Effect of Addation Anise (*Pimpinlla Anisum L.*) and Caraway (*Carum Carvi, L.*) on Retard the Rancidity of El-Mewled El-Nabawy Sweets with Sweetener

Tolba, K. H.; Ginat, El-sherif and M. A. El-sherif

Food Technology Res. Institute, Agric. Res. Center, Giza, Egypt
ginat.elsherif@yahoo.com

Abstract: The natural antioxidants of anise (*Pimpinlla anisum L.*) and caraway (*Carum carvi, L.*) were extracted, determined and added to refined sunflower oil at different concentrations to evaluate their effect on the oxidation activity of the oil by Rancimat assay and determination of the peroxide value. Phenols and flavonoids compounds were identified and quantified using HPLC method. The obtained results indicated that total polyphenols were 64.63 mg/100g (as galic acid), total carotenoids were 23.33mg/100g and total tannins were 83.31mg/100g (as tannic acid) in anise ethanolic extract, while, in caraway ethanolic extract the same compounds value were 77.86, 103.42 and 40 mg/100g, respectively. HPLC-analysis of ethanolic extracts of anise and caraway showed presence of a large number of phenols and flavonoids compounds (14 compounds). The possibility of their application as powders and extracts by different concentrations as natural antioxidant in El-mewled El-nabawy sweets (sesames and folia) with sweetener and fortified by full cream milk powder was evaluated for retarding the rancidity of fat/oil in the sweets to recode a large storage time.

[Sahar Ahmed Shafik and Afaf Salah Abd El-Mohsen. **Occupational health: Health Promotion Program to Improve Health Workers in Tourah Cement Factory]** Journal of American Science 2012; 8(4):310-318].(ISSN: 1545-1003). http://www.jofamericanscience.org. 42

Key words: Anise – caraway – natural antioxidants – antioxidant activity – herbs – El-mewled El- nabawy sweets – sweetner.

1. Introduction

Many antioxidant compounds, occurring naturally in plant sources have been identified as free radical or active oxygen scavengers (**Duh, 1998**). It's found that interest has considerably increased in finding naturally occurring antioxidant for use in foods or medicinal materials to re-place synthetic antioxidants, which are being restricted due to their side effects such as carcinogenicity (**Zheng and Wang, 2001**). Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods (Lai *et al*., 2001).

Significantly numbers of natural antioxidants have been identified. Among them, rosemary and vitamin E have commercial significance, B-carotene and many spices, herbs and cereal extracts have been found to be promising natural antioxidants (Patro *et al*., 2005). Herbs have been used for a large range of purposes including medicine, nutrition, flavoring, beverages, dyeing, repellents, fragrances, cosmetics, charms, smoking and industrial uses. Since prehistoric times, herbs were the basis for nearly all medicinal therapy until synthetic drugs were developed in the nineteenth century. Today, herbs are still found in 40 % of prescription drugs (**Smith and Winder, 1996**). Culinary herbs have been grown and used for hundreds of years, and they have flavors of a wide variety of foods (**Zheng and Wang, 2001**).

Caraway (*Carum carvi, L.*) is one of the commonly used spices for food preparations. Similarly to other spices, its primary active constituent represents volatile oil (4-6 % on average), which itself consists from carvone and limonene. Caraway aldehyde was found to be the main component of oil (53.6 %) (**El-Sawi and Mohamed, 2002**). Caraway seed oils may retain more natural beneficial components of the seeds, including natural antioxidants, and are free of chemical contamination (**Liangli *et al*., 2005**). Caraway seed have a characteristic agreeable odor, and aromatic, pleasant, warm, sharp test. Caraway seed is used in many of baked goods-breads, roast goose, sea foods and cabbage, potato soups (**Hannan Al-Sayed, 2008**).

Anise (*Pimpinlla anisum L.*) umbelliferae is an annual herb and a grassy plant with white flowers and small green to yellow seeds, which grows in Turkey, Indian, Egypt and many other warm regions of the world (**Pourgholami, *et. al*., 1999**). *P. anisum* is primarily grown for its fruits, commercially called "seeds" that are currently used for flavoring. The essential oil from *P. anisum* fruits is also valuable in perfumery and in medicine (**Santos *et al*., 1998 and Gülçin *et al.*, 2003).**

The objective of this work was to:-

(1) Evaluate the effect of two aromatic herbs (Caraway and Anise) as antioxidant compared with synthetics antioxidant.
(2) Develop chromatographic procedures to identify and quantify phenolic antioxidants in selected herbs by high-performance liquid chromatography (HPLC).
(3) Study the possibility of their application at the best selected concentration as natural antioxidant in El-mewled El-nabawy sweets with sweetener and fortified by full cream milk powder (sesames and folia).

