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CARROT LEAVES: ANTIOXIDATIVE AND NUTRITIVE VALUES

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ABSTRACT

This study was carried out for chemical assess of yellow carrot leaves and possibility to use its methanol and acetone extracts as a new source of natural antioxidants instead of artificial antioxidants due to its negative effect on human health.

So, chemical composition of carrot leaves were estimated, as well as methanol and acetone extracts content of phenolic compounds using liquid chromatography and also, antioxidant activity of both extracts were studied prior to the sunflower oil stored in opened bottles stored at 63°C as an oxidation accelerated conditions for 60 days using acid number, peroxide value, antioxidant effectiveness and thiobarbituric acid value changes.

Obtained results showed that carrot leaves could be considered as a source of carbohydrates and protein, which represented 61.36% and 20.27% (dry weight), respectively. Also, it was a good source of some minerals such as potassium (975.00 ppm). It was observed that extraction efficiency of methanol was higher than acetone where, it contained 82.07 mg/ml total phenols as galic acids. Both of two extracts had antioxidant effect. It was found that there were no significant differences between control treatment and all treatment up to 45 days of storage and the best antioxidant effectiveness was observed for D treatment (0.15% acetone extract) compared with other treatments.

The results also showed that thiobarbituric acid values did not show significant differences between all treatments up to 30 days of storage and it ranged between 0.515 to 0.788 mg malonaldehyde/kg oil. Therefore, it was recommended to use carrot leaves as a new source of natural antioxidants, as well as source of some minerals to fortify some food products.

Keywords: carrot leaves, chemical composition, phenolic compounds, natural antioxidants.

INTRODUCTION

Concerning the commercial synthetic phenolic compounds in food system such as butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT) and their negative health effect *(*Williams *et al.,* 1990*)*. For that reason, natural compounds as antimicrobial and antioxidants were widely used against food-borne microorganisms or retard the development of rancidity and extent the shelf-life of many foods *(*Friedman*,* 2007 and Beverlya *et al.,* 2008*)*.

Cultivars of carrot differ in their essential oil contents and leaves have higher amount of essential oils than the roots. The principal compounds in the leaves were β-myrcene, α-asarone, methyl isoeugenol, β-caryophyllene, (E)-β-farnesene, limonene and sabinene *(*Habegger and Schnitzler*,* 2000*)*.

Many vegetable leaves, including those of carrot (*Daucus carota L.*), are wasted. Carrot leaves are very rich in both nutrients such as vitamin C, β-

carotene, fibers and several minerals such as Na, P, K, Ca, Mg, Mn, Zn, and Fe *(*Pereira *et al.,* 2003*)*.

They have a pleasant taste and characteristics suitable for processing. They may be used as a raw basis for the preparation of several foods. The use of the byproducts of the vegetable industry has presented technological viability, and they have been used for the formulation of cream soups made of dehydrated vegetable stalks *(*Couto *et al.,* 2004*)*.

Mammals who feed on these plants convert LNA by the same sequential desaturation and elongation enzyme systems. It results in the production of long chain-polyunsaturated fatty acids (n-3 LC-PUFA) which are reported to play an important role in human health. Carrot leaves, like others green leafy vegetables, are a good source of essential fatty acids, being LNA the predominant fatty acid. In the process for dehydration of carrot leaves aiming the conservation LNA the fresh leaves are converted in dry product rich in essential fatty acids that might be used as complementary food in the human nutrition *(*Leonard *et al.,* 2004*)*.

Allam *et al. (2008)* found that Fig leaves extract (60 mg/ml water) contained higher content of total phenols than other doses (20 and 40 mg/ml water), which was found to be 9.14±0.09 mg/g extract.

