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INVESTIGACIÓN

Antioxidant activity of alcohol extracts of chamomile flowers, anise seeds and dill seeds in two vegetable oils and two animal fats

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RESUMEN

Actividad antioxidante de extractos alcohólicos de flores de camomila, semillas de anís y eneldo en dos aceites vegetales y dos grasas animales.

Se investigó la actividad antioxidante de extractos alcohólicos de flores de camomila (CFE), semillas de anís (ASD) y eneldo (DSE) en aceites de maíz y soja, sebo y grasa de mantequilla anhidra (ABF), almacenados a 65°C. El seguimiento de la oxidación se realizó mediante el índice de peróxidos. Además, se determinó el poder reductor de los mismos. La actividad antioxidante de los extractos alcohólicos de estas plantas (3 g k^{-1}) resultó más eficaz que la del BHA (0.2 g kg^{-1}), en cambio, resultaron menos eficaces en el sebo y ABF. El extracto de manzanilla resultó ser mejor antioxidante para los aceites de maíz y soja que los extractos ASE y DSE, ambos mostraron una actividad similar. Sin embargo, en el sebo y la ABF todos los extractos revelaron una actividad antioxidante parecida. El poder reductor de estos extractos mostró un comportamiento diferente. El CFE que fue el extracto más activo como antioxidante presentó el menor poder reductor, además, mientras que los extractos ASE y DSE presentaron una actividad antioxidante similar, el primero de ellos mostró un poder reductor inferior, indicando que la actividad antioxidante no se correlaciona linealmente con el poder reductor de estos extractos.

PALABRAS-CLAVE: Antioxidante - Aceite vegetal - Flor de camomila - Grasa animal - Oxidación de grasas - Semilla de anís - Semilla de eneldo.

SUMMARY

Antioxidant activity of alcohol extracts of chamomile flowers, anise seeds and dill seeds in two vegetable oils and two animal fats.

The antioxidant activity of alcohol extracts of chamomile flowers (CFE), anise seeds (ASE) and dill seeds (DSE) in corn oil, soybean oil, beef tallow and anhydrous butter fat (ABF) was investigated during storage at 65°C. The extent of oxidation was followed by peroxide value (PV). In addition, reducing power of these extracts was determined. Alcohol extracts of these plants at 3 g kg^{-1} were more effective as antioxidant than BHA (0.2 g kg^{-1}) in corn and soybean oils, while they were less effective in beef tallow and ABF. CFE was more effective in retarding fat oxidation in corn and soybean oil than ASE and DSE, which both showed similar activity. However, in beef tallow and ABF all extracts showed similar activities. On the other hand, the reducing power of these extracts showed different behavior. CFE, which was the most active as antioxidant among the extracts showed the lowest reducing power. Furthermore, ASE and DSE, which exhibited similar antioxidant activity, the former has lower reducing activity than the latter, indicating that the antioxidant activity didn't correlate linearly with the reducing power of these extracts.

KEY-WORDS: Animal fat - Anise seeds - Antioxidant - Chamomile flowers - Dill seeds - Fat oxidation - Vegetable oils.

1. INTRODUCTION

Lipid oxidation is a chemical change in foods, which depends on the level of oxygen, degree of unsaturation of fatty acids, energy (heat/light) and presence of metals. Lipid oxidation products are responsible for the development of rancidity by the production of low molecular weight compounds that cause undesirable flavors, thus affecting quality and limiting the shelf-life of food products (Frankel, 1985; Frankel et al., 1987). In addition, some of the oxidation products of fat oxidation products are related to aging, heart diseases and cancer (Marx, 1987; Eriksson, 1987; Addis and Warner, 1991; Kubow, 1992; Esterbauer, 1993).

The addition of antioxidants to food is effective in retarding fat oxidation. The most commonly used antioxidants at the present time are BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene). However, these antioxidants are believed to possess carcinogenic activity (Imadia et al., 1983; Hauman, 1990). This finding together with consumer preference to natural products has resulted in increased work on natural antioxidants. Studies on lipid oxidation demonstrated that the extracts of a number of plants have potent antioxidant activity compared to BHA and BHT. These include rosemary (Karin et al., 1992; Frankel et al., 1996; Marie-Elisabeth et al. 1996) lavender (Economou et al., 1991), sage (Marie-Elisabeth et al. 1996, Yildirim et al., 2000), ginger (Jitoe et al., 1992), tea (Cao et al. 1996) and oregano (Economou et al., 1991; Vekieri et al. 1993). Furthermore, antioxidants prepared from rosemary are being marketed commercially (Quirin and Gerard, 1994).

