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EFFECT OF SAGE (*Salvia officinalis*) EXTRACTS ON THE STABILITY OF CORN OIL AND ITS TOCOPHEROLS CONTENT DURING HEATING PROCESS.

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ABSTRACT

The activity of hexane- extract (H.E) and ethanol extract (E.E) of sage leaves as a natural antioxidant, in a comparison with butylated hdroxytoluene (BHT), as a synthetic one in reducing the oxidative changes during intermittent heating of corn oil was studied. Changes in some constants, oxidized products, the fatty acids and tocopherols contents of corn oil free or containing hexane (H.E) and ethanol extract (E.E) of sage leaves and (BHT) as antioxidants were tested. Refractive index, acid value, peroxide value, total carbonyls and absorbance at 234, 270, 400 and 450 nm increased progressively during the intermittent heating process, while iodine value decreased. Addition of 0.1% of (E.E) and 0.05% (BHT) showed clear effect in reducing these changes, whereas (H.E) was less effective. The addition of (E.E) and (BHT) reduced markedly the loss of linoleic acid after 10 hr of intermittent heating. γ - tocopherol represented the major tocopherol fraction in corn oil, while β- tocopherol was not detected. The losses of α-, γ-,δ- and total tocopherols were 70.9%, 50.6%, 20.3% and 53.9% respectively after 4 hr of intermittent heating of corn oil without additives. (E.E) reduced these losses to 17.7%, 4.5%, 5.5% and 7.2% respectively for the same tocopherols mentioned above and under the same conditions comparing with BHT, which decreased these losses to 19.0% , 5.0%, 7.7% and 8.0% respectively, while H.E had less effect. So, (E.E) had nearly 50% of the efficiency of BHT as antioxidant.

INTRODUCTION

In food processing which include almost thermal treatments lipidic oxidation not only causes a loss in nutritional and gustative quality of foods but also generates oxidized products such as free radicals which lead to various undesirable products (Pamela, 1991, Sanchez- Muniz *et. al.*, 1993 and Abd-Allah, 1997). To avoid or delay this autooxidation processes antioxidants have been used for over 50 years. The use of synthetic antioxidants such as BHT and BHA has been decreased because of their suspected action as promoters of carcinogenoesis, as well as the general consumer rejection of synthetic food additives (Chen *et. al.*, 1992).

The spices, notably the Labiatae family, are well known for their antioxidative properties. Two plants: (rosemary and sage) have been reported to have strong characteristics (Cort, 1974; Watanabe, and Ayano 1974; Gerhardt and Schroter, 1983; Houlihan and Ho, 1985).

Rosemary has been extensively studied for its antioxidative principles as well as for its industrial and commercial exploitation (Chang *et. al.*, 1977, 1987; Wu *et. al.* 1982; Löliger, 1989, Schuler, 1990; Chen *et. al.*, 1992; Madsen *et. al.*, 1996).

The current hypothesis is that sage contains the same antioxidants as rosemary (Nakatani, 1989, cuvelier *et. al.*, 1994, Baricevic *et. al.*, 2001, and Kavvadias *et. al.*, 2003).

Sage was shown to possess a strong antioxidative efficiency comparable to rosemary when tested against methyllinoleate oxidation in an polar medium (Cuvelier *et. al.*, 1991, 1994). Cuvelier *et. al.*, 1996 reported that ethanolic sage extract is more active as antioxidant than sage hexane extract. They mentioned that the ethanolic extract of sage pilot plant contains rosemarinic acid, carnosol, caffiec acid and carnosic acid whereas carnosol, rosemadial, carnosic acid and methyl-carnosate represent the main components of its hexan extract. Generally, caffiec acid, rosemarinic acid, carnosol and carnosic acid were the most active compounds as antioxidants.

Therefore, this investigation deals with the antioxidant efficiency of both crude hexane and ethanol extracts of sage leaves against corn oil which used in a large scale in food processing and cooking during intermittent heating for different periods in comparison with butylated hydroxytoluene (BHT) as a synthetic antioxidant. The effects of these extracts on the changes of some physical and chemical properties of corn oil as well as their effect on the losses of its tocopherols content during heating process was studied.