2. Materials and Methods

Materials:

1- Caraway (*Carum carvi, L.*), anise (*Pimpinlla anisum L.*), sesame (*Sesamus indicum*), peanut (*Arachis hypogeae, L.*), full cream milk powder (NIDO) and sucrose were purchased from a local market at Cairo, Egypt.

2- All chemicals reagents were purchased from El-Gomhogoria Co. at Cairo, Egypt**.**

3- Sugar alternative sweetener (SWIST) manufactured by: MEPACO Medi Food, Egypt. M. O. H. Reg. No.: 4795/2009.

Methods:

1- Preparation of extracts and its yield of tested spices:

Twenty five grams of sample fine powder was mixed with 500 ml ethanol and the extracted was filtered and the residue was re-extracted until extraction solvents became colorless. The obtained extracts were filtered over Whatman No.1 paper and the filtrate was collected, and then ethanol was removed by a rotary evaporator at 50°C to obtain dry extract. The extracts were placed in a plastic bottles, and then stored at refrigerator until used (Gülçin ***et al.*, 2003).**

2-Dtermination of total phenolic compounds

Total soluble phenolics in caraway and anise extracts were determined with Folin-Ciocalteu reagent using gallic acid as standard phenolic compound and using an equation obtained from the standard gallic acid graph: Absorbance=0.0028 x gallic acid (Mg) as the method described by **(Slinkard and Singleton, 1977)**.

3-Determination of Total Carotenoids

The total carotenoids were determined according to **Heinonen and Marina (1989)**.

4- Determination of tannins (as tannic acid)

Total tannins were determined calorimetrically as described by **A.O.A.C (2000)**.

5- Separation of polyphenols and flavonoids by HPLC

High performance liquid chromatography (HPLC) technique was applied using HPLC Agilent 1100 Series equipped with Quaternery pump, set at flow 1 ml/ min. Auto sampler, degasser, column compartment set at 35° C and variable wavelength detector set at 330 for flavonoid compounds and 280 for phenolic compounds, column: Hypersil ODS 5 um, 250 x 4 mm was used. Pure phenolic compounds: p. coumaric, pyrogallol, catechein, salycilic, coumarin, protocatchoi, chlorogenic, vanillic, cinnamic, syrinic, caffeic, chrisin, ferullic, caffeine, p. OH. Penzoic and naringnin and pure flavonoid compounds: kampferol, Apegnin, luteolin, Quecettin and Hypersoid were used as standard obtained from El-Gomhoria-Chemical Company, Egypt.

6- Antioxidant efficiency determination

a.Determination of antioxygenic activity using the peroxide value:-

Samples (100g) of refined sunflower oil, both with and without 0.25g, each of caraway and anise as well as 200 ppm of Rutin and 200 ppm of butylated hydroxyl anisole (BHA). All treatments were placed in an oven at 60° C for 3 hours daily. The experiment was repeated for 7 days (**Tolba and Azouz, 2006**). Peroxide value was determined for each sample according to the **A.O.A.C. (2000)** method. Antioxygenic activity was calculated as the ratio between the peroxide value of control and the peroxide value of sample (**Gerard and Roberts, 2004**).

$$\% \text{ anti oxygenic activity} = \frac{\text{peroxide value of control}}{\text{peroxide value of sample}}$$

b.Oxidation systems by Rancimate

Different concentrations of caraway and anise ethanolic extract (1.5, 3 and 4.5 ml/ 25 ml refined sunflower oil), Rutin and BHA (200 ppm) were individually added to refined sunflower oil (25g) to study their antioxidant efficiency. The designation of induction period by Rancimate instrument was taken as a tool to compare the effectiveness of spices extract fractions on refined sunflower oil stability according to the method of **Mendez *et al.* (1996)**. 679 Rancimate (Metrobm Ltd. CH.9100 Herisau, Switzerland) was used for the determination of the oxidative stability of refined sunflower oil mixed with spices extract. The 679 Rancimate comprises of control unit and wet section containing 6 reactions vessels.

The induction period was used as a mean for measuring the antioxidant activity of the various spices extracts added individually to refined sunflower oil and compared with the antioxidants used. The control sample was refined sunflower oil without any antioxidants.

Preparation of El-mewled El-nabawy sweets (sesames and folia):-

1. Roasting of sesames and peanut:

Sesames and peanut were roasted at 160 ^{0}C for 20 – 30 min. (**Refaat, 1988**) and the peanut hulls were removed.

2. Processing procedures of oriental sweets:

Sugar and water were heated with stirring until light caramel is obtained and the consistency of the syrup became thick, then, other ingredients were added and thoroughly mixed with the hot syrup. The mass was left to cool partially, then re-stirred and poured on a marble surface (recoated lightly with oil) and left for cooling to ambient temperature. The mass was extended with a rolling pin to 1.5 cm. thickness and cut with a knife into bars which were packed in tightly closed cellophane pouches and were stored. The used formula is given in Table (1).