In many of Middle East countries, many of herbs are used such as tea, chamomile and anise seeds as beverages. In addition, it has been reported that chamomile is used to treat teething symptoms in infants and to allay irritability and anxiety (Bruenton, 1999) and its acetone extract showed antioxidant activity in rapeseeds oil stored at 40°C for 70 days. Anise is used to paliate cough and lozenges (Nicholson, 1981) and its essential oils exhibited antioxidants activity in butter stored at refrigeration temperature (Farag et al., 1989). Dill seeds and anis seeds are also used as spices.

The present work was conducted to evaluate the antioxidant activity of alcohol extracts of chamomile (*Matricaria chamomilla*) flowers, anise seeds (*Pimpinella anisum*) and dill seeds (*Anethum graveolens*) in some oils and fats, and comparing such activity with that of synthetic antioxidants.

2. MATERALS AND METHODS

The selected plants were purchased from a local market. The taxonomic identity of plants was confirmed by comparing these plants with those of known identity in the Herbarium of the Department of Biological Science, University of Jordan (Table I).

2.1. Preparation of extracts

The alcohol extracts of chamomile flowers, anis, dill seeds were prepared under reflux conditions for a period of 2 hours. The extracts were concentrated using a rotary evaporator. The yields of a 50 g of the dried flower of chamomile, anise and dill seeds were 12.2, 10.1and 11.4 g, respectively. The alcohol extracts were then dissolved in alcohol for the evaluation of antioxidant activity.

2.2. Preparation of anhydrous butter fat

ABF was prepared at laboratory following the traditional method (Amr, 1991): Durum wheat bulgur grits (75 g kg^{-1} butter) were added to the unsalted cow's butter, prepared from soured milk and obtained from local producer. The mixture was then heated until a clear butter liquid was obtained (about 30 min.), which indicates the doneness of the products. The temperature did not exceed 117 °C and at which the bulgur starts to gather and precipitate at the bottom of the container. Anhydrous butter fat was decanted and filtered through a piece of cheesecloth, filled in glass jars and left to solidify.

2.3. Preparation of beef tallow

Beef tallow purchased from a local market was mixed with about 400 ml of water and steam rendered in an autoclave at 120 (15 psig) for 30 min. The rendered fat was strained to get rid of meat pieces and left to settle at room temperature. The layer of the fat was skimmed and then decanted and kept for the accelerated oxidation.

2.4. Evaluation of the antioxidant activity.

Calculated quantities of the extracts of Chamomile flowers (CFE), anise seeds (ASE) and dill seeds (DSE) were added to beef tallow, anhydrous butter fat, corn and soybean oils obtained from commercial refining plant and contained no additives. The mixtures were stirred for 30 min at 35°C. Comparative treatment was conducted with butylated hydroxy toluene (BHA) at a level of 0.2 g kg^{-1}. Control samples contained no additives were prepared under the same condition. The samples were oxidized at 65 ± 2°C, in darkness. Oxidative stability was evaluated by analyzing samples periodically for peroxide number according to the AOAC, 1990, The percentage inhibition of oil oxidation, 100%-[(PV increase of sample/PV increase of control) x 100%] was used as a measure of the present antioxidant activity (Pin-Der, and Gow-Chin, 1997). All analysis were run in duplicate and averaged.

2.5. Reducing Power

The reducing power of the alcohol extracts was determined following the method of Yildirim et al. (2003): One ml of each extract solutions was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (K_3 Fe $(CN)_6$; 10g l^{-1}). The mixture was incubated at 50°C for 30 min. After that, 2.5 ml of trichloroacetic acid (100g l^{-1}) was added and the mixture centrifuged at 1650x g for 10 min, 2.5 of the supernatant was treated with 2.5 ml of water and 0.5 ml of FeCl3 (1 g l^{-1}), and then the

Table I
Ethnobotanical data of studied plants

Botanical Name	Family	Part Used
Matricaria aurea (Loefi.) Schultz Bip.	Compositae	Flowers
Pimpinella anisum L.	*Apiaceae*	Seeds
Anethum graveolens L.	*Apiaceae*	Seeds

absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

2.6. Statistical analysis

Data were analyzed using the Statistical Analysis System (1885) program. Significant differences between means were determined by Duncan's multiple-range tests.