Different extracts of carrot seeds, leaves and roots were evaluated for their effectiveness inhibition on some groups of microorganisms fungal growth and reducing the production of aflatoxins. Ethanol, chloroform and water extracts showed effects against some groups of microorganisms which contaminated wheat products. Water and chloroform extracts of yellow carrot leaves showed the most effect against coliform group. Water, ethanol and chloroform extracts from carrot seeds, leaves and root reduced the fungal growth rate. No aflatoxin was produced by fungi in wheat samples treated by extracts of carrot seeds, leaves and roots *(*Emara *et al.,* 2010*)*.

El-Shehawy *et al. (*2010*)* reported that total phenolic compounds (as gallic acid) of black berries leaves methanolic extract was 100.54 mg/ml. While, black berries leaves acetonic extract contained 31.43 mg/ml. Both of white or black berries leave acetone or methanol extracts exhibited antioxidant activity. Sunflower oil treated with white berries leave acetone extract (0.20%) had the best peroxide value of 36.06 and also, the percentage of antioxidant effectiveness ranged from 7.05% at zero time to 82.62% at the end of storage period (60 days). TBA value of 0.20% black berries leave methanol extract treated sunflower oil had the highest value (2.512) after 15 days of storage.

Abou-Arab and Abu-Salem *(*2010*)* concluded that *Stevia rebaudiana* is considered natural antioxidants, which contained phenolic compounds, flavonoids as well as gallic acid. These substances have been suggested to play a preventive role for human health. Also, the results indicated that stevia leave and callus extracts may be an ideal candidate for further research to their uses for food preservation.

So, the main goal of such study was directly to evaluate the nutritive and antioxidative values of carrot (*Daucus carota L.*) leaves and their solvent extracts.

MATERIALS AND METHODS

Materials:

Carrot (*Daucus carota L.*) leaves were collected from Posat village farms, Talkha, Egypt.

Sunflower oil and TBHQ were kindly obtained from Misr Oil and Soap company, Mansoura, Egypt.

Solvents, other reagents and dark glass bottles were purchased from El-Gomhoria for chemicals company, Mansoura, Egypt.

Methods:

Extraction of antioxidant compounds: The antioxidant compounds were extracted according to the method described by Adegoke and Gopala *(*1998*)* with some modification as follows: Carrot leaves firstly, were dried at 45-50°C for 8-10 hours using air drying oven (Officine specializzate, GARBUIO, essiccatioi, TREVISO, ITALY). Then, it was extracted using a method of maceration with methanol and acetone (Duh *et al.,* 1992) (100g dried carrot leaves/500 ml solvent) for 24 hours at room temperature. After maceration, the extracts were collected, filtered with Whatmann No. 1 filter paper and evaporated with vacuum rotary evaporator at 45-50°C (BÜCH, RE 111, SWIZERLAND). The evaporated extracts were collected in dark glass bottles and stored at 5-7°C until using.

Experiment design: 100 ml of sunflower oil was put in dark glass bottles without caps. Then, TBHQ , carrot leaves extracts were added to sun flower oil samples with concentration of 200 ppm for TBHQ ; 0.10%, 0.15% and 0.20% for both acetone and methanol extracts. After that all treatments (8 samples), were stored in accelerated oxidation conditions at 63°C for 60 days, according to El-Shawaf (2000), in digital incubator (INCUBATOR ISOTEMP, TURKEY) and were chemically analyzed every 15 days.

Chemical analysis:

Moisture, ash, crude protein and crude fat contents were determined according to the method described by *AOAC (2000)*. Carbohydrates were calculated by difference.

Carrot leaves were prepared for minerals determination by digestion in perchloric acid and nitric acid. Phosphorus (P), Calcium (Ca), Iron (Fe), Zinc (Zn) and Potassium (K) contents were determined using Atomic Absorption Spectrophotometer according to the method of Pupsa *et al.* (1994).

