

ANTIOXIDANT PROPERTIES OF SOLVENT EXTRACTS FROM SOME PLANT SOURCES

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Keywords: Fenugreek, Soybean, Canola Seeds, Ginger roots, Conjugated diene method

ABSTRACT

This study was conducted to evaluate the antioxidative activity of extracts from fenugreek, soybean, canola seeds and ginger roots. The antioxidant properties of 90% ethanol and 90% methanol were used as solvents. Antioxidative effects measured by the conjugated diene method. The effects of these extracts on the TBA values and rancid odor of beef patties during cold storage at ~ 5 °C for 12 days were also evaluated. The extract of ginger roots had a higher antioxidant activity than extracts of fenugreek, soybean and canola seeds as well as BHA. The ethanol extracts of fenugreek and soybean seeds exhibited high antioxidant activity than that of methanol extracts. While, the methanol extracts of canola seeds and ginger roots had higher antioxidant activity than that of their ethanol extracts. The antioxidant in fenugreek, soybean, canola seeds and ginger roots extracts were fairly heat-stable and showed 51.15-64.32 % activity after 100 min heating at 100°C. The maximum antioxidant activity of all extracts was found at pH 7.0. Extracts stored in the dark at ~5°C, ~25°C and ~37°C for 24 day did not show any change in the antioxidant activity. However, extracts stored in light at ~25°C showed a significant reduction in the antioxidant activity after 3 days of

storage. Pyrogallol was the highest phenolic compounds in fenugreek seeds and ginger roots extracts. However, hydroquinone and salicylic were the highest phenolic compounds in soybean and canola seeds extracts, respectively. Addition of antioxidant extracts to beef patties were reduced the TBA values and rancid odor during cold storage.

INTRODUCTION

Lipid oxidation is a major cause of muscle food deterioration, affecting color, flavor, texture and nutritional value (Lee *et al* 1986; Kanner *et al* 1991; Chan *et al* 1993; Rhee *et al* 1996; Yin and Cheng, 1997).

Lipid oxidation not only produces undesirable characteristics of odors and flavors, but also decreases the nutritional quality and safety of food by the formation of secondary reaction products during cooking and processing (Frankel, 1993).

Lipid oxidation via free radical chain reaction may be a cause of numerous diseases such as atherosclerosis, ischemia, inflammation, carcinogenesis and aging (Aruoma and Halliwell, 1991).

The addition of antioxidants is effective in retarding oxidation of fats. Labuza (1971) noted that antioxidants can increase shelf-life of foods by 15 – 200%. Addition of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ) can control lipid oxidation in foods (Khalil and Mansour, 1998).

(Received August 25, 2009)

(Accepted October 19, 2009)

However, the use of these synthetic antioxidants has begun to be restricted because of their health risks and toxicity (Buxiang and Fukuhara, 1997 and Hirose *et al* 1998).

Therefore, there is a strong need for effective antioxidants from natural sources to prevent deterioration of foods. Naturally occurring materials may provide advantage over synthetic compounds because they may be safer to humans (Kikuzaki and Nakatain, 1993). The importance of replacing synthetic antioxidants with natural ingredients from oil seeds, spices and other plant materials has increased greatly. Some components of extracts isolated from fruits and vegetables have been proven in model systems to be as effective antioxidants as synthetic antioxidants (Loliger, 1989; Pralt and Hudson, 1990; Pabadopoulos and Boskou, 1991; Rodriguez *et al* 1994 and Al-Saikhan *et al* 1995).

The present study addresses the utilization of economic plant materials such as fenugreek, soybean, canola seeds and ginger roots as sources of natural antioxidants.

MATERIALS AND METHODS

Materials

The materials used in this investigation and their sources were:

Fenugreek seeds (*Trigonella foenum-graecum*, Giza 30), soybean seeds (*Glycine max*, Giza 111) and canola seeds (*Brassica napus*) were obtained from Agricultural Research Center (Mars 2001), Ministry of Agricultural, Giza, Egypt.

Ginger roots (*Zingiber officinalis*) were obtained from the local market, Cairo, Egypt. Butylated hydroxyanisole (BHA, Sigma) was obtained from Agricultural Research Center, Ministry of Agricultural, Giza, Egypt. Linoleic acids, thiobarbituric acid (TBA), trichloroacetic acid (TCA) and other chemicals used were obtained from Sigma Chemical Co., St. Louis, Mo, USA.