MATERIALS AND METHODS

Refined corn oil free from any additive antioxidants was obtained from Cairo factory for oils and soaps production. Dried sage (*Salvia officinalis*) leaves were purchased from the local market Cairo, Egypt. Butylated hydroxytoluene (BHT) and standard kits of α, β, γ and δ-tocopherols were obtained from E. Merck, Germany. All solvents and other chemicals were analytical grade.

Preparation of sage extracts:

It was prepared according to method of Chen *et. al.*, (1992), but the ground sage leaves were extracted three times with each solvent (n- hexane or ethanol 95%) in a ratio of 1:5 W/V at 60 °C for 2 hr. the samples were filtered after each extraction. The combined extract was bleached with 10g of active carbon at 60 °C and then filtered to yield light brown filtrate. The solvents was removed at 45 °C by a vacuum rotary evaporator to obtain crude sage extracts.

Before heating process of corn oil, it was divided in the following manner:

1- Corn oil free from sage extracts or butylated hydroxy toluene (BHT) as a control.
2- Corn oil containing 0.05% BHT.
3- Corn oil containing 0.1% hexane sage extract (H.E)
4- Corn oil containing 0.1% ethanol sage extract (E.E)

Heating process: Oil samples were heated for 2 hr at 180 °C in open beaker then cooled to room temperature (25 °C) and stored uncovered for about 22 hr in dark place. This process was repeated for 5 days to reach 10 hours intermittent heating. Samples of oil were taken after each 2 hr heating for analysis.

Analytical methods: Acid value, peroxide value, iodine value, and refractive index were determined according to AOAC (1990).

The absorbance of 1% oil in chloroform was measured at 450 and 400 nm to monitor the changes in oil colour during heating. The absorbance of 1% oil in octane at 270 and 234 nm was determined to detected the formation of conjugated triene and diene, respectively (Abdel- Aal and Karara, 1986). Total carbonyl compounds were colorimetrically estimated at 480 nm as described by Lappin and Clark (1951). Apye unicam Sp-800 recording spectrophotometer was used for these measurements.

Fatty acids were methylated as mentioned by Vogel (1975) with diazomethane then analyzed by gas liquid chromatography Model GC 42 CM, Shimadzu.

Saponification and extraction of tocopherols were done as described by konning *et. al.,* (1996). TLC technique was used to separate tocopherols as reported by Gogolewski (1973). Quantitative determination of the TLC separated tocopherols was followed by the Emmerie and Engel reaction (1953).

RESULTS AND DISCUSSION

The changes in the physical and chemical parameters in treated and untreated corn oil with antioxidants during intermittent heating are presented in Table (1) and Figures (1, 2, 3 and4). In general, an increase in refractive index, acid value, peroxide value, and total carbonyls was noticed, whereas iodine value decreased gradually during heating. The increase in refractive index and peroxide value is possibly attributable to conjugation known precede hydroproxide formation in the secondary stage and polymerization of partially oxidized fats in tertiary state of autooxidation (Arya *et. al.*, 1969). The increase in acide value is due to oxidation and hydrolysis which produce free fatty acids during deep fat frying (Fritisch, 1981), while the decrease in iodine value indicates the consumption of double bonds by oxidation and polymerization (Chang *et al.*, 1978).The progressive increase of total carbonyl compounds during heating refers to the breakdown of peroxides resulting in the formation of secondary products (Alexander, 1978). BHT and the ethanolic extract of sage leaves (E.E) reduced the changes in the before mentioned values otherwise retarded the appearance of carbonyl compounds, whereas sage hexane extract was less effective.

The changes in the ultraviolet spectrum of corn oil during heating were used as a relative measure of oxidation. The increase in the extinctions at 234 and 270 nm (Table 2 and Figures 5,6) showed that conjugated dienes and ketodienes were formed and that they were propotional to the increase in heating time. Addition of (BHT) and (E.E) reduced clearly the promotion of these compounds, while (H.E) was less effective.