Total phenolic compounds were analyzed at Bio-technology Lab., Plant Pathology Institute, Agricultural Research Center, Giza, Egypt. Analysis was performed with a liquid chromatograph "HP1050" equipped with a 4.6 mm x 150 mm ODS C_{18} column with UV detector and the injection volume was 5µl. The mobile phase yielded results of 40% methanol : 60% distilled water. The wave length in the UV detector was 230 nm, total run time for the separation was approximately 15 min. at a flow rate of 0.60 ml/min according to the proposed method of Waskmundzka *et al*. (2007).

Acid value (AV) and peroxide value (PV) of treated sunflower oil samples were determined as described in AOAC (2000).

Thiobarbituric acid value (TBA) was determined as described by Tarladgis *et al.* (1960). TBA value was expressed as mg malonaldehyde/kg oil with the following equation:

$$TBA=7.8 \times O.D.$$

Where: O.D. = optical density at 538 nm using Spekol 11, Carl Zeiss Jena, German.

Antioxidant effectiveness (AE) was calculated from peroxide value according to Adegoke and Gopala (1998) using the following equation:

$$AE\ \% = 100(PV_{blank} - PV_{treatment})/ PV_{blank}$$

Statistical analysis (ANOVA) was done using SPSS (2008) version 17 program for windows.

RESULTS AND DISCUSSION

Table (1) show approximate chemical composition of dried carrot leaves. From these results, it could easily be seen that carbohydrate represented the high percentage of this waste components (61.35%), followed by protein which was 20.27%. Ash percentage came in the third order, where it was 15.00% and contained some of important elements such as Potassium (975ppm), Calcium (15.1ppm), Phosphor (7.80ppm), Iron (3.63ppm) and Zinc (0.88ppm). Moisture content was 9.15% and crude fat was 3.37%. These results refer to the high nutritive value of carrot leaves which were considered as wastes especially the content of crude protein and a variety of elements. So, it is possible to utilize carrot leaves in some products such as cakes or extracts some valuable components as natural antioxidants. These results agreed with those obtained by Pereira *et al.* (2003).

Table (1): Approximate chemical composition of dried carrot leaves:

Components	Concentration
Carbohydrates %	61.36
Crude protein %	20.27
Moisture %	9.15
Crude fat %	3.37
Ash %	15.00
K (ppm)	975.00
Ca (ppm)	15.10
P (ppm)	7.80
Fe (ppm)	3.63
Zn (ppm)	0.88

The content of phenolic compounds in carrot leave extracts were presented in Fig. (1). The total content of phenolic compounds in carrot leave extracts were clearly correlated with the solvent used, where there was higher amount of total phenols in methanol extract (82.07mg/ml as gallic acid). Also, methanol extract had high content of other phenolic compounds when compared with acetone extract, which recorded 40.62, 22.08, 12.31, and 397.5mg/ml for galic, salicylic, qumarin and querecetine, respectively.

While, acetone extract contained ferulic and salicylic only (1.51 and 3.3 mg/ml, respectively). These results confirmed with El-Shehawy *et al.* (2010) who reported that methanol could extract more phenolic compounds than acetone.