Meat and fat were obtained from local market in Shibin El-Kom City, Minufiya Governorate, Egypt.

Methods

Preparation of antioxidant extracts

Fenugreek seeds, soybean seeds, canola seeds and ginger roots were ground separately, passed through a 60 mesh screen. Antioxidant

extracts were prepared as described by Mansour and Khalil (2000) as follows: One hundred grams of each material were defatted by shaking three times with four volumes of petroleum ether in a rotary shaker (Julabo D-7632 Seelbach, Germany) for 1 h. The residues obtained after filtration was dried overnight under a hood until all traces of petroleum ether were removed. The dried residues from each material were extracted three times with four volumes of 90% ethanol or 90% methanol by shaking for 1 h, and filtered. The combined filtrates from each material were concentrated in a rotavapor (Rotvac evaporator RVO-64, Gzechoslovakia) and placed under a hood to remove the residual ethanol or methanol solvent. The obtained aqueous extracts were frozen overnight and freeze-dried at -60°C (Labconco Freeze Dry 64312, Kansas, MO). The freeze-dried extracts were stored in air-tight containers at 5°C until used for the determination of antioxidant activity.

Determination of antioxidant activity

The antioxidant activity was determined by the conjugated diene method (Lingnert *et al* 1979). Each extract (1–5 mg / ml) in methanol (100 µl) was mixed with 2 ml of 10 mM linoleic acid emulsion (pH 6.5) in test tubes and placed in darkness at 37°C to accelerate oxidation. After being incubated for 15 h, 6 ml of 60% methanol in deionized water was added, and the absorbance of the mixture was measured at 234 nm in a spectrophotometer (Jenway 6305 uv/vis, Model Voltage 230 / 115 V-power 50 VA-Serial No. 1169, Frequency 50/60 Hz, England). The antioxidant activity (AOA) was calculated as follows:

$$AOA (\%) = \frac{(\Delta A_{234} \text{ of control} - \Delta A_{234} \text{ of sample})}{(\Delta A_{234} \text{ of control})} \times 100\%$$

An AOA value of 100% indicates the strongest antioxidant activity

Evaluation of heat, pH and storage stability

The antioxidants stability of extracts was determined as described by Mansour and Khalil (2000).

Heat stability

Extracts were pre-incubated at different temperatures in the range of 40 – 100°C for 30 min. Antioxidant activity was determined as previously mentioned.

To evaluate the effect of boiling time on the antioxidant activity, the extracts were heated in boiling water (100°C) both for 0, 30, 60, 90 and 120 min and the residual antioxidant activity was determined.

pH stability

Extracts were pre-incubated at pH values in the range of 4.0 – 9.0 for 30 min. The residual antioxidant activity was determined.

Storage stability

Extract from each material was divided into four (10 ml) aliquots. The first three aliquots were stored in dark condition under refrigeration (~ 5°C), room temperature (~ 25°C) and 37°C, while the fourth aliquot was stored in light condition at room temperature (~ 25°C). Antioxidant activity was determined periodically over 3 weeks for each aliquot.

Preparation and identification of phenolic compounds using HPLC

To separate and identification the phenolic compounds, extracts were performed with HPLC (Hewlett Packard Series 1100, USA) equipped with UV 254 nm detector. Injection and detector temperature was 25°C. Two carrier were used, mobile phase A (0.5 ml acetic acid + 99.5 ml distilled water) and mobile phase B (0.5 ml acetic acid + 99.5 ml acetonitrile, CH₃CN) with a flow rate of 0.3 ml / min. Column (Hypersil BDS 5 µm C 18). Phenolic compounds were identified and quantified by comparing the retention time and peak area of the unknowns with those of the phenolic compounds standards.

Beef patties formulation

Beef patties were prepared from fresh leanbeef and kidney fat. Leanbeef were obtained from boneless round and trimmed from all subcutaneous and intramuscular fat as well as thick, visible connective tissue. The leanbeef and kidney fat sources were separately ground in a Hobart meat grinder (Model No. 4046, Hobart Manufacturing Co., Troy, OH). Representative samples from the trimmed leanbeef and fat were initially analyzed for fat content prior the manufacture of beefburgers.