Table (1) Formula of El-mewled El-nabawy sweets (sesames and folia) samples

Ingredients	Weight (gm)
Sesame or peanut	800
Dry milk powder	200
Sugar alternative sweetener(SWIST)	5
Water	100 ml
Arabic gum	30
Antioxidant addition:- *As extracts *As powder of caraway or anise	 5 and 10 ml 5 and 10 gm/500g

3. Results and Discussion

1. Determination of total phenolic compounds:-

Because phenolic compounds may contribute to overall antioxidant activities (**Liangli *et al.*, 2005**), it was determined in caraway and anise and the obtained results are shown in Table (2). The results indicated that, ethanolic extract of caraway contained the greatest contents of phenolic compounds, carotenoids and tannins (77.86 mg as gallic acid, 103.42 and 40 mg as tannic acid, respectively) than ethanolic extract of anise, which contained 64.63 mg of phenolic compounds as gallic acid and 83.31 mg/100g of carotenoids as well as 23.33 mg of tannins as tannic acid. These results are in agreement with those reported by **Gülçin *et al.* (2003) and Padmashree *et al.* (2007).**

Table (2): Total phenols, carotenoids and tannins in ethanolic extracts of caraway and anise

Components	Caraway	Anise
Total phenols (as mg gallic acid)	77.86	64.63
Carotenoids (mg/100g)	103.42	23.33
Tannins (mg as tannic acid)	40.00	83.31

2. Identification of poly phenols and flavonoids by HPLC:

Since phenols and flavonoids possess similar spectroscopic and chromatographic properties, HPLC is an invaluable means of separating and analyzing them. Tentative identification of these antioxidants can be deduced from their chromatographic behavior, and corroborative data may be provided by an analysis of their absorption spectra. HPLC analysis of ethanolic extracts of caraway and anise showed a large number of phenolic compounds and flavonoids which were present in significant amounts (Table 3 and Fig. 1). As seen the response intensity as well as the total area of phenolic compounds under chromatogram for caraway extract was higher than total area of flavonoid compounds for the same extract. On the other side, the total area of phenolic compounds under chromatogram for anise extract was lower than the total area of flavonoid compounds for the same extract. On the other hand, some components such as protocatchoi, caffeic, vanillic, caffeine and ferullic were presented in caraway extract but it were absent in anise extract. As seen in the same table, catechein, p. coumaric, salycilic, cinnamic and kampferol were absent in caraway extract but it were found in anise extract.

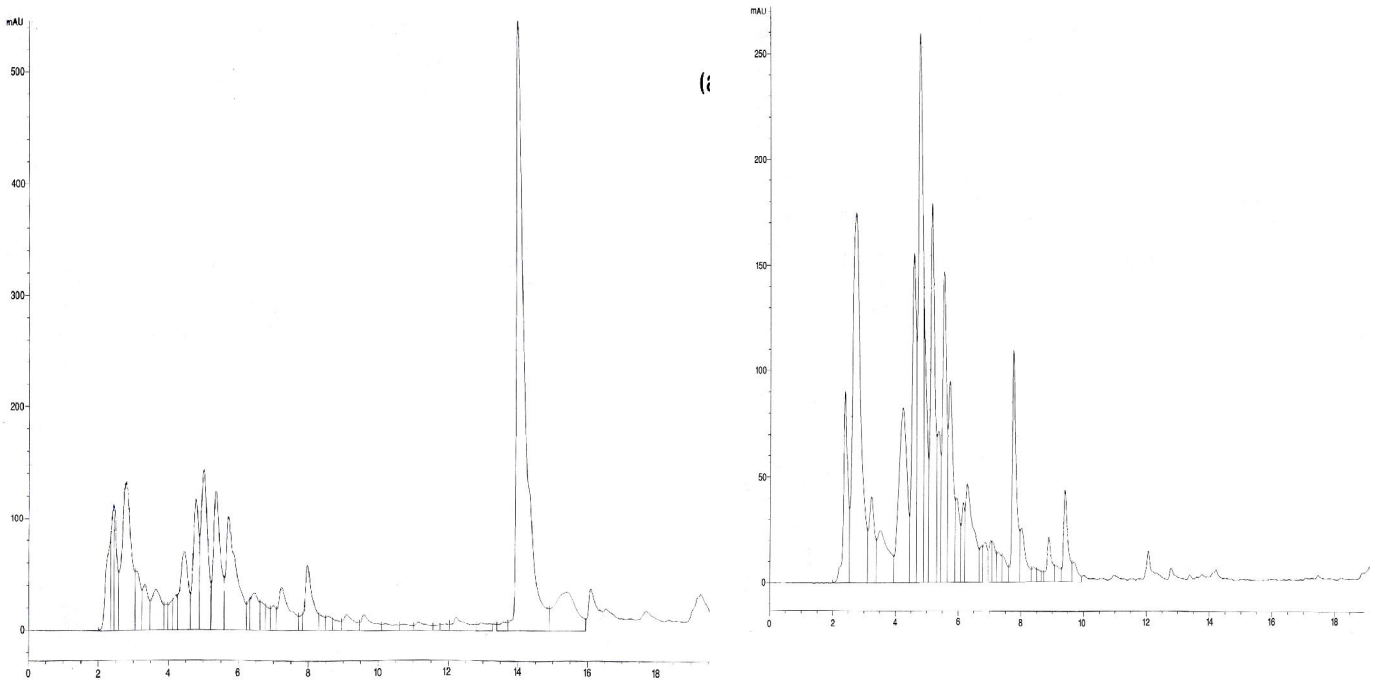
Table (3): HPLC-analysis of polyphenolic compounds in ethanolic extracts of caraway and anise

