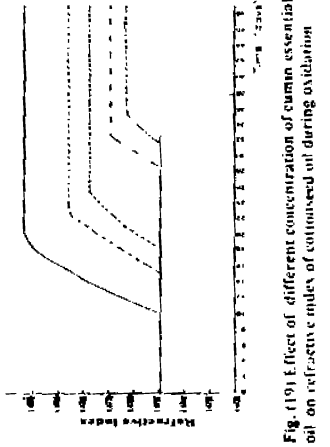

Fig. (19) Effect of different concentration of cuman essential oil on refractive index of cottonseed oil during oxidation

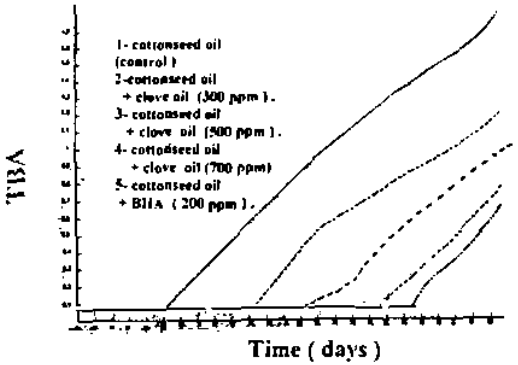

Fig.(21) Effect of different concentration of clove essential oil on TBA of cottonseed oil during oxidation .

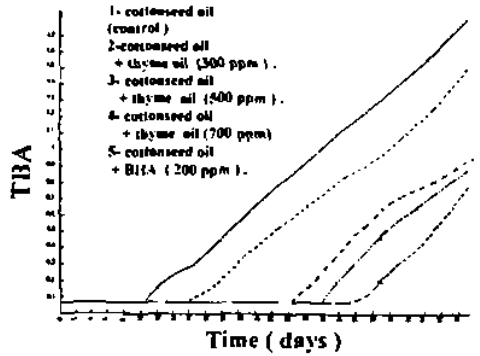

Fig.(22) Effect of defferent concentration of thyme essential oil on TBA of cottonseed oil during oxidation .

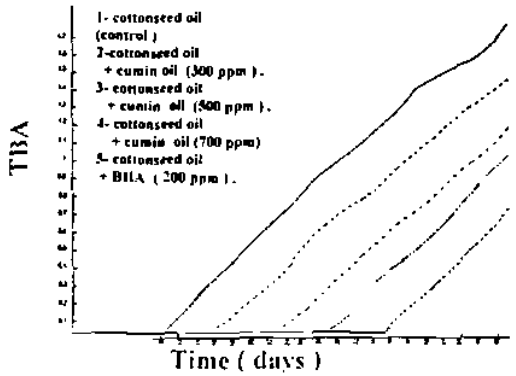

Fig.(23) Effect of different concentration of cumin essential oil on TBA of [illegible] oxidation .

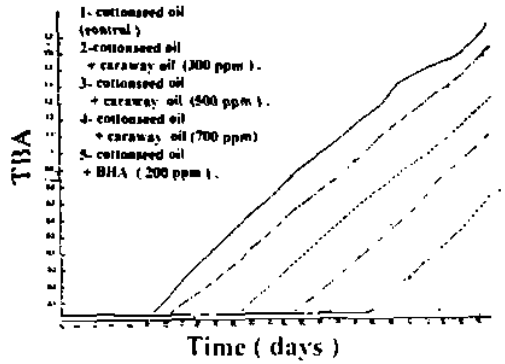

Fig.(24) Effect of defferent concentratioo of caraway essential oil on TBA of cottonseed oil during oxidation .

The same data indicated that the addition of the essential oils to cottonseed oil with various concentration 300-700 ppm led to a clear decrease in the peroxide number of cottonseed oil. The data also indicated that clove oil had the highest antioxidant effect during oxidation period. On the contrary, caraway essential oil was the lowest. Cumin essential oil had a moderate effect.

In general, the antioxidant effect of the four essential oils followed the sequence:

Clove > thyme > cumin > caraway.