The ground lean beef (65%), kidney fat (20%), salt (2%), spices mixture were consisted of 60 gm

dehydrated onion, 60 gm dried ginger, 40 gm black pepper, 40 gm dehydrated garlic, 40 gm cinnamon, 30 gm Marjoram, 30 gm cumin, 25 gm cloves and 25 gm nutmeg, (1.5%), sugar (1.0%), sodium tripolyphosphate (0.2%), ascorbic acid (0.3%) and water (10%) were thoroughly mixed manually and then passed twice through a 4 mm plate to be ground. Control treatment was formulated without antioxidant. The other treatments were prepared by adding three levels (0.01, 0.05 and 0.1%) from each antioxidant extract (Fenugreek seeds, soybean seeds, canola seeds and ginger roots) and BHA.

After forming the beefburgers, the beefburgers were located on plastic foam meat trays, wrapped with polyethylene film and kept at -18°C in deep freezer (Model up 270, W. Alaska, Egypt) until further analysis. The other parts of beefburgers were kept in a refrigerator at ~ 5°C for 12 days.

Thiobarbituric acid (TBA)

TBA values were determined spectrophotometrically according to the procedure described by *Siu and Draper (1982)*. Ten grams of sample were homogenized in 25 ml distilled water, and then mixed with 25 ml of 10% trichloroacetic acid. The mixture was vortex-mixed and filtered. One milliliter of 0.06 M thiobarbituric acid was added to 4 ml aliquots of the filtrate and heated in boiling water both (10 min) for color development. The absorbance was measured at 532 nm using a spectronic 2000 spectrophotometer. The TBARS values of antioxidant-treated patties were compared to control patties (without antioxidant). The TBARS values were expressed as mg malonaldehyde / kg sample.

Sensory properties

Sensory evaluation of patties was conducted to determine the presence of rancid meat odor. Evaluation was performed by ten panelists who were graduate students and staff members in the Department of Nutrition and Food Science, Faculty of Home Economics, Minufiya University. Shibin El-Kom, Egypt. Panelists were selected on the basis of their interest and availability. Panelists were trained in two / hour sessions in which they were served patties from a wide variety of treatments to familiarize them with a wide range of odor. Freshly prepared controls were made on the day testing to be used as a reference odor. Three repetitions from each treatment were served to

each of the panelists during six separate sessions. Sensory scores were recorded utilizing a 10 point descriptive odor score. Descriptive terms used were absent, very slight, slight, strong and very strong. Numerical values were ranged from 10 (absent) to 0 (very strong).

Statistical analysis

The data were analyzed using a completely randomized factorial design (SAS, 1988) when a significant main effect was detected, the means were separated with the Student-Newman-Keuls Test. Differences between treatments of ($P \leq 0.05$) were considered significant.

RESULTS AND DISCUSSION

Table (1) shows the effect of solvent type on the antioxidant activity of extracts from fenugreek, soybean, canola seeds, ginger roots and BHA. The methanol and ethanol extracts of ginger roots exhibited high ($p \leq 0.05$) antioxidant activity than methanol and ethanol extracts of fenugreek, soybean, and canola seeds. The methanol extract of ginger roots had a higher ($p \leq 0.05$) antioxidant activity than BHA. While the ethanol extract of ginger root had a lower ($p \leq 0.05$) antioxidant than BHA. The ethanol extracts of fenugreek seeds and soybean seeds exhibited high ($p \leq 0.05$) antioxidant activity than that of methanol extract. However, methanol extracts of canola seeds and ginger roots had stronger ($p \leq 0.05$) antioxidant activity than that of ethanol extracts. These results are in agreement with those for mustard (*Brassica juncea*) reported by **Kaur and Kapoor (2002)**. However, these results differ from those in fenugreek seeds (71.4) and ginger rhizomes (77.4) reported by **Mansour and Khalil (2000)** and soybean seeds (66) reported by **Shih et al (2002)**. This difference may be due to the interspecies variation and or the methods of antioxidant determination.

The methanol extracts of canola seeds and ginger roots and the ethanol extracts of fenugreek seeds and soybean seeds were used for evaluating the stability of antioxidant under the different conditions and separating the phenolic compounds present in these extracts.

Table 1. Effect of solvent type on the antioxidant activity of extracts from fenugreek, soybean, canola seeds, ginger roots and BHA¹.