3. RESULTS AND DISCUSION

3.1. Dose effect relationship

The data of corn oil oxidation, measured as peroxide value at 65°C after the addition of the alcohol extracts of chamomile flowers (CFE), dill seeds (DSE) and anise seeds (ASE) are reported in Tables II-IV, respectively. The concentration of the added extracts varied from 0-3 g Kg^{-1}.

It is evident that all extracts showed antioxidant activity, which increased with increasing their concentration and best results were found at 3 g $Kg^{-1}$of all extracts. Furthermore, this dose of the extracts did not affect the sensory properties of the oil. These results agree with many workers who found that the antioxidant activity of natural extracts was concentration dependent (Economou et al., 1991; Pin-Der and Gow-Chin, 1997; Guohua et al., 1996). It appears also that the length of the induction period (the period during which the rate of oxidation is slow) in corn oil upon the addition of CFE at all doses extended to 6 days instead of 3 days found for the control (Table II). However, the addition of DSE and ASE did not affect the length of this period when compared to that of the control (Tables III & IV). It can be observed also that shifting to the propagation stage of oxidation was faster in the control and it was concentration dependent in the other treatments, the higher the extracts concentration, the lower the shifting to the

Table II
The effect of chamomile extract dose and BHA (0.2 gkg^{-1}) on peroxide value (meq $O2Kg^{-1}$) in corn oil during storage at 65°C

	Dose of chamomile flower extract (g kg^{-1})					
Storage time (days)	**Control**	**1**	**2**	**2.5**	**3**	**BHA**
0	Nd[a,b]	Nd	Nd	Nd	Nd	Nd
3	3.0[c]	1.9[d]	1.8[d]	1.9[d]	1.7[d]	1.8[d]
6	20.0[c]	2.8[d]	2.5[d]	2.8[d]	2.5[d]	3.5[d]
8	46.6[c]	15.2[d]	6.2[de]	5.1[e]	5.1[e]	15.0[d]
10	92.3[c]	37.6[d]	23.0[e]	14.7[f]	8.8[g]	40.8[d]
13	146.1[c]	63.8[d]	38.8[e]	26.7[f]	14.4[g]	70.5[d]
15	195.3[c]	106.1[d]	77.6[e]	49.5[f]	27.9[g]	107.1[d]

[a]Mean of two replicates.
[b]Means within a row with the same upper case letters are not significantly different ($P \leq 0.05$).

Table III
The effect of anise seeds extract dose and BHA (0.2 gkg^{-1}) on peroxide value (meq $O2Kg^{-1}$) in corn oil during storage at 65°C.

	Dose of anise seeds extract (g kg^{-1})					
Storage time (days)	**Control**	**1**	**2**	**2.5**	**3**	**BHA**
0	Nd[a,b]	Nd	Nd	Nd	Nd	Nd
3	1.6[e]	2.0[de]	1.9[de]	2.3[d]	2.9[c]	1.6[de]
6	20.0[c]	15.9[d]	7.1[e]	5.5[ef]	3.2[f]	3.5[f]
8	46.6[c]	37.6[d]	23.0[e]	13.9[f]	6.1[g]	15.0[f]
10	92.4[c]	65.1[d]	45.2[e]	30.2[g]	17.3[h]	40.8[f]
13	146.1[c]	96.0[d]	63.3[ef]	54.5[f]	32.4[g]	70.5[e]
15	195.3[c]	126.4[d]	103.3[e]	74.6[f]	58.9[g]	107.1[e]

[a]Mean of two replicates.
[b]Means within a row with the same upper case letters are not significantly different (P =0.05).

Table IV
The effect of dill seeds extract dose and BHA (0.2 g kg^{-1}) on peroxide value (meq O_2Kg^{-1}) in corn oil during storage at 65°C

	Dose of dill seeds extract (g kg^{-1})					
Storage time (days)	**Control**	**1**	**2**	**2.5**	**3**	**BHA**
0	Nd[a, b]	Nd	Nd	Nd	Nd	Nd
3	1.6[d]	1.9[cd]	1.9[cd]	2.2[c]	2.2[c]	1.8[cd]
6	20.0[c]	12.2[d]	10.0[e]	9.2[e]	4.4[f]	3.5[f]
8	46.6[c]	27.9[e]	34.5[d]	18.0[f]	10.5[h]	15.0[g]
10	92.4[c]	67.9[d]	51.4[e]	37.8[f]	24.9[g]	40.8[f]
13	146.1[c]	91.9[d]	76.4[e]	61.9[f]	42.6[f]	70.5[ef]
15	195.3[c]	121.8[d]	102.4[e]	82.0[f]	67.9[f]	107.1[f]

[a]Mean of two replicates.
[b]Means within a row with the same upper case letters are not significantly different ($P \leq 0.05$).