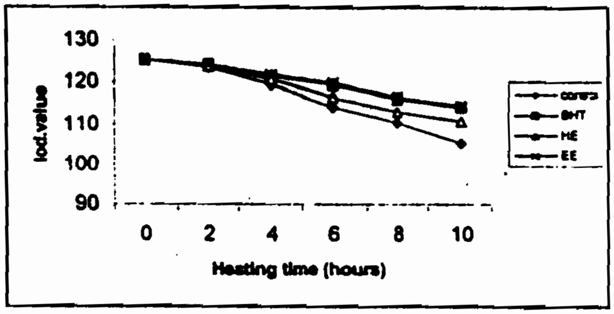

Fig. (1) : Effect of sage extract on Iodine value changes in corn oil during heating process

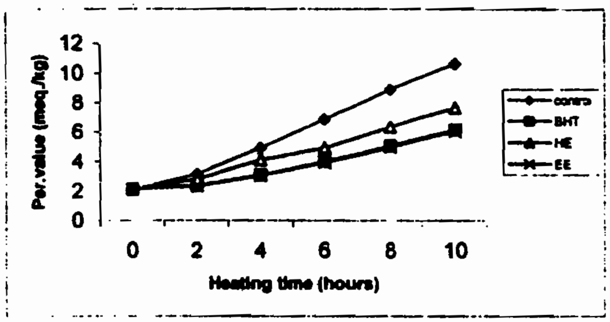

Fig. (2) : Effect of sage extract on peroxide value changes in corn oil during heating process

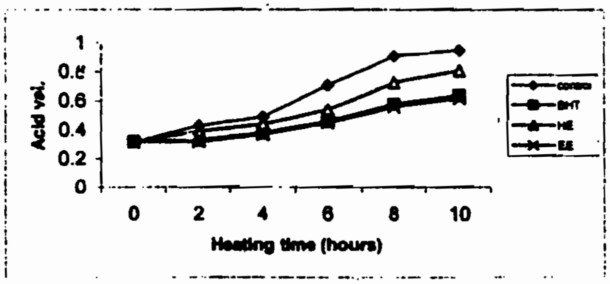

Fig. (3) : Effect of sage extract on acid value changes in corn oil during heating process

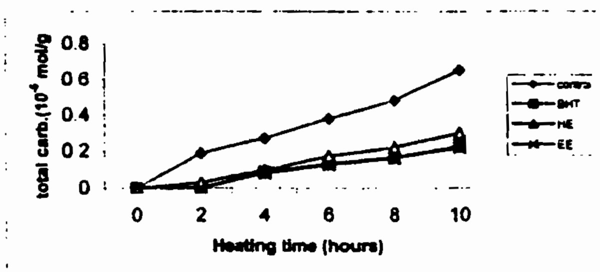

Fig. (4) : Effect of sage extract on total carbonyl groups changes in corn oil during heating process

Table (1): Effect of sage leaves extracts on some corn oil constants during heating .

Constants	Corn Oil	Heating time (hr)					
		0.0	2	4	6	8	10
Refractive Index at 25 °C	Control	1.4735	1.4736	1.4740	1.4742	1.4746	1.4751
	BHT	1.4735	1.4735	1.4737	1.4738	1.4740	1.4744
	H.E	1.4735	1.4735	1.4738	1.4740	1.4742	1.4747
	E.E	1.4735	1.4735	1.4737	1.4738	1.4739	1.4743
Acid Value	Control	0.32	0.43	0.49	0.71	0.91	0.95
	BHT	0.32	0.33	0.38	0.46	0.58	0.64
	H.E	0.32	0.39	0.44	0.54	0.73	0.81
	E.E	0.32	0.32	0.37	0.45	0.56	0.62
Peroxide Value (meq/1kg oil)	Control	2.14	3.17	4.93	6.87	8.91	10.63
	BHT	2.14	2.39	3.11	4.03	5.04	6.14
	H.E	2.14	2.82	4.12	4.92	6.34	7.65
	E.E	2.14	2.35	3.06	3.94	4.98	6.03
Iodine Value (gI_2/100g oil)	Control	125.27	123.25	119.32	114.33	110.25	105.21
	BHT	125.27	124.06	121.51	119.23	116.42	114.21
	H.E	125.27	123.78	120.66	116.63	113.24	110.82
	E.E	125.27	124.10	121.91	119.68	116.85	114.46
Total carbonyl Groubs (10^{-3} mol/g)	Control	0.00	0.189	0.0296	0.378	0.481	0.562
	BHT	0.00	0.00	0.085	0.132	0.168	0.224
	H.E	0.00	0.03	0.098	0.173	0.221	0.298
	E.E	0.00	1.00	0.081	0.127	0.163	0.219

Control: Corn oil without any additives. BHT = Corn oil + BHT.
H.E: Corn oil + Hexane exract of sag leaves
E.E: Corn oil + Ethanolic extract of sage leaves.