Tested material	² Antioxidant activity %	
	Methanol	Ethanol
Fenugreek seeds	27.10 ^b	37.52 ^a
Soybean seeds	20.75 ^b	52.04 ^a
Canola seeds	58.32 ^a	21.05 ^b
Ginger roots	94.20 ^a	65.11 ^b
BHA	84.50 ^a	84.50 ^a

¹ Butylated hydroxyanisole

² Means in the same row with different letters are significantly different ($p \leq 0.05$), LSD = 2.73

Heat stability

Data in **Table (2)** showed that the antioxidant activity was constant for all extracts when incubated at a temperature ranging from 40 to 60°C for 30 min. Similar results was reported by **Mansour and Khalil (2000)** for ginger roots extract. However, for fenugreek seeds extract, the antioxidant activity was stable when incubated at 40 – 80°C for 30 min.

The antioxidant activity of the extracts was decreased ($p \leq 0.05$) by increasing the temperature. Heating at 100°C for 30 min reduced ($p \leq 0.05$) the antioxidant activity of ginger roots, canola seed, soybean seed and fenugreek seed extracts by 17.6, 12.4, 14.7 and 9.0%, respectively. Higher values were reported by **Mansour and Khalil (2000)** for ginger roots (25%) and fenugreek seed (15%) extracts. There are a significantly ($p \leq 0.05$) differences among all extracts in the antioxidant activity. Ginger roots extract had the highest ($p \leq 0.05$) antioxidant activity followed by canola seeds extract, soybean seeds extract and fenugreek seeds extract.

Data in **Table (3)** showed that the antioxidant activity of all extracts was affected by boiling treatment. Increasing the boiling time resulted in a significant ($p \leq 0.05$) decrease in the antioxidant activity of extracts. These results agree well with those reported by **Mansour and Khalil (2000)** for freeze-dried extracts from ginger roots and fenugreek seeds. Boiling the extracts for 60 min caused a reduction ($p \leq 0.05$) in the antioxidant activity of

Table 2. Effect of temperature on the antioxidant activity of extracts from fenugreek, soybean, canola and ginger roots

Temperature (°C)	Antioxidant activity %				Mean ¹
	Fenugreek	Soybean	Canola	Ginger roots	
40	38.50	53.30	58.23	94.50	61.13 ^a
50	38.50	53.30	58.23	94.50	61.13 ^a
60	38.30	53.40	58.23	94.50	61.11 ^a
70	38.30	52.80	57.95	92.07	60.28 ^b
80	35.40	52.05	57.60	89.63	58.67 ^c
90	33.15	48.80	52.71	87.80	55.62 ^d
100	29.50	38.55	45.85	76.83	47.68 ^e
Mean ²	35.95 ^d	50.31 ^c	55.54 ^b	89.98 ^a	

¹ Means in the same column with different letters are significantly different ($p \leq 0.05$), LSD = 0.568

² Means in the same row with different letters are significantly different ($p \leq 0.05$), LSD = 0.429

Table 3. Effect of boiling for different time on the antioxidant activity of extracts from fenugreek, soybean, canola seeds and ginger roots

Boiling time (min)	Antioxidant activity %				Mean ¹
	Fenugreek	Soybean	Canola	Ginger roots	
0	38.49	53.30	58.23	94.50	61.13 ^a
30	27.47	44.23	41.77	74.69	47.04 ^b
60	22.53	34.28	30.23	59.15	36.55 ^c
90	16.51	23.31	20.38	43.90	26.03 ^d
120	10.36	13.24	14.74	27.44	16.45 ^e
Mean ²	23.07 ^d	33.67 ^b	33.07 ^c	59.94 ^a	

¹ Means in the same column with different letters are significantly different ($p \leq 0.05$), LSD = 0.629

² Means in the same row with different letters are significantly different ($p \leq 0.05$), LSD = 0.562

ginger roots, canola seed, soybean seed and fenugreek seed extracts by 62.59, 51.15, 64.32 and 58.53%, respectively. The corresponding values for boiling for 120 min were 29.04, 25.31, 24.84 and 26.92%, respectively. Ginger roots had a higher ($p \leq 0.05$) antioxidant activity than other antioxidant types. Mansour and Khalil (2000) reported that the antioxidant in fenugreek seed extract was fairly heat-stable with 58.0% activity after 120 min heating at 100°C. However, the remaining antioxidant activity in ginger roots extract was about 28% after 120 min. heating at 100°C.

pH stability

The antioxidant activity of extracts was affected ($p \leq 0.05$) by pH values Table (4). The antioxidant activity of extracts gradually increased ($p \leq 0.05$) till pH 7.0 followed by continuous decrease ($p \leq 0.05$) at alkaline pH. Lee *et. al* (1986) reported that the antioxidant activity of ginger roots extracts increased with pH between 5 and 7.