Table V
Peroxide value (meq O_2 Kg^{-1}) of corn oil treated with 3 g kg^{-1} of the alcohol extracts of chamomile flowers (CFE), anise seeds (ASE) and dill seeds (DSE) extracts and BHA (0.2 g kg^{-1})

Time (days)	Control[a,b]	CFE[a]	ASE[a]	DSE[a]	BHA[a]
0	ND	Nd	Nd	ND	ND
3	3.0[c]	1.5[d]	1.7[d]	1.8[d]	1.8[d]
6	20.0[c]	2.5[d]	3.2[d]	3.7[d]	3.5[d]
8	46.6[c]	5.1[e]	6.1[e]	6.9[e]	15.0[d]
10	92.3[c]	8.8[f]	17.3[e]	18.3[e]	40.8[d]
13	146.1[c]	15.4[f]	32.4[e]	35.1[e]	70.5[d]
15	195.3[c]	27.9[f]	58.9[e]	60.1[ce]	107.1[d]

[a]Mean of two replicates.
[b]Means within a row with the same upper case letters are not significantly different ($P \leq 0.05$).

Table VI
Inhibitory effect of the alcohol extracts (3 g kg^{-1}) expressed as inhibition % on corn oil oxidation.

Time (days)	CFE[a,b]	ASE[a]	DSE[a]	BHA[a]
0	0.0[c]	0.0[c]	0.0[c]	0.0[c]
3	50.0[c]	43.3[cd]	40.0[d]	40.0[d]
6	87.5[c]	84.0[d]	81.5[f]	82.5[e]
8	89.1[c]	87.1[d]	85.0[e]	67.8[f]
10	90.5[c]	81.6[d]	80.1[d]	56.8[e]
13	89.5[c]	77.9[d]	76.0[d]	51.8[de]
15	85.7[c]	69.8[d]	69.2[d]	45.2[ce]

[a]Mean of two replicates.
[b]Means within a row with the same upper case letters are not significantly different ($P \leq 0.05$).

propagation stage. Tables II-IV also show that the addition of the extracts at doses of 2.5 and 3 g Kg^{-1} were more effective in depressing the formation of peroxides than BHA. This result agrees with those reported by Nawar (1996) that BHA and BHT show low antioxidant activity in vegetable oils. However, the activity of BHA and the extracts at dose of 2 g Kg^{-1} were comparable.

3.2. Corn Oil

The progress of the oxidative rancidity in corn oil samples treated with the three alcohol extracts (3 g kg^{-1}) and BHA (0.2 g kg^{-1}) measured by PV during storage at 65°C is shown in Table V. It appears that the addition of alcohol extracts and BHA to corn oil extended the length of the induction period of

oxidation to 6 days compared to 3 days observed in the control. Significant differences between the control and the other treatments in their PV values started to appear after 3 days of incubation and after 6 days among these treatments. The data in Table V also show that the propagation stage was higher and faster in the control followed by BHA and then in other treatments. Among the other treatments, the samples treated with CFE showed slower propagation stage than ASE and DSE.

It is also evident that BHA was significantly less effective as antioxidant than the extracts. CFE was more effective in depressing the oxidative rancidity of corn oil than ASE and DSA, which showed similar effectiveness. PV values of the samples treated with BHA, CFE, ASE and DSA, after 15 days of storage were 107.1, 27.9, 58.9 and 60.1 which represents 54.8, 14.3, 30.2 and 30.8%, respectively of that of the control (195.3 meq O_2 kg^{-1}). Considering the maximum allowed level of 10 meq O_2 kg^{-1} oil set up by Jordanian Standards for refined vegetable oils, it can be concluded that the addition of the CFE, ASE, DSE and BHA to corn oil extended its keepability for 10, 8, 8 and 6 days, respectively compared to 3 days observed in the control or the samples treated with BHA.