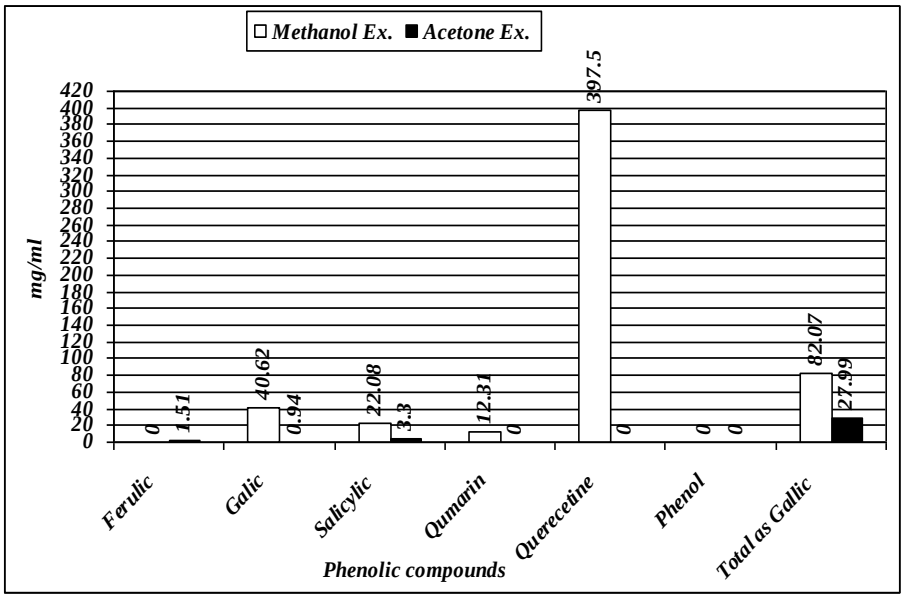

Fig. (1): Phenolic compounds content (mg/ml) in carrot leaves methanol and acetone extracts.

Fig. (2) showed the effect of carrot leaves extracts on sunflower oil acid value (AV), which indicated weather there were hydrolytic rancidity or not, stored at 63°C for 60 days. Acid value of blank treatment ranged from minimum value of 0.136 mg KOH/g oil at zero time and maximum value of 0.525 at the end of storage period. But oil sample containing 200ppm TBHQ had the highest acid value of 0.337 after 60 days of storage. From the same figure, it could be easily observed that acid value of the six examined oil sample showed increments and decrements in the same time. However, the highest acid value of all treatment was noticed after 15 days of storage which recorded 0.641 for 0.15% carrot leave acetone extract treated oil. Then, acid value of all treatments decreased after 30 days of storage and relatively increased again after 45 days of storage.

At the end of storage period, acid value of oil treatments had clear increase where it recorded the highest value of 0.622 for 0.20% carrot leave acetone extract treated oil. From these results, it could be concluded that carrot leave extract treated oil samples were in good quality, from hydrolytic rancidity view, until 45 days of worm storage. Also, both carrot leave extracts had no clear effect on acid values of all treatments.

Data in Table (2) and Fig. (3) showed peroxide value changes of sunflower oil samples treated with carrot leave acetone and methanol extracts and 200ppm TBHQ as an artificial antioxidant compared with blank treatment during storage at 63°C for 60 days.