Table 4. Effect of pH on the antioxidant activity of extracts from fenugreek, soybean, canola seeds and ginger roots

pH	Antioxidant activity %				Mean ¹
	Fenugreek	Soybean	Canola	Ginger roots	
4	25.34	19.46	19.35	17.07	20.31 ^f
5	30.69	40.84	38.34	65.94	43.95 ^d
6	34.35	45.37	47.38	78.46	51.39 ^b
7	38.50	53.28	58.23	94.50	61.13 ^a
8	35.44	43.78	48.28	74.39	50.47 ^c
9	28.08	32.92	32.28	50.31	35.90 ^e
Mean ²	32.07 ^d	39.28 ^c	40.64 ^b	63.45 ^a	

¹ Means in the same column with different letters are significantly different ($p \leq 0.05$), LSD = 0.137

² Means in the same row with different letters are significantly different ($p \leq 0.05$), LSD = 0.303

The reduction of antioxidant activity at alkaline pH might be attributed to either the loss of antioxidant property of the extracts or the enhancement of lipid oxidation (Mansour and Khalil, 2000). The mean value of antioxidant activity of ginger roots extract (63.45%) was the highest ($p \leq 0.05$) compared with other extracts.

Storage stability

Antioxidant activity of the extracts did not affect ($p \leq 0.05$) by storage in the dark at -5°C , -25°C and -37°C for 24 days Table (5). Similar results were reported by Mansour and Khalil (2000) who found that the freeze-dried extracts from ginger roots and fenugreek seeds stored in the dark at -5°C , -25°C and -37°C over a 21 day period did not show any change in the antioxidant activity.

Extracts stored in light at -25°C showed a significant ($p \leq 0.05$) reduction in the antioxidant activity after 24 days of storage Table (6). The mean value of reduction was 21.20% after 24 days of storage. This reduction is attributed to the light effect (Mansour and Khalil, 2000). There was a significant ($p \leq 0.05$) difference in the antioxidant activity among extract types. Ginger roots extract had the lowest ($p \leq 0.05$) reduction (15.24%) in the antioxidant activity followed by canola seeds extract (21.28%), fenugreek seeds extract (24.45%) and soybean seeds extract (29.30%).

Separation and identification of phenolic compounds

Data in Table (7) showed that methanolic extract of canola seeds contained the highest total phenolic compounds followed by methanolic extract of ginger roots, ethanolic extract of fenugreek seeds and ethanolic extract of soybean seeds. Pyrogallol was the highest phenolic compounds in fenugreek seeds and ginger roots extracts. However, hydroquinone and salicylic were the highest phenolic compounds in soybean seeds and canola seeds extracts, respectively. On the other hand, protocatechuic, rutin, p-OH-benzoic and cinnamic were the lowest phenolic compounds in fenugreek seeds, soybean seeds, canola seeds and ginger roots extracts, respectively. Dawoud *et al* (2003) found that ethyl acetate extract of fenugreek seeds had a higher chlorogenic acid as compared with other phenolic compounds.

TBA values

Data in Table (8) showed that TBA values of raw beef patties were affected ($p \leq 0.05$) by storage period, whereas TBA values of all antioxidants increased ($p \leq 0.05$) as storage period progressed. Similar results were reported by Mansour and Khalil (2000) who found that TBA values of raw patties containing freeze-dried extracts from ginger roots and fenugreek seeds stored at 5°C for 12 days increased ($p \leq 0.05$) as storage period increased.

Table 5. Effect of different storage conditions in dark for 24 days on the antioxidant activity of extracts from fenugreek, soybean, canola seeds and ginger roots