Table VI reports the inhibition % of corn oil oxidation throughout the storage period as a result of the addition of the extracts or BHA to the oil. It can be observed that the inhibition % of the oil in all treatments increased gradually to reach a maximum value after 10, 8, 6 and 6 days in the samples treated with CFE, ASE, DSE and BHA with inhibition % of 90.5, 87.1, 85.0 and 82.5 %, respectively. After that, a decrease in these values was observed throughout the rest of the storage period. It can be observed that CFE was the most effective and BHA was the least in the inhibition of oxidative rancidity of corn oil. Therefore, the antioxidant activities of these additives were in the following order of decreasing activity: CFE ASE = DSE BHA.

3.3. Soybean oil

The data in Table VII show the development of PV values of the different soybean oil treatments during storage at 65°C. Significant differences started to appear between the control and the other treatment after 3 days of incubation. As was noticed in case of corn oil, the length of the induction period in the treated oil samples was extended to 6 days versus three days found in the control, indicating the effectiveness of these extracts in depressing the oxidative rancidity of soybean oil during the storage period. As also observed in corn oil, BHA was less effective as antioxidant than the extracts confirming

Table VII

Peroxide value (meq O_2 Kg^{-1}) of soybean oil treated with 3g kg^{-1} of the alcohol extracts of chamomile flowers (CFE), anise seeds (ASE) and dill seeds (DSE) extracts and BHA (0.2 g kg^{-1})

Time (days)	Control[a,b]	CFE[a]	ASE[a]	DSE[a]	BHA[a]
0	ND	ND	ND	ND	ND
3	4.6[a]	1.3[c]	2.7[bc]	2.4[c]	2.9[b]
6	30.6[a]	4.5[c]	7.5[bc]	8.4[bc]	9.4[b]
8	83.4[a]	9.7[d]	14.4[c]	16.4[c]	25.6[b]
10	128.6[a]	23.3[d]	34.4[c]	35.8[c]	50.2[b]
13	188.7[a]	54.5[d]	66.4[c]	67.4[c]	119.0[b]

[a]Mean of two replicates.
[b]Means within a row with the same upper case letters are not significantly different ($P \leq 0.05$).

Table VIII

Inhibitory effect of the alcohol extracts (3 g kg^{-1}) expressed as inhibition% on soybean oil oxidation

Time (days)	CFE[a,b]	ASE[a]	DSE[a]	BHA[a]
0	0.0[c]	0.0[c]	0.0[c]	0.0[c]
3	71.7[c]	41.3[cd]	47.8[c]	37.0[d]
6	85.3[c]	74.5[d]	72.6[d]	69.3[d]
8	88.4[c]	82.7[d]	80.3[e]	69.3[f]
10	81.9[c]	73.3[cd]	72.2[c]	61.0[d]
13	71.1[c]	64.8[c]	64.3[c]	37.0[d]

[a]Mean of two replicates.
[b]Means within a row with the same upper case letters are not significantly different ($P \leq 0.05$).

the fact that BHA has low antioxidant activity in vegetable oils (Nawar, 1996). On the other hand, CFE showed higher antioxidant activity than ASE and DSE, which showed similar activities. It also appears that the addition of CFE to soybean oil kept PV values below the maximum allowed level of 10 meq O_2 kg^{-1} for 8, while the addition of BHA and the other two extracts kept it below this level for 6 days when compared to 3 days in the control. After 13 days of storage, PV values in the sample treated with CFE, ASE, DSE and BHA were 54.5, 66.4, 67.4 and 119 meq O_2 kg^{-1}, which represent 28.9, 35.2, 35.7 and 63.1%, respectively of that found in the control (188.7 meq O_2 kg^{-1}).

Table VIII shows that there were significant differences in the inhibition % (P =0.05) of fat oxidation in all treated samples. The inhibition % reached maximum values for all treatment after 8 days of incubation followed by gradual decrease toward the end of the incubation period, which indicates that the rate of oxidative rancidity became less affected by the extracts or BHA. The maximum inhibition % observed for CFE, ASE, DSE and BHA were 88.4, 82.7, 80.3 and 69.3%, respectively and significant differences ($P \leq 0.05$) were found among them. It also appears that the extracts were significantly more effective in the retardation of soybean oxidation than BHA. Furthermore, CFE seemed to be the most effective in retarding oil oxidation.

The oxidative rancidity measured in soybean was higher than that found in corn oil, indicating that the extracts were less effective in the former. This may be attributed to the fact that soybean oil has higher degree of unsaturation than corn oil, especially in linolenic acid, which makes it more susceptible for oxidation (Nawar, 1996).