The data in figs. (13 –16). (17-20) and (21 – 24) of specific gravity , refractive index and TBA of the essential oils showed the same trend of the four essential oils as antioxidant agents .

It appears that there is a relationship between the antioxidant activity of the oil and its chemical composition. Clove and thyme oils had the highest antioxidant activity due to their phenolic compounds such as eugenol in clove and thymol in thyme oils, respectively. These compounds had the highest antioxidant action due to the presence of phenolic OH . This group prevent the hydrogen atom formation from the fatty acid leads to the decrease of hydro peroxide formation which as reported by Torel et al., (1986). These structural requirements were supported by the powerful antioxidant activity of the well known BHA.

In case of caraway essential oil, it had a little antioxidant activity, due to the absense of aromaticity, EL – Baroty (1988).

As for cumin oil, it had a moderate antioxidant activity due to its containing an aromatic terpene cuminaldhyde in great quantities and aromatize hydrocarbon *p*-cymene .

Similar findings have been reported by other investigators of using such essential oil as antioxidant agents in food such as farag *et al.*, (1988) , EL. Baroty (1988) and Mier *et al.*, (1995).

D) Sensory evaluation

A Set of experiment was conduct to detect the acceptability of Cottonseed oil mixed with clove or thyme or cumin or caraway essential oils for friend food such as friend fish as example of hot treatment and addition cumin oil to cottonseed oil used in cold bean dish as example of cold treatment. the ranking test and the Data are presented in tables (4,5,6 and 7).

Analysis of variance for the overal acceptability data reveald that no significant differences between the food prepared with such oils . Thus , The addition of these oils to cottonseed oil as an antioxidant agent did not alter the acceptability of cottonseed oil used for processing food .

Table (4) Mean *sensory* scores and standard errors for fish fried in cottonseed oil mixed with clove oil

Number of persons	Concentration							
	control	mean	300 PPM	mean	500 PPM	mean	*700 PPM*	*mean*
1	10	10	9	8.0	7	8.1	7	8.1
2	10		9		7		8	
3	10		8		9		7	
4	10		8		9		7	
5	10		7		8		9	
6	10		9		9		9	
7	10		9		9		8	
8	10		7		8		8	
9	10		7		8		9	
10	10		7		7		9	

The intensity of acceptability **was described according to following scales :**

10 = Non (acceptable as coontrol) .

8 = weak (order different from control) .

5 = Medium (*acceptable odar from control*) .

0 = strong (unecceptable from control) .

Table (5) Mean sensory scores and standard errors for fish fried in cottonseed oil mixed with thyme oil

Number of persons	Concentration							
	control	mean	300 PPM	mean	500 PPM	mean	*700 PPM*	*mean*
1	10	10	9	8.3	9	8.2	9	8.2
2	10		9		7		8	
3	10		7		9		8	
4	10		9		9		8	
5	10		9		8		7	
6	10		8		9		9	
7	10		8		9		9	
8	10		7		7		9	
9	10		9		7		7	
10	10		8		8		8	

Table (6) Mean sensory scores and standard errors for fish fried in cottonseed oil mixed with cumin oil

Number of persons	Concentration							
	control	mean	300 PPM	mean	500 PPM	mean	700 PPM	mean
1	10	10	9	8.8	9	8.5	9	8.5
2	10		9		8		9	
3	10		9		9		8	
4	10		8		9		9	
5	10		8		7		8	
6	10		9		9		9	
7	10		9		9		8	
8	10		9		9		8	
9	10		9		7		9	
10	10		9		9		8	

Table (7) Mean sensory scores and standard errors for bean meal prepared by mixing cottonseed oil with cumin oil

Number of persons	Concentration							
	control	mean	300 PPM	mean	500 PPM	mean	700 PPM	mean
1	10	10	9	8.9	8	8.6	9	8.4
2	10		9		8		8	
3	10		9		8		8	
4	10		9		9		8	
5	10		9		8		8	
6	10		9		9		8	
7	10		9		9		9	
8	10		8		9		8	
9	10		9		9		9	
10	10		9		9		9	