Compound	Caraway			Anise		
	R_t	Area (%)	ppm	R_t	Area (%)	ppm
Protocatchoi	2.454	1.8860	291.784	-	-	-
Catechein	-	-	-	2.467	1.2923	3.310
Pyrogallo	2.441	0.0712	26.475	2.494	1.4354	6.391
Chlorogenic	2.782	8.0452	216.256	2.785	4.0396	2.875
Caffeic	3.316	1.5781	15.399	-	-	-
Vanillic	3.625	2.2867	4.101	-	-	-
Syrinic	3.929	0.5054	5.715	3.933	1.0083	0.137
Caffeine	4.438	3.5573	59.663	-	-	-
p. coumaric	-	-	-	6.090	0.6257	0.060
ferullic	6.317	0.4919	3.128	-	-	-
salycilic	-	-	-	6.541	2.3829	1.704
coumarin	6.763	0.5376	44.036	6.679	1.3413	0.132
cinnamic	-	-	-	8.447	0.2376	0.017
chrisin	11.750	0.1663	1.767	11.708	0.2359	1.285
Hypersoid	4.893	4.0221	129.337	4.936	3.9599	47.103
Quecettin	7.417	0.6014	23.284	7.384	1.2037	17.239
Luteolin	7.744	4.7237	21.990	7.756	1.8574	159.300
Kampferol	-	-	-	8.757	0.5232	7.177

Apegnin	8.879	1.0999	62.522	8.860	0.3272	6.880
Total area of phenolic compounds	$3.07381x10^4$			7360.32203		
Total area of flavonoid compounds	$2.06451x10^4$			7681.33273		

Antioxygenic activity of caraway and anise powders:

Test systems that evaluate the ability to inhibit lipid or other sensitive component oxidation commonly involve deterioration tests in which oxidation is accelerated sometimes as a result of the action of light or UV radiation, but more frequently by elevated temperatures. For instance, in monitoring antioxidant activity in a food, potential measurements include peroxide value (**Antolovich *et al.*, 2002**).