3.4. Beef Tallow

The development of the PV values in beef tallow during storage at 65°C is reported in Table IX. It is evident that the level of the rate of ABF oxidation is much lower than that observed in vegetable oils. This maybe due to the fact that beef tallow contains high level of saturated fatty acids, which render it more resistant to oxidation. Significant differences between the control and the other treatments in their PV values started to appear after 8 days and after 13 days between the extracts and BHA treatments. It also appears that throughout the storage period, PV values in all treatments, except the control, did not

Table IX

Peroxide value (meq O_2 Kg^{-1}) of beef tallow treated with 3gkg^{-1} of the alcohol extracts of chamomile flowers (CFE), anise seeds (ASE) and dill seeds (DSE) extracts and BHA (0.2 gkg^{-1}).

Time (days)	Control[a,b]	CFE[a]	ASE[a]	DSE[a]	BHA[a]
0	ND	ND	ND	ND	ND
3	1.0	0.6[c]	0.6[d]	0.8[d]	ND[e]
8	2.1[c]	0.8[de]	1.0[d]	1.0[d]	0.5[e]
13	3.5[c]	1.7[d]	1.8[d]	1.8[d]	1.0[e]
20	7.8[c]	2.9[d]	3.1[d]	3.1[d]	1.0[e]
28	17.6[c]	4.7[d]	4.5[d]	4.7[d]	1.5[e]

[a]Mean of two replicates.
[b]Means within a row with the same upper case letters are not significantly different ($P \leq 0.05$).

Table X

Inhibitory effect of the alcohol extracts (3 g kg^{-1}) expressed as inhibition% on beef tallow oxidatio

Time (days)	CFE[a,b]	ASE[a]	DSE[a]	BHA[a]
0	0.0	0.0	0.0	0.0
3	40.0[e]	50.0[d]	40.0[e]	100.0[c]
8	61.9[d]	54.3[d]	52.4[d]	76.2[c]
13	51.4[d]	50.0[d]	48.6[d]	71.4[c]
20	62.8[d]	61.5[d]	60.3[d]	87.2[c]
28	73.3[d]	73.3[d]	73.4[d]	91.5[c]

[a]Mean of two replicates.
[b]Means within a row with the same upper case letters are not significantly different ($P \leq 0.05$).

Table XI

Peroxide value (meq O_2 Kg^{-1}) of ABF treated with 3g kg^{-1} of the alcohol extracts of chamomile flowers (CFE), anise seeds (ASE) and dill seeds (DSE) extracts and BHA (0.2 g kg^{-1})

Time (days)	Control[a,b]	CFE[a]	ASE[a]	DSE[a]	BHA[a]
0	ND	ND	ND	ND	ND
3	1.8[c]	0.7[d]	0.7[d]	0.8[d]	0.5[d]
8	2.3[c]	1.1[de]	1.1[d]	1.2[d]	0.5[e]
13	4.2[c]	1.9[d]	2.1[d]	2.3[d]	1.3[e]
20	7.9[c]	3.4[d]	4.0[d]	4.2[d]	1.5[e]
24	15.9[c]	5.5[d]	5.3[d]	5.4[d]	2.1[e]
28	32.5[c]	7.7[d]	7.2[d]	7.4[d]	2.4[e]

[a]Mean of two replicates.
[b]Means within a row with the same upper case letters are not significantly different ($P \leq 0.05$).

Table XII

Inhibitory effect of the alcohol extracts (3 g kg^{-1}) expressed as inhibition % on ABF oxidation

Time (days)	CFE[a]	ASE[a]	DSE[a]	BHA[a]
0	0.0[b]	0.0	0.0	0.0
3	61.1[d]	61.1[d]	55.6[d]	72.2[c]
8	52.2[d]	52.1[d]	47.8[d]	78.3[c]
13	54.8[d]	50.0[e]	45.2[f]	64.3[c]
20	57.0[d]	49.4[de]	46.8[e]	76.6[c]
24	65.4[d]	66.7[d]	66.0[d]	86.8[c]
28	76.3[d]	77.9[d]	77.2[d]	92.6[c]

[a]Mean of two replicates.
[b]Means within a row with the same upper case letters are not significantly different ($P \leq 0.05$).

exceed 5 meq O_2 kg^{-1}, indicating that the extracts were efficient to extend the keepability of the products for at least 28 days compared to about 20 days observed in the control. BHA was more effective in depressing the development of peroxides than the extracts. This result agrees with those found by many workers that BHA and mixtures of BHA/BHT were more effective as antioxidants in animal fats than natural antioxidants (Shahidi and Wanasundara, 1992, Buck, 1991). On the other hand, no significant differences between the extracts in their PV values during the entire period of storage were observed.