Table (2): Effect of sage leaves extracts on UV absorption of the Heated corn oil.

Wave length	234 nm				270 nm			
Oil Heating time (hr)	Control	BHT	H E	E.E	Control	BHT	H.E	E.E
0.0	0.059	0.059	0.059	0.59	0.084	0.084	0.048	0.048
2	0.069	0.67	0.86	0.066	0.91	0.086	0.089	0.086
4	0.075	0.70	0.072	0.69	0.96	0.87	0.092	0.087
6	0.087	0.79	0.082	0.078	0.105	0.093	0.098	0.092
8	0.107	0.89	0.95	0.87	0.124	0.107	0.115	0.105
10	0.154	0.121	0.132	0118	0.164	0.124	0.141	0.122

Control = Corn oil without any additives. BHT = Corn oil + BHT.
H.E = Corn oil + Hexane extract of sage leaves.
E.E = corn oil + Ethanolic extract of sage leaves.

Table (3) and Figures (7,8) demonstrate the changes in color intensity of the free and treated corn oil with antioxidants. Extinctions at 400 and 450 nm increased simultaneously with the increase in heating time.

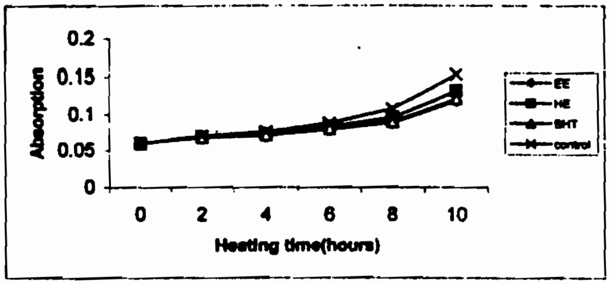

Fig. (5) Effect of sage leaves extracts on UV (234nm) Absorption of the heated corn oil

Fig7-8

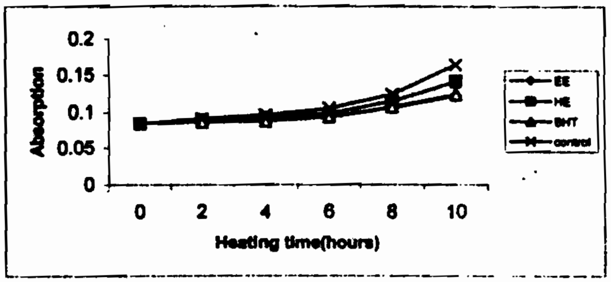

Fig. (6) Effect of sage leaves extracts on UV(270nm) Absorption of the heated corn oil

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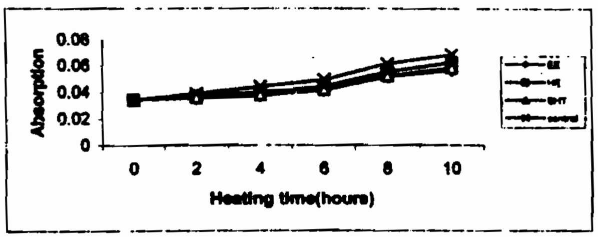

Fig. (7) Effect of sage leaves extracts at (400nm) Absorption of the heated corn oil

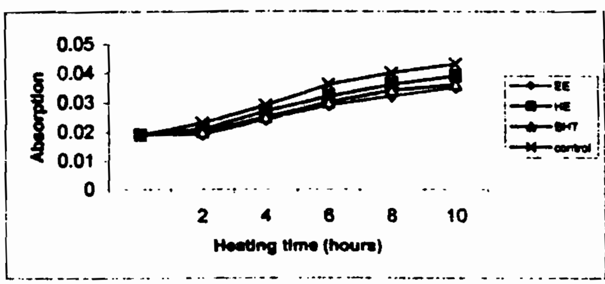

Fig. (8) Effect of sage leaves extracts at (450nm) Absorption of the heated corn oil

Table (3): Effect of sage leaves extracts on UV extinction of the heated corn oil at 400 and 450 nm.