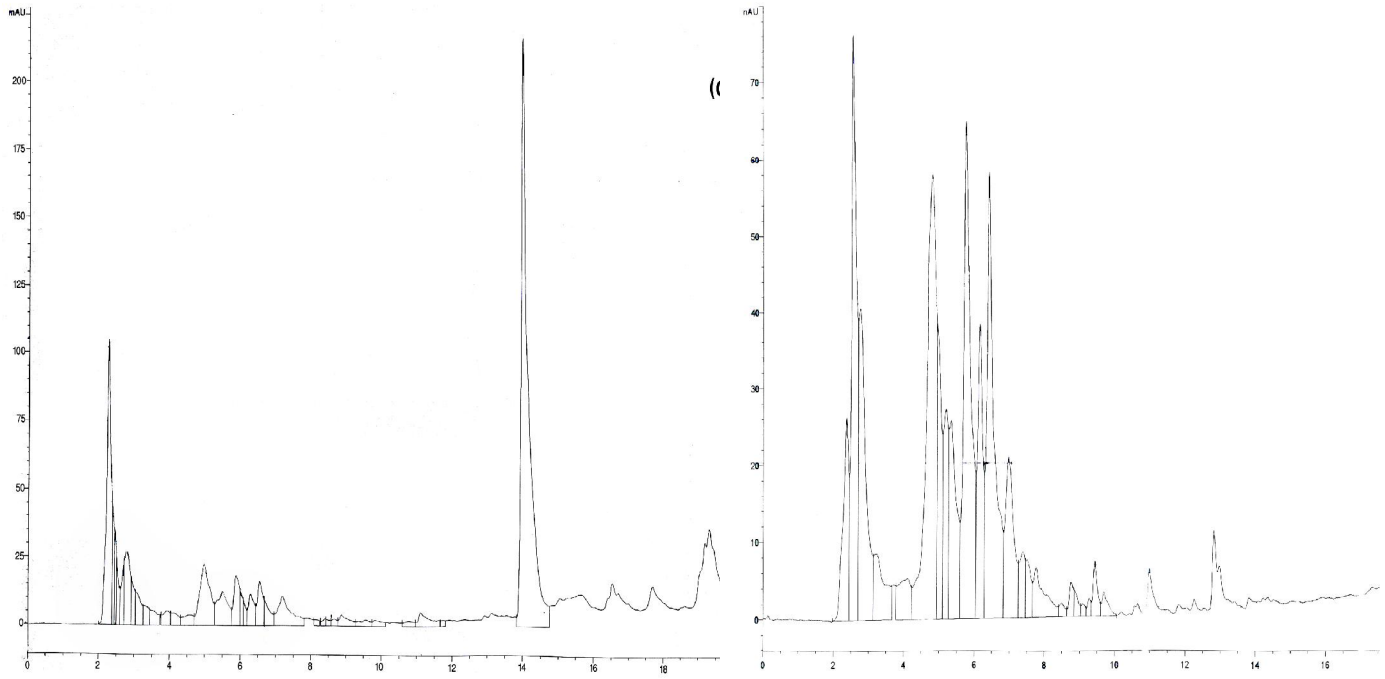


Fig. (1): Polyphenols and flavonoids contents of ethanolic extracts of caraway and anise

(a) Phenol compounds of caraway, (b) Flavonoids compounds of caraway, (c Phenol compounds of anise and (d) Flavonoids compounds of anise.

The antioxygenic activity of caraway and anise powders as well as Rutin on peroxide formation in refined sunflower oil in comparison to butylated hydroxyl anisole (BHA) were tabulated in Table (4). As seen, the control gave higher peroxide values than did samples containing caraway and anise powders. It was found that, caraway spice gave strong effect as antioxidants than anise spice in refined sunflower oil. Both caraway and anise powders exhibited strong antioxygenic activity. Earlier, **Padmashree, *et. al*., (2007)** also found the antioxygenic activity of stare-anise and black caraway powders when added to sunflower oil.

Table (4) Peroxide value and antioxygenic activities of caraway and anise powders, Rutin and BHA in refined sunflower oil

Stored period (days)	Peroxide value of control	Caraway powder		Anise powder		Rutin		BHA	
		Peroxide value	Antioxygenic activity	Peroxide value	Antioxygenic activity	Peroxide value	Antioxygenic activity	Peroxide value	Antioxygenic activity
0	0.66	0.66		0.66		0.66		0.66	
1	5.25	1.07	4.91	1.31	4.01	1.03	5.10	1.02	5.15
2	5.95	2.47	2.41	4.84	1.23	2.89	2.06	2.34	2.54
3	8.08	4.71	1.72	6.07	1.33	4.73	1.71	5.78	1.40
4	10.08	6.05	1.66	8.53	1.18	7.91	1.27	6.52	1.55
5	15.39	10.03	1.53	12.75	1.21	8.91	1.79	7.85	1.96
6	27.42	10.53	2.60	16.60	1.65	17.75	1.54	9.46	2.90
7	29.43	23.28	1.26	21.93	1.34	25.81	1.14	19.99	1.47

*Antioxygenic activity values > 1 indicate Antioxygenic activity and <1 indicate pro-oxygenic activity.
*Antioxygenic activity = peroxide value of control / peroxide value of sample

Measuring the effect of polyphenols on the stability of refined sun flower oil by Rancimate method:

The ethanolic extracts from caraway and anise powders and Rutin as well as BHA were added to refined sunflower oil to study the stability of refined sunflower oil by Rancimate method. Table (5) shows the addition levels of concentrate ethanolic extracts, Rutin and BHA as well as it shows the induction

periods by hours. As seen, the induction period for sunflower oil (control) was 9.43 hrs. On the other side, the synthetic antioxidant (BHA) increased the induction period to 10.20 hrs in the same oil. While, the levels of herbs ethanolic extracts gave lower induction period compared with synthetic antioxidant, but high levels gave data nearly from control sample. On the other hand, the highest levels from herbs extracts gave high slight protective factor. However, caraway extract was more effective than anise extract. Although, synthetic antioxidant and natural antioxidant showed approximately similar results, the consumers prefer natural products, than synthetic additives. These data are in the line with **Suhaj, 2006.**

Table (5): Effect of adding different concentrations of caraway and anise extracts, Rutin (200 ppm) and BHA (200 ppm) on the oxidative stability of refined sunflower oil.