The inhibition % of beef tallow oxidation during storage is reported in Table X. It can be observed that inhibition % varied during the storage period. However, BHA showed higher inhibition % than the extracts, which showed comparable values.

3.5. Anhydrous Butter fat (ABF)

The data reported in Table XI show the progress of ABF oxidation during storage at 65°C. As in the case of beef tallow, peroxides were undetected at the onset of storage period, which indicates the good quality of the products. Furthermore, the heat treatment used during the preparation of ABF had little or no influence on PV. It is evident that the addition of the extracts and BHA exhibited significant effect in depressing the progress of ABF oxidation throughout storage period. Significant differences between the control and the other treatment in their PV were observed after 3 days of storage. After 24 days of storage, only the PV value of the control was above the maximum allowed level (5 meq O_2 Kg^{-1}) set up by the Jordanian Standards for ABF. Furthermore, PV values of the samples treated with CFA or BHA remained below 5 meq O_2 kg^{-1} during the entire period of storage (28 days), while they were less than 7 meq O_2 kg^{-1} in the samples treated with DSE and ASE. As observed in the case of beef tallow, BHA was the most effective in depressing the oxidative rancidity in ABF followed by CFE, while DSE and ASE showed similar activities.

Inhibition % of oxidation of ABF by the extracts and BHA varied during the storage but reached maximum values at the end of the experiment time (Table XII). It appears that BHA was the most effective in the inhibition of ABF oxidation, while the extracts showed similar activities.

Although ABF contain higher level of saturated fatty acid than that in beef tallow, it showed higher level of oxidative rancidity under the condition used in the present study. This might be due to the lower viscosity of ABF, which may result in relatively higher

Table XIII
Comparison of the reducing powers of various amounts of alcohol extracts of chamomile flowers (CFE), anise seeds (ASE) and dill seeds (DSE)

	Absorbance (700 nm)		
Concentration ((mg ml^{-1})	**CFE[a]**	**ASE[a]**	**DSE[a]**
0.5	0.021[e,b]	0.132[c]	0.098[d]
1.0	0.050[e]	0.311[c]	0.210[d]
1.5	0.141[e]	0.443[c]	0.338[d]
Ascorbic acid (0.5 mg ml^{-1})		1.712	

[a]Mean of two replicates.
[b]Means within a row with the same upper case letters are not significantly different ($P \leq 0.05$).

rate of movement of ABF molecules to the surface of the product resulting in higher level of oxidation.

3.6. Reducing Power

The reducing power of the alcohol extracts is given in Table XIII. The reducing power increased as the amount of extracts increased. It is evident that ASE was the most effective and CFE was the least one. It can be observed also that ascorbic aid at 500 mgml^{-1} exhibited a greater reducing power than the extracts at all used concentrations. It is well known that ascorbic acid is a reducing agent (Shimada et al., 1992). It was interesting to find that although CFE had the highest antioxidant activity, it was the least effective in reducing power. This disagrees with the results of Mier et al. (1995) who reported that the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, these results agree with those of Yildirim *et al*. (2000) who found that although the water extract of sage (*Salvia tribola* L.) had the lowest antioxidant activity among black tea and linden (*Tilia argentea Desf.ex Dc*) flowers, it was the most effective in reducing power. This could be attributed to the various mechanisms that could explain the antioxidant activity; among these are prevention of chain initiation, decomposition of peroxides, prevention of continual hydrogen abstraction and radical scavenging (Yildirim et al., 2003; Diplock, 1997). Therefore, we assume that there is no linear relationship between total antioxidant activity and reducing power.

4. CONCLUSION

It was found that the alcohol extracts of chamomile flowers, anise seeds and dill seeds at 3g kg^{-1} have antioxidant activity which is higher than BHA (0.2 g kg^{-1}) in vegetable oils and lower in beef tallow and anhydrous butter fat. Chmomile flowers extract at the used dose was more effective as antioxidant than Dill and anise seeds extracts in vegetable oils. However, they showed similar activities in animal fats.

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