Wave length	400 nm				450 nm			
Oil Heating time (hr)	Control	BHT	H.E	E.E	Control	BHT	H.E	E.E
0.0	0.034	0.039	0.034	0.034	0.019	0.019	0.019	0.019
2	0.039	0.036	0.37	0.035	0.023	0.020	0.021	0.019
4	0.044	0.038	0.040	0.037	0.029	0.025	0.027	0.024
6	0.049	0.042	0.044	0.041	0.036	0.030	0.032	0.029
8	0.061	0.052	0.055	0.051	0.040	0.034	0.036	0.032
10	0.098	0.058	0.062	0.056	0.043	0.036	0.039	0.035

Control = Corn oil without any additives. BHT = Corn oil + BHT.
H.E = Corn oil + Hexane extract of sage leaves.
E.E = corn oil + Ethanolic extract of sage leaves.

Table (4) indicate the changes in the fatty acids composition of free and antioxidants containing oils before and after 10 hr of intermittent heating. A marked decrease in linoliec and relatively slight one in oleic acid was noticed after heating of the control oil. The ratio of total unsaturated fatty acids to the saturated ones was decreased from 4.94 for unheated oil to 3.30, 4.03 , 3.7 and 4.07 for control, (BHT), (H.E)and (E.E) respectively referring to the effectiveness of ethanolic sage extract (E.E) as well as (BHT) in reducing the consumption of unsaturation during heating.

Table (4) Effect of sage extracts on the fatty acids percentage of corn oil after intermittent heating for 10 hours.

Fatty acids	Fresh oil	Heated oil			
		Control	BHT	H.E	E.E
C 14:0	0.08	0.12	0.09	0.11	0.09
C 16:0	12.57	16.54	14.82	15.24	14.73
C 18:0	3.80	6.16	4.62	5.13	4.54
C 18:1	37.32	36.14	37.70	37.29	37.77
C 18:2	45.94	40.71	42.41	41.84	42.50
C 20:0	0.32	0.43	0.36	0.39	0.37
Tu	83.17	79.75	80.11	79.13	80.27
Ts	16.85	23.25	19.89	20.73	19.73
Tu/Ts	4.94	3.30	4.03	3.79	4.07

Control: Corn oil without any additives
BHT: Corn oil + BHT
H.E: Corn oil + Hexane extract of sage leaves.
E.E: Corn oil + Ethanol extract of sage leaves.
Tu: Total unsaturated fatty acids.
Ts : Total saturated fatty acids.

The changes in tocopherols content during intermittent heating of corn oil free and containing 0.05% (BHT), 0.1% (H.E) and 0.1% (E.E) are reported in Table (5) and Figures (9,10,11 and 12). The concentration of α -,

γ- and δ-tocopherols in corn oil was 12.43, 45.21, and 1.82 mg /100 gm oil respectively, while β- tocopherol was not detected. After 4 hr heating about 70.9 % from α- tocopherol, 50.6 % from γ- tocopherol and 20.3 % of δ- tocopherol were consumed. The rapeseed oil with no additives showed obviously that α- tocopherol was consumed significantly faster than the β- or γ -tocopherol (Gordon and Kourimska 1995).

Table (5): Effect of sage extracts on tochopherols cntent of corn oil during heating process.

Tocopherol Fractions	Corn Oil Samples	Heating time (hr)					
		0.0	2	4	6	8	10
α Tocopherol (mg/100g oil)	Control	12.43	8.64	3.62	1.82	0.96	0.65
	BHT	12.43	11.65	10.07	7.89	5.66	3.91
	H.E	12.43	10.12	6.87	7.68	3.37	2.32
	E.E	12.43	11.72	10.23	8.02	8.85	4.01
γ Tocopherol (mg/100g oil)	Control	45.21	35.61	22.32	16.27	12.16	7.93
	BHT	45.21	44.12	42.94	29.78	21.23	14.54
	H.E	45.21	42.32	33.13	22.58	16.89	11.98
	E.E	45.21	44.31	43.17	30.22	21.95	14.92
δ - ocopherol (mg/100g oil)	Control	1.82	1.68	1.45	1.24	0.87	0.59
	BHT	1.82	1.82	1.68	1.43	1.27	1.09
	H.E	1.82	1.75	1.57	1.35	1.11	0.92
	E.E	1.82	1.82	1.72	1.49	1.32	1.12
Total Tocopherols (mg/100g oil)	Control	59.46	45.93	27.39	19.33	13.99	9.08
	BHT	59.46	57.59	54.69	39.10	28.16	19.54
	H.E	59.46	54.21	41.57	28.61	21.37	15.22
	E.E	59.46	57.85	55.12	39.73	29.12	20.05