Items	Oxidative stability (Induction periods = hrs)	Protective factor
Refined sunflower oil (control)	9.43	1.00
Refined sunflower oil +200 ppm BHA	10.2	1.02
Refined sunflower oil +200 ppm Rutin	9.63	1.08
Refined sunflower oil (25 ml) + 1.5 ml anise extract	9.00	0.95
Refined sunflower oil (25 ml) + 3 ml anise extract	9.58	1.02
Refined sunflower oil (25 ml) +4.5 ml anise extract	9.62	1.02
Refined sunflower oil (25 ml) +1.5 ml caraway extract	9.03	0.96
Refined sunflower oil (25 ml) +3 ml caraway extract	9.62	1.02
Refined sunflower oil (25 ml) +4.5 ml caraway extract	9.70	1.03

*protective factor= Induction period of sample/ Induction period of control

Effect of addition of anise and caraway as powder or extracts on oxidative stability of El-mewled El-nabawy sweets (sesames and folia):-

The primary products of lipid/ oil peroxidation are hydroperoxidations (**Hannan Al-sayed, 2008**). Therefore, determining the concentration of peroxides is one clear index of lipid/ oil peroxidation. Changes occurring in peroxide values in extracted lipid/ oil from sweets containing the chosen herbs (as powder or polyphenol extracts) during storage are shown in figures (2 and3). The peroxide values of all sweets which contained natural antioxidants were lower than that of control. The samples which contained antioxidant extracts gave better effect than that contained powders of herbs. Sesames sweets showed lower peroxide values compared with folia sweets. That may be due to sesame hulls which contain phenolic compounds and sesame oil contain natural antioxidants (sesamol and sesamoline) more than in peanut oil (**Awatif *et al*., 2004**). These results are in accordance with **Raddy, *et.,al*. (2005)** who mentioned that peroxide value of biscuits containing plant extract increased with increasing storage time.

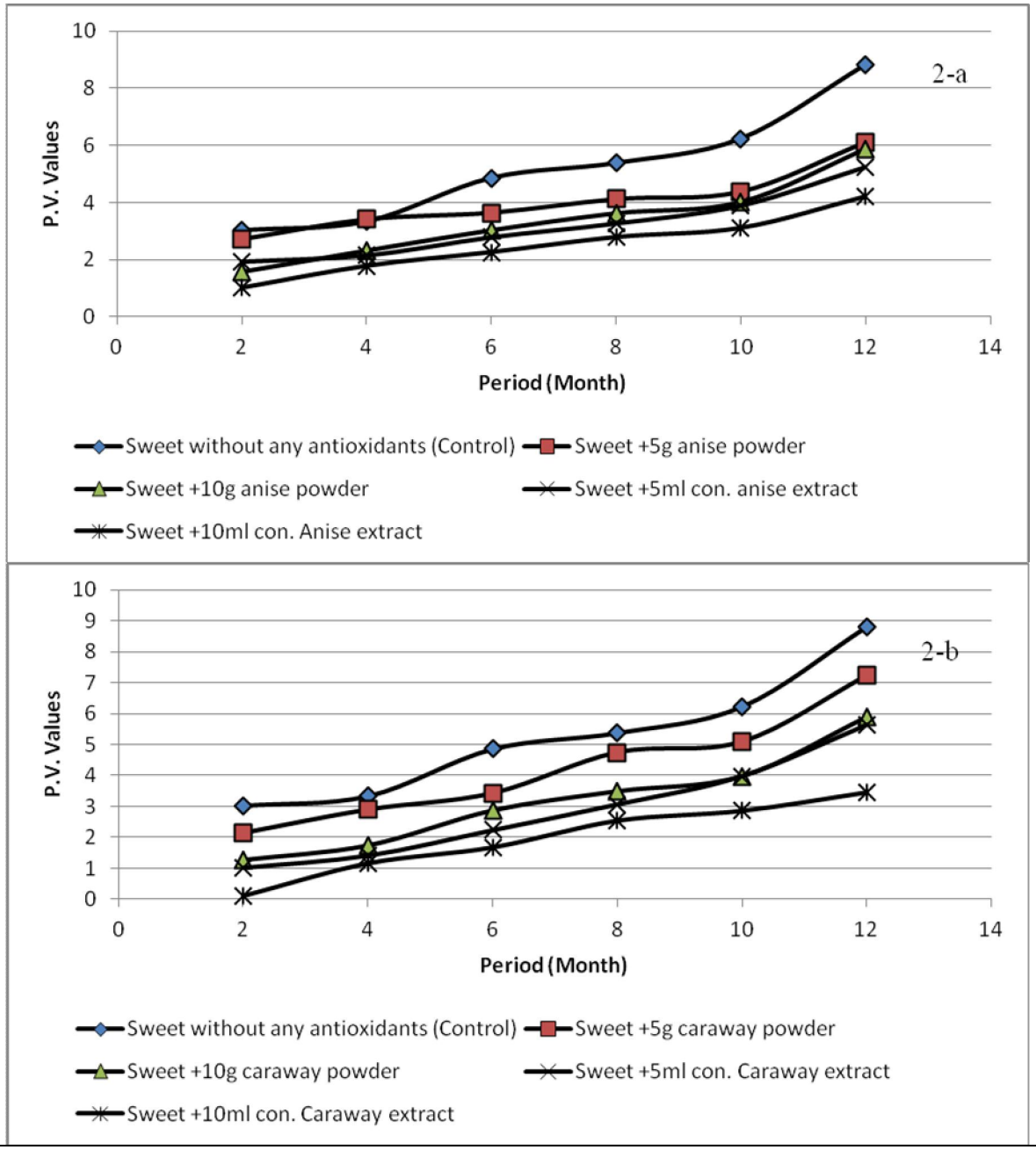


Fig. (2): Effect of added anise and caraway ((a) as powder and (b) as extracts) on peroxide value of sesames sweets