Storage period (days)	Antioxidant activity %												Mean ¹
	~ 5°C				~ 25°C				~ 37°C				
	Fenugreek	Soybean	Canola	Ginger roots	Fenugreek	Soybean	Canola	Ginger roots	Fenugreek	Soybean	Canola	Ginger roots	
0	38.61	53.28	58.23	94.40	38.61	53.28	58.23	94.40	38.61	53.28	58.23	94.40	61.13 ^a
3	38.61	53.17	58.23	93.90	38.61	53.17	57.66	94.50	38.61	53.39	57.67	93.90	60.95 ^a
6	38.61	53.17	58.23	93.30	38.61	52.94	58.32	94.50	38.61	53.39	58.23	93.90	60.98 ^a
9	38.61	53.17	58.23	94.50	38.61	52.94	58.32	94.50	38.61	53.39	58.23	93.90	61.08 ^a
12	38.61	52.94	58.23	94.50	38.61	52.70	58.32	93.90	38.61	52.94	58.23	93.90	60.96 ^a
15	38.61	52.94	58.23	93.90	38.61	52.94	58.04	93.29	38.61	52.82	58.05	93.90	60.83 ^a
18	38.61	52.94	58.23	93.90	38.61	52.70	58.04	93.29	37.12	52.70	57.66	93.80	60.63 ^a
21	38.61	52.94	58.04	93.90	38.07	52.40	57.66	92.07	37.12	52.15	57.68	92.68	60.28 ^a
24	37.89	51.80	56.78	90.85	37.88	49.09	55.88	87.80	37.88	48.31	55.15	82.93	57.69 ^a
Mean ²	60.79 ^a				60.48 ^a				60.24 ^a				

¹ Means in the same column with different letters are significantly different ($p \leq 0.05$), LSD = 18.90

² Means in the same row with different letters are significantly different ($p \leq 0.05$), LSD = 10.91

Antioxidant properties of some solvent extracts

Table 6. Effect of storage in light for 24 days at ~25°C on the antioxidant activity of extracts from fenugreek, soybean, canola seeds and ginger roots

Storage period (days)	Antioxidant activity %				Mean ¹
	Fenugreek	Soybean	Canola	Ginger roots	
0	38.61	53.28	58.23	94.40	61.13 ^a
3	37.15	52.72	57.41	92.68	59.99 ^a
6	34.59	48.31	56.51	91.46	57.72 ^b
9	33.29	47.63	55.61	90.55	56.77 ^b
12	32.89	46.04	53.89	89.02	55.46 ^c
15	32.70	45.82	53.76	88.41	55.17 ^c
18	32.64	45.25	53.44	87.94	54.82 ^c
21	32.31	45.02	53.39	87.80	54.63 ^c
24	29.17	37.67	45.84	80.01	48.17 ^d
Mean²	33.71^d	46.86^c	54.23^b	89.14^a	

¹ Means in the same column with different letters are significantly different ($p \leq 0.05$), LSD = 1.219

² Means in the same row with different letters are significantly different ($p \leq 0.05$), LSD = 0.813

Table 7. Phenolic compounds (mg / 100 g) in extracts from fenugreek seeds, soybean, canola and ginger roots

Phenolic compound	Fenugreek	Soybean	Canola	Ginger roots
Pyrogallol	0.2343165	0.1700674	0.6360734	2.1231183
Hydroquinone	0.0987981	0.1796497	0.1622296	1.1564817
Gallic	-	-	0.0250355	0.0198402
Resorcinol	-	-	0.151809	0.7316782
Protocatechuic	0.0012083	-	0.1025375	0.0105
P-OH-benzoic	-	-	0.0054968	0.0049481
Chlorogenic	-	-	0.0538996	0.0151857
Catechin	-	-	0.1330461	0.0697035
Phenol	-	-	0.9953723	0.8651081
Vanillic	0.009671	-	0.994555	0.0191962
P-coumaric	0.0205216	-	1.0410403	0.0188348
Ferulic	-	-	0.7165412	0.0169454
Salicylic	-	-	4.9075138	0.1049015
Rutin	-	0.0004236	0.3010387	0.0171373
O-coumaric	-	-	0.3004063	0.0129756
Coumarin	-	-	0.2432572	0.0073791
Myristin	-	-	0.0683716	0.0018461
Cinnamic	-	-	0.1610154	0.0029384
Quercetin	-	-	0.0453057	0.0067429
Kaempferol	-	0.0008975	-	0.0062646
Total phenolic	0.3645155	0.3510382	11.044545	5.2117257

Table 8. TBA values of beef patties as affected by antioxidant types and cold storage at ~5°C for 12 days

Storage period (days)	Control	TBA (mg malonaldehyde / kg)					Mean ¹
		Antioxidant types					
		Fenugreek	Soybean	Canola	Ginger roots	BHA	
0	0.858	0.827	0.793	0.837	0.835	0.816	0.83 ^d
3	1.115	0.956	0.922	0.909	0.915	1.003	0.97 ^c
6	1.392	1.087	1.171	1.038	1.082	1.162	1.16 ^b
9	1.540	1.188	1.256	1.144	1.175	1.263	1.26 ^{bc}
12	1.583	1.286	1.407	1.227	1.349	1.390	1.37 ^a
Mean ²	1.30 ^a	1.07 ^b	1.11 ^b	1.03 ^b	1.07 ^b	1.13 ^b	