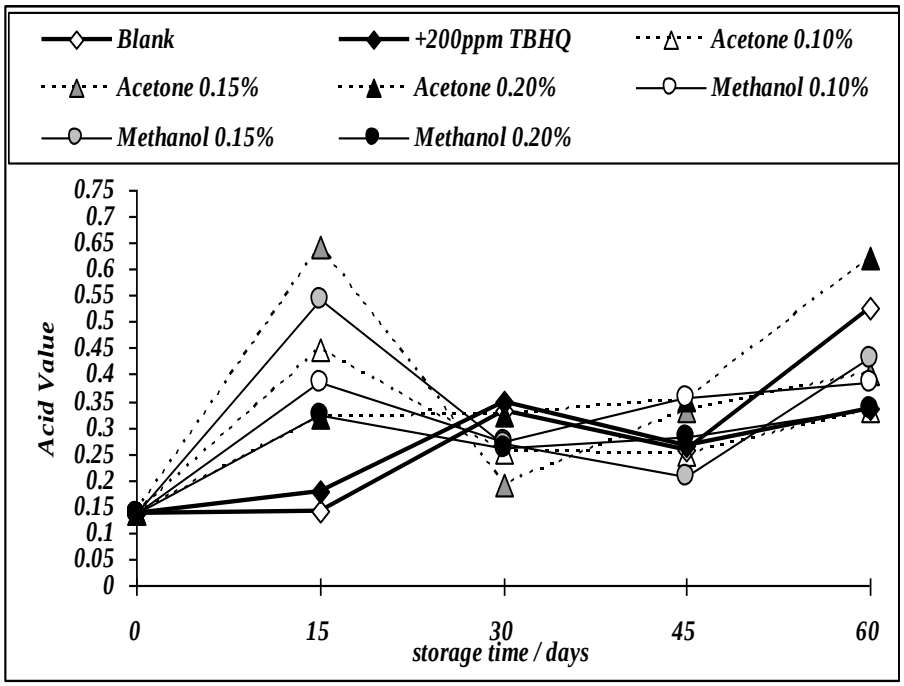

Fig. (2): Acid value (mg KOH/g oil) changes of sunflower oil samples treated with carrot leaves extracts during storage at 63°C for 60 days.

Table (2): Peroxide value (PV) "meq./Kg oil" changes of sunflower oil samples treated with carrot leaves extracts during storage at 63°C for 60 days.

Code	Treatments			Storage period/day				
				0	15	30	45	60
A	Blank (Sunflower oil)			5.05	8.09^{b}	40.95^{e}	101.96^{f}	207.44^{h}
B	Control (Blank+200ppm TBHQ)			5.05	5.66^{a}	28.17^{c}	32.01^{a}	33.69^{a}
C	Carrot leaves	Acetone	0.10%	5.05	11.41^{c}	32.71^{cd}	35.10^{b}	102.19^{b}
D			0.15%	5.05	7.39^{b}	33.33^{d}	37.69^{c}	128.04^{c}
E			0.20%	5.05	12.59^{d}	32.96^{cd}	82.59^{e}	170.92^{e}
F		Methanol	0.10%	5.05	7.85^{b}	32.07^{c}	56.18^{d}	197.40^{g}
G			0.15%	5.05	12.26^{d}	22.47^{a}	36.25^{b}	158.28^{d}
H			0.20%	5.05	20.24^{e}	25.97^{b}	37.61^{c}	174.61^{f}

Means of treatments having the same letter(s) within a column are not significantly different (P> 0.05).

It could be noticed that there were significant differences between blank, control and treated oil samples after 15 days of storage. But after 30 days of storage, there were no significant differences between control and most of treated samples. Peroxide values gradually increased until reached the maximum value of 207.44 meq./Kg oil in blank and the minimum value of 33.69 for control treatment at the end of storage period.

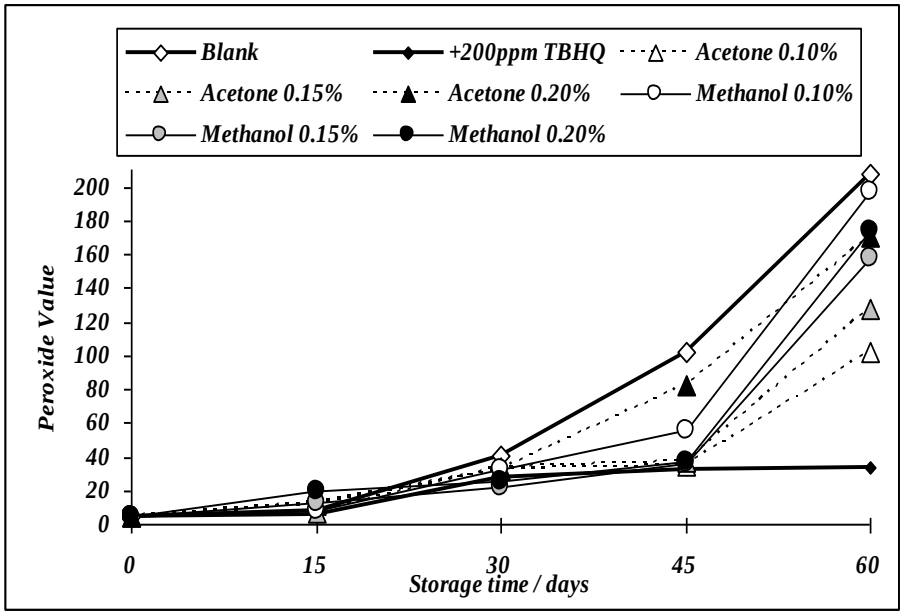

Fig. (3): Peroxide value (meq./Kg oil) changes of sunflower oil samples treated with carrot leaves extracts during storage at 63°C for 60 days.