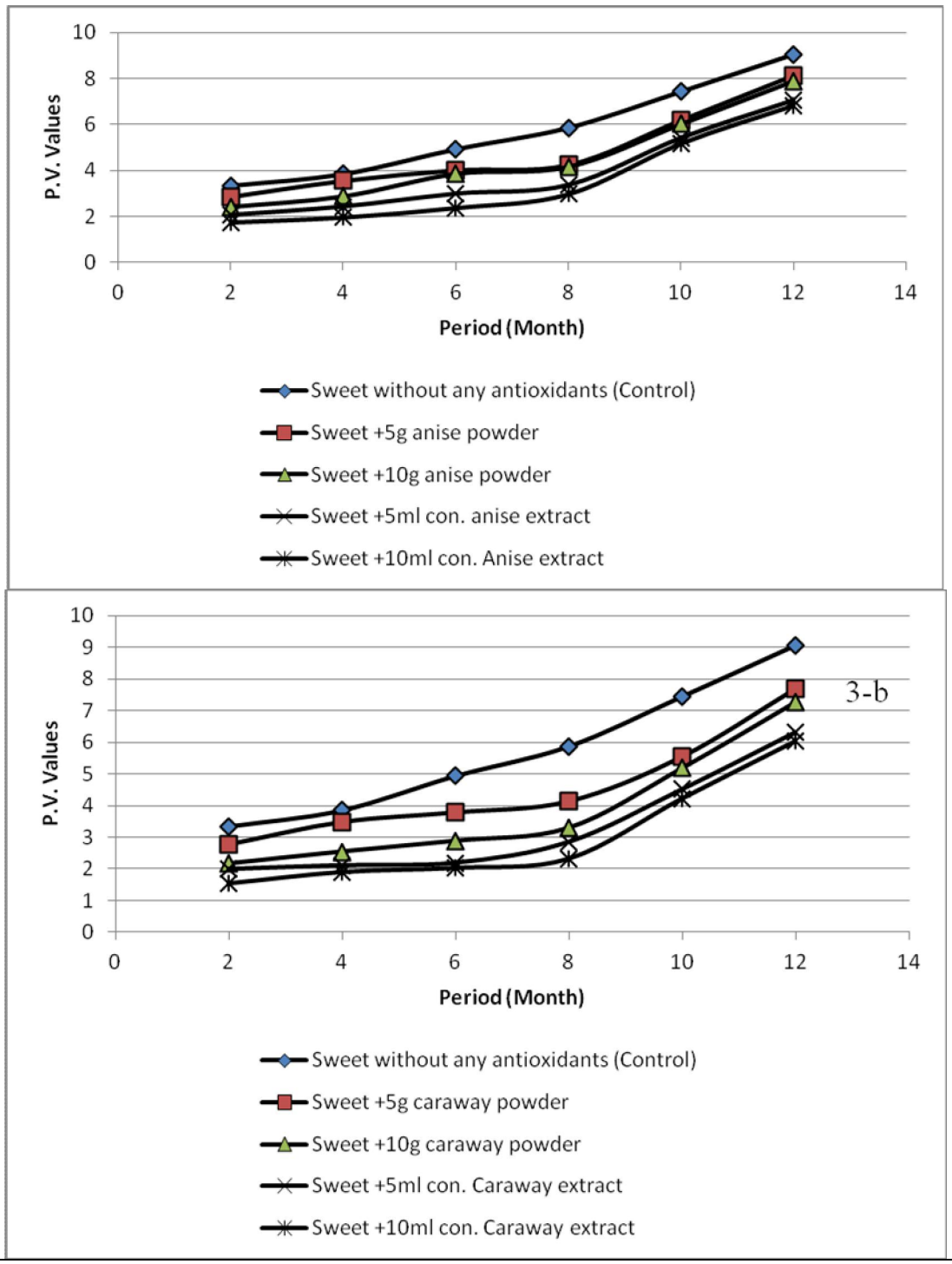


Fig. (3): Effect of added anise and caraway ((a) as powder and (b) as extracts) on peroxide value of Folia sweets

Corresponding author
Ginat, El-sherif
Food Technology Res. Institute, Agric. Res. Center, Giza, Egypt
ginat.elsherif@yahoo.com

4. References:

Antolovich, M.; Prenzler, P. D.; Patsalides, E.; McDonald, S. and Robards, K. (2002). Method for testing antioxidant activity. Analyst, 127: 183-198.