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دراسة على بعض الزيوت النظرية كمواد حافظة طبيعية في الأغذية

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هذه الدراسة قد أجريت لدراسة التأثير المانع للأكسدة لأربعة زيوت عطرية على زيت بذرة القطن واستعمال هذه الزيوت كمواد حافظة للأغذية . وهذه الزيوت هي : زيت القرنفل – زيت الزعتر – زيت الكمون وزيت الكراوية . ولقد تم استخلاص هذه الزيوت الأربعة بطريقة الاستخلاص بالبخار من مصادرها المختلفة . وتم تقدير الصفات الطبيعية والكيماوية لهذه الزيوت وكذلك تركيبها الكيماوي بطريقة التحليل الكروماتوجرافي والتعرف على المركبات الكيماوية الرئيسية بها . كما تم إجراء الاختبارات الخاصة لهذه الزيوت من حيث تأثيرها كمضادات أكسدة على زيت بذرة القطن باستعمال ثلاث تركيزات مختلفة ٣٠٠ ، ٥٠٠، ٧٠٠ جزء في المليون ومقارنتها بتأثير مضاد الأكسدة الصناعي بيوتيلايتد هيدروكس انيسول بتركيز ٢٠٠ جزء في المليون وهذا تم عن طريق إجراء خمس اختبارات هامة وهي اختبار مدى الثبات في الفرن – التغير في رقم البيروكسيد – التغير في الوزن النوعي – التغير في معامل الانكسار – تقدير التغير في حمض الباربانيوريك .

وقد أسفرت هذه الدراسة على النتائج الآتية .

١- أن لكل من الزيوت الأربعة السابقة تأثير مضاد للأكسدة واضح على زيت بذرة القطن إذا قورن بالصيغة الكونترول ويزداد هذا التركيز لكل زيت بزيادة التركيز من ٣٠٠ – ٧٠٠ جزء في المليون .

٢- إن زيتي القرنفل والزعتر كانوا من أقوى الزيوت كمضادات أكسدة حيث مثلوا ٩٣,٧ % ، ٨٧,٧ % من تأثير مضاد الأكسدة بيوتيلاند هيدروكس اينسول لكل من الزيتين على الترتيب .

٣- زيت الكراوية كان من أقل الزيوت تأثيرا كمضاد أكسدة وهذا يرجع إلى تركيبه الكيماوي حيث مثل ٦٢,٥ % فقط من تأثير مضاد للأكسدة الصناعي .

٤- إن زيت الكمون فكان له تأثير متوسط حيث مثل ٧٥ % من تأثير مضاد الأكسدة الصناعي

٥- إن تأثير هذه الزيوت يرجع إلى تركيبها الكيميائي وكلما اقترب التركيب الكيميائي للزيت من التركيب الكيميائي كمضاد للأكسدة الصناعي كلما كان الزيت أقوى كمضاد للأكسدة

٦- عند إضافة الزيوت القرنفل ، الزعتر ، الكمون إلى الزيت المستعمل في تحمير السمك كمثال للوجبة الساخنة بنفس التركيزات التي استعمل فيها مضاد للأكسدة (٣٠٠ ، ٥٠٠ ، ٧٠٠) واجراء اختبارات حسية على مدى قابليته بالنسبة للمستهلك لم تؤثر إضافة أحضان هذه الزيوت على طعم السمك الناتج ولم توجد فروق معنوية بين هذه التركيزات .

٧- عند إضافة زيت الكمون بنفس التركيزات السابقة للزيت المضاف إلى الفول المدمس وجبة باردة لم تؤثر على مدى قابلية المستهلك ولم توجد فوق معنوية بالتركيزات المستخدمة لدي المستهلك .

٨- وبالتالي يمكن استعمال هذه الزيوت كمضادات للأكسدة وإدخالها في بعض الوجبات الساخنة والباردة دون أن تؤثر على ذوق المستهلك .