¹ Means in the same column with different letters are significantly different ($p \leq 0.05$), LSD = 0.120

² Means in the same row with different letters are significantly different ($p \leq 0.05$), LSD = 0.120

On the other hand, there were a significant ($p \leq 0.05$) differences in TBA values between the control and antioxidant types. TBA values of raw patties containing antioxidants from fenugreek seeds, soybean seeds, canola seeds, ginger roots and BHA were lower ($p \leq 0.05$) than that of the control. Also, there were no significant ($p \leq 0.05$) difference in TBA values among beef patties treated with antioxidants. Similar results were reported by **Shih and Dajgle (2003)** they found that addition of the ethanolic extracts of milled-rice co-products to ground beef inhibited lipid oxidation effectively and thus prolonged storage stability of the product.

Data in **Table (9)** indicated that TBA values of beef patties containing different levels of antioxidants were lower ($p \leq 0.05$) than the control. Also, there were no significant ($p \leq 0.05$) differences in TBA values among all levels. These results agree with those reported by **Mansour and Khalil (2000)** who showed that there were no significant difference in TBA values between 500 and 1000 ppm for all freeze-dried extracts from ginger roots and fenugreek seeds.

Odor

Data in **Table (10)** indicated that the rancid odor increased ($p \leq 0.05$) as the storage period progressed. At the end of storage (12 days), pat-

ties treated with antioxidants had scores in the range of very slight rancid odor, while control had score in the range of strong rancid odor. Data indicated that the addition of antioxidants to beef patties reduced ($p \leq 0.05$) the rancid odor compared to the control. These results are in agreement with those obtained by **Mansour and Khalil (2000)** who reported that addition of freeze-dried extracts from fenugreek seeds and ginger roots to beef patties reduced the rancid odor scores as compared with the control.

On the other hand, beef patties treated with antioxidants from fenugreek seeds were effective in ($p \leq 0.05$) reducing the rancid odor compared to BHA. There were no significant ($p \leq 0.05$) difference in the odor among soybean seeds, canola seeds, ginger roots and BHA.

Patties treated with different level of antioxidants had lower ($p \leq 0.05$) rancid odor than the control **Table (11)**. Also, there were no significant ($p \leq 0.05$) difference in the odor scores among patties treated with different levels of antioxidant. These results indicated that addition of different level of extracts from fenugreek seeds, soybean seeds, canola seeds, ginger roots to beef patties were effective in reducing the oxidative deterioration of fat in beef patties throughout the cold storage. Similar results were reported by **Mansour and Khalil (2000)**.

Table 9. TBA values of beef patties as affected by antioxidant levels and cold storage at -5°C for 12 days

Storage period (days)	TBA (mg malonaldehyde / kg)				Mean ¹
	Antioxidant levels (%)				
	Control	0.01	0.05	0.10	
0	0.858	0.824	0.825	0.817	0.83 ^e
3	1.115	0.958	0.938	0.927	0.98 ^d
6	1.392	1.122	1.106	1.096	1.18 ^c
9	1.540	1.212	1.209	1.195	1.29 ^b
12	1.583	1.337	1.343	1.316	1.39 ^a
Mean ²	1.30 ^a	1.09 ^b	1.08 ^b	1.07 ^b	

¹ Means in the same column with different letters are significantly different ($p \leq 0.05$), LSD = 0.037

² Means in the same row with different letters are significantly different ($p \leq 0.05$), LSD = 0.031

Table 10. Odor of beef patties as affected by antioxidant types and cold storage at -5°C for 12 days

Storage period (days)	Control	Antioxidant types					Mean ¹
		Fenugreek	Soybean	Canola	Ginger roots	BHA	
0	9.5	9.7	9.4	9.6	9.8	9.2	9.50 ^a
3	6.4	8.0	7.3	8.0	7.8	7.8	7.55 ^b
6	5.0	6.9	7.0	6.8	7.0	6.3	6.50 ^c
9	4.8	7.6	7.1	6.6	7.0	7.1	6.70 ^c
12	2.9	6.8	6.7	6.9	6.9	6.5	6.12 ^d
Mean ²	4.77 ^c	7.80 ^a	7.50 ^{ab}	7.58 ^{ab}	7.70 ^{ab}	7.38 ^b	