A. O. A. C. (2000). Official Methods of Analysis of the Association of Official Analytical Chemists, 17th ed., edited by W. Hormitz, A. O. A. C., Washington, D. C.

Awatif, I. Ismeal; Amany M. M. Basuny and Dalia, M. M. Mostafa (2004). Effect of some processing on the quality of sesame oil and sesame hulls as a natural antioxidant. Minia J. of Agric. Res. And Develop., 24 (3):457-468.

Duh, P.D. (1998). Antioxidant activity of burdock (*Arctium lappa linne*): its scavenging effect on free radical and active oxygen. J. of the American oil Chemist's Soiety, 455-465.

El-Sawi, S. A. and Mohamed, M. A.(2002). Cumin herb as a new source of essential oils and its response to foliar spray with some micro-elements. Food Chemistry, 77: 75-80.

Gerard, A. A. and Roberts, J. S. (2004). Microwave heating of apple mash to improve juice yield and quality. Lebensmittel-Wissenschaft and Technologie, 37:551-557.

Gülçin, I.; Oktay, M.; Kireçci, E. and Küfreviõğlu, Ő. İ. (2003). Screening of antioxidant and antimicrobial activities of anise (*Piminella anisum L.*) seed extracts. Food Chemistry, 83:371-382.

: Hannan, M. A. Al-Sayed (2008). Evaluation of antioxidant activity of some spices and their application in croissant and filling cream. Arab. Univ. J. Agric. Sci., 16 (1): 97-114.

Heinonen, I. and Marina, A. (1989). Carotenoids and provitamin A activity of carrot. J. Agric., Food Chem., 38: 609- 612.

Lai, L.S.; Chou, S. T. and Chao, W. W. (2001). Studies on the antioxidative activities of Hsian-tsao (*Mesona procumbens Hemsl*) leaf gum. J. of Agric. And Food Chem., 49:963-968.

Liangli Lucy Yu; Kequan Kevin Zhou and John Parry (2005). Antioxidant properties of cold-pressed black caraway, carrot, cranberry and hem seed oils. Food Chemistry, 91: 723-729.

Mendez, F.; Sanhueza, T.; Speisky, H. and Valenzuela, A. (1996). Validation of the rancimat test for the assessment of the relative stability of fish oils. J. Am. Oil Chem. Soc., 73:1033-1037.

Padmashree, A.; Roopa, N.; Semwal, A. D.; Sharma, G. K.; Agathian, G. and Bawa, A. S. (2007). Star-anise (*Illicium verum*) and black caraway (*Carum nigrum*) as natural antioxidants. Food Chemistry, 104:59-66.

Patro, B. S.; Bauri, A. K.; Mishra, S. and Chattopadhyay, S. (2005). Antioxidant activity of myristica Malabarica extracts and their constituents. J. of Agric. And Food Chem., 53: 6912-6918.

Pourgholami, M. H.; Majzoob, S.; Javadi, M.; Kamalinejad, M.; Fanaee, G. H. R. and Sayyah M. (1999). The seeds essential oil of *Pimpinella anisum* extracts anticonvulsant effects in mice. J. of Ethropharmacology, 66: 211-215.

Refaat, O. G. A. (1988). Nutrition and chemical studies on candy, oriental sweets and bakery products. Ph. D. Thesis faculty of Home Economics, Helwan Univ.

Reddy, V.; Urooj, A. and Kumar, A. (2005). Evaluation of antioxidant activity of some plant extracts and their application in biscuits. Food Chemistry, 90:317-321.

Santos, P. M.; Figueiredo, A. C.; Olivera, M. M.; Barroso, J. G.; Pedro, L. G.; Deans, S. G.; Younus, A. K. K. M. and Secheffer, J. C. (1998). Essenial oils from hairy root cultures and from fruits and roots of *Pimpinella anisum*. Phytochemistry, 46 (3): 455-460.

Smith, R. J. and Winder, M. L. (1996). Medicinal Gaeden. In the National Herb Garden Guidebook; Ober, R., Ed.; The Herb Society of America, Inc. Springfield, VA; pp. 69-93.

Slinkard, K. and Singleton, V. L. (1977). Total phenol analysis: Automation and comparison with manual methods. American J. of Enology and Viticulture, 28:49-55.

Suhaj, M. (2006). Spice antioxidants isolation and their antiradical activity: a review. J. of Food Composition and Analysis, 19:531-537.

Tolba, K. H. and Azouz, A. (2006). Anthocyanin from Acalypha leaves as a multifunction food additives. J of Home Economics, Minufiya Univ., 16 (1/2): 157-168.

Zheng,W. and Wang, S. Y. (2001). Antioxidant activity and phenolic compounde in selected herbs. J. of Agric. And Food Chem., 49: 5165-5170.