Control: Corn oil without any additives. BHT: Corn oil + BHT
H.E: Corn oil + Hexane extract of sag leaves
E.E: Corn oil + Ethanolic extract of sage leaves.

This difference in stability of tocopherols was also evident in the oil sample containing sage extracts and (BHT). However, sage ethanolic extract (E.E) in comparison with (BHT) increased the stability of tocopherols by a considerable extent, where only 17.7% of α- tocoplerol, 4.5% of γ- tocopherol and 7.2% of δ- tecopherol were consumed after 4 hr heating. The sage hexane extract (H.E) had less effect.

After 8 hours 92.3 % , 73.1 %, 52.2 %, and 79.5% of α-, γ-, δ- and total tocopherols respectively consumed where as the addition of sage leaves ethanolic extract (E.E) reduced the losses of these compenents into 52.9 %, 51.4 %, 27.5 %, and 51.1% from α-, γ-, δ- and total tocopherols. Relatively the sage hexane extract was less effective.

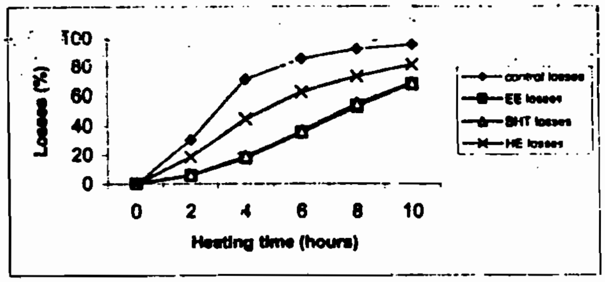

Fig. (9) : Effect of sage leaves extracts on losses (%) of α-Tocopherol in corn oil during intermittent heating

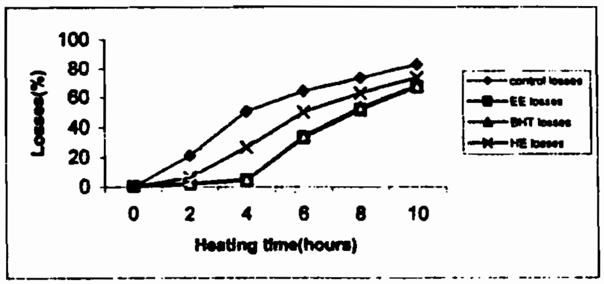

Fig. (10) : Effect of sage leaves extracts on losses (%) of gama γ - Tocopherol in corn oil during intermittent heating

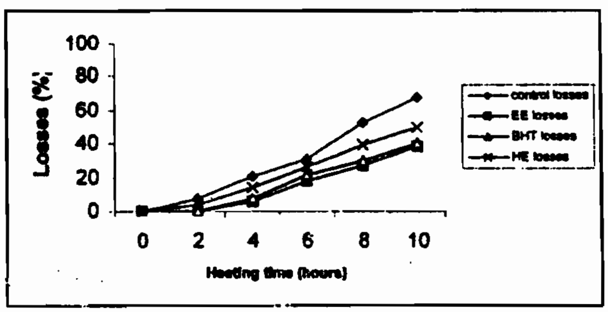

Fig. (11) : Effect of sage leaves extracts on losses (%) of δ-Tocopherol in corn oil during intermittent heating

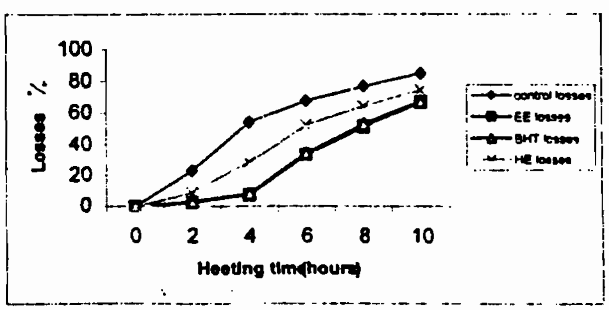

Fig. (12) Effect of sage leaves extracts on losses (%) of total Tocopherols in corn oil during intermittent heating