¹ Means in the same column with different letters are significantly different ($p \leq 0.05$), LSD = 0.320

² Means in the same row with different letters are significantly different ($p \leq 0.05$), LSD = 0.320

Table 11. Odor of beef patties as affected by antioxidant levels and cold storage at -5°C for 12 days

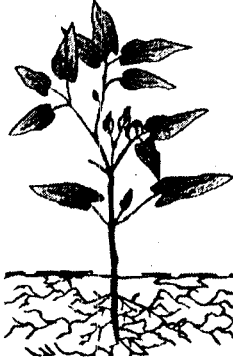
Storage period (days)	Antioxidant levels (%)				Mean ¹
	Control	0.01	0.05	0.10	
0	9.5	9.3	9.5	9.4	9.43 ^a
3	6.4	7.8	7.8	7.6	7.40 ^b
6	5.0	6.6	6.9	7.0	6.40 ^c
9	4.8	7.1	7.3	7.2	6.60 ^c
12	2.9	6.9	6.7	6.7	5.80 ^d
Mean ²	4.77 ^b	7.54 ^a	7.64 ^a	7.58 ^a	

¹ Means in the same column with different letters are significantly different ($p \leq 0.05$), LSD = 0.330

² Means in the same row with different letters are significantly different ($p \leq 0.05$), LSD = 0.270

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خصائص مضادات الأكسدة للمذيبات المستخلصة من بعض المصادر النباتية

[٣٠]

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- أظهر الميثانول المستخلص من بذور الكانولا وجذور الزنجبيل نشاطا أعلى كمضاد للأكسدة من الإيثانول المستخلص من هذه البذور .

- نشاط مضادات الأكسدة في كل من مستخلصات الحلبة ، فول الصويا ، بذور الكانولا وجذور الزنجبيل بالكاد ثابتا حراريا ، وتراوح قيمه بين ٥١,١٥ - ٦٤,٣٢% بعد ١٠٠ دقيقة من التسخين عند ١٠٠° م .

- وجد أن أعلى نشاط لمضادات الأكسدة في كل المستخلصات عند ٧,٠ pH .

- وعند تخزين هذه المستخلصات في الظلام عند درجات حرارة ٢٥° م ، ٣٧° م لمدة ٢٤ يوم ، لم يحدث أي تغيير في نشاط مضادات الأكسدة .

- عند تخزين المستخلصات في الضوء عند درجة حرارة ٢٥° م حدث انخفاض معنوي في نشاط مضادات الأكسدة بعد ثلاثة أيام من التخزين .

- البيروجاليك هو الأعلى معنويا لمركبات الفينول في كل من مستخلصات الحلبة وجذور الزنجبيل .

- الهيدروكينون ومركب السالسليك كانوا الأعلى كمركب فينولي في مستخلصات فول الصويا وبذور الكانولا على التوالي .

- بإضافة مستخلصات مضادات الأكسدة لقطائر الليف أدى إلى حدوث انخفاض في قيم TBA ورائحة التزنخ أثناء التخزين على البارد .

الكلمات الدالة: الحلبة ، فول الصويا ، بذور الكانولا، جذور الزنجبيل ، خصائص مضادات الأكسدة وأسلوب ديان المترافق

الموجز

التجربة

أجريت هذه الدراسة لتقييم نشاط مضادات الأكسدة المستخلصة من كل من بذور الحلبة ، فول الصويا ، بذور الكانولا وجذور الزنجبيل . واستخدم خواص مضادات الأكسدة ٩٠% إيثانول و ٩٠% ميثانول كمذيبات . ويقدر تأثير هذه المستخلصات بطريقة مترافق ديان ، وقد تم تقدير تأثير هذه المستخلصات على كل من قيم TBA ورائحة التزنخ بأقرص الليف خلال التخزين على تبريد بأقل من ٥° م لمدة ١٢ يوم .

النتائج المتحصل عليها

- بذور الزنجبيل لها نشاط أعلى كمضاد للأكسدة من مستخلصات كل من الحلبة ، فول الصويا ، بذور الكانولا بالإضافة إلى مركب BHA .

- أظهر الإيثانول المستخلص من الحلبة وبذور فول الصويا نشاطا أعلى كمضاد للأكسدة من الميثانول المستخلص من هذه البذور .