It can be concoluded that the ethanoilc extract of sage leaves (0.1%) has nearly the same antioxidative effect of BHT (0.05%), while the hexane extract of the same leaves was clearly less effective. This means that the ethanolic extract of sage leaves possesses about 50 % of the BHT efficacy, while the hexane extract of the same leaves showed less efficiency. The difference in the efficacy of the two extracts may be due to that ethanolic extract contains the most effective components as antioxidants (rosmarinic and carnosic acid) in higher ratio than those presented in hexane extract of sage leaves (Cuvelier *et. al.*, 1996).

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تأثير مستخلصات أوراق المريمية على ثبات زيت الـــذرة وعلـــى مكوناتـــه مـــن التوكوفيرولات أثناء عملية التسخين المتقطع.

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في هذا البحث تم دراسة مستخلص أوارق المريمية بكل من مذيبى الهكســان وكحــول الإيثانول كمضادات أكسدة طبيعية مقارنة بـ البيوتيلاتد هيدروكسي تلـــوين (BHT) كمضــاد أكسدة صناعى, في تقليل التغيرات التأكسدية أثناء عملية التسخين لفترات متقطعة لزيت الـــذرة. وقد تم اختيار زيت الذرة في هذه الحالة لأنه يستعمل على نطاق واسع في تصنيع الأغذيـــة وفـــي الطهى. ولقد قدرت التغيرات في بعض الثوابت وكذلك نواتج الأكسدة وكذلك نســـبة الأحمـــاض الدهنية والتوكوفيرولات في زيت الذرة فقط وزيت الـــذرة المضـــاف إليـــه مســـتخلصات أوراق المريمية والـــ BHT وكانت لنتائج كالآتى:

١- زادت أرقام الحموضة والبيروكسيد ومعامل الانكسار والامتصـــاص الضوئـــى علـــى الأطوال الموجيه ٢٣٤، ٢٧٠، ٤٠٠، ٤٥٠ نانوميتير تدريجيا بينما قل الرقم اليودى أثنـــاء عملية التسخين. إضافة ٠,١% من مســـتخلص الايثـــانول لأوراق المريميـــة وكذلـــك ٠,٠٥% من الـــ BHT اظهر تأثيراً كبيراً في تقليل هذه التغيرات بينما كان مســـتخلص هذه الأوراق بالهكسان أقل تأثيراً .

٢- نقص حمض اللينوليك موضوح بعد ١٠ ساعات من التسخين المتقطـــع ولكـــن اضافـــة المستخلص الكحولى لأوراق المريمية والـــ (BHT) قلل بوضوح من هذا النقص.

٣- جاما توكوفيرول كان يمثل الكون الرئيسى للتوكوفيرولات في زيت الذرة بينما لم يوجـــد بيتا – توكوفيرول.

٤- الفقد في الفا وجاما وزلتا توكوفيرول وكذلـــك التوكوفـــيرولات الكليـــة كـــانت نســـبة ٢٠,٣، ٥٣,٩، ٧٠,٩، ٥٠,٦ % على التوالى. بعد ٤ ساعات من التسخين المتقطـــع فـــي زيت الذرة , ولكن اضافة المستخلص الكحولى لأوراق المريمية قللت هذا الفقد إلـــى ١٧,٧، ٤,٥، ٥.٥، ٧,٢ % لنفس التوكوفيرولات المذكورة سابقا على التوالى وكذلك تحت نفس الظروف. وكذلك اضافة الـــ BHT قللت هذه الفقد إلى , ١٩,٠، ٥.٠، ٧.٧، ٨.٠% لنفس أنواع التوكوفيرولات على التوالى, وايضا بعد ٤ ساعات من التســـخين المتقطـــع بينما كان تأثير المستخلص الهكسانى لأوراق المريمية أقل مما سبق. من ذلك يتضـــح أن المستخلص الكحولى لأوراق المريمية يحوز على أكثر من ٥٠% من كفاءة الـــ BHT كمضاد للأكسدة.