So, the highest antioxidative effect was observed in all carrot leave extracts treated oil samples after 45 days of storage except for E and F samples, where the highest peroxide values were found which were 82.59 and 56.18, respectively. But at the end of storage period, there were significant differences between control and all treated oil samples in peroxide values.

Data in Table (3) showed the percentage of antioxidant effectiveness of sunflower oil treated with acetone and methanol carrot leave extracts compared with synthetic antioxidant (TBHQ) during storage for 60 days at 63°C.

Table (3): Antioxidant effectiveness (AE) during storage at 63°C of treated sunflower oil samples.

Code	Treatments			Storage period/day				
				0	15	30	45	60
A	Blank (Sunflower oil)			-	-	-	-	-
B	Control (Blank+200ppm TBHQ)			-	30.04	31.21	68.61	83.76
C	Carrot leaves	Acetone	0.10%	-	-41.04	31.21	65.57	50.74
D			0.15%	-	8.65	20.12	63.03	38.28
E			0.20%	-	-55.62	18.61	19.00	17.61
F		Methanol	0.10%	-	2.97	19.51	44.90	4.84
G			0.15%	-	-51.55	21.68	64.45	23.70
H			0.20%	-	-150.19	45.13	63.11	15.83

These results showed that the highest values of antioxidant effectiveness were observed at 45 days of storage for all treated oil. But 0.15% acetone carrot leave extract treated oil had gradual increases of antioxidant effectiveness value during storage period where, it recorded 8.65, 20.12, 63.03 and 50.74 at 15, 30, 45 and 60 days, respectively.

Also, 0.10% acetone and 0.15%, 0.20% methanol carrot leave extract treated oil samples had nearly equal value of antioxidant effectiveness of control sample (65.57, 64.45 and 63.11, respectively) after 45 days of storage. So, D treatment could be considered the best treatment compared with control.

Results in Table (4) and Fig. (4) showed changes of TBA values in acetone and methanol carrot leave extracts treated sunflower oil during storage at 63°C for 60 days. Data illustrated in Fig (4) indicated that addition of carrot leave extracts showed lower TBA value than blank sample in most treatments after 30days of storage. But at 15 days of storage, there were no significant differences between blank, control and 0.10%, 0.15% methanol carrot extracts.

Table (4): Thiobarbituric acid value (TBA) "mg malonaldehyde/Kg oil" changes of sunflower oil samples treated with carrot leaves extracts during storage at 63°C for 60 days.

Code	Treatments			Storage period/day				
				0	15	30	45	60
A	Blank (Sunflower oil)			0.515	0.835^{ab}	0.694^{a}	1.661^{cd}	5.585^{b}
B	Control (Blank+200ppm TBHQ)			0.515	0.601^{a}	0.530^{a}	1.170^{abc}	0.445^{a}
C	Carrot leaves	Acetone	0.10%	0.515	1.942^{de}	0.515^{a}	0.749^{a}	7.075^{b}
D			0.15%	0.515	1.505^{cd}	0.788^{a}	1.334^{ab}	5.694^{b}
E			0.20%	0.515	2.044^{e}	0.788^{a}	1.841^{d}	7.121^{b}
F		Methanol	0.10%	0.515	1.092^{abc}	0.523^{a}	1.474^{bcd}	6.201^{b}
G			0.15%	0.515	1.287^{bcd}	0.538^{a}	1.287^{abcd}	6.006^{b}
H			0.20%	0.515	1.622^{cd}	0.632^{a}	1.006^{ab}	5.491^{b}

Means of treatments having the same letter(s) within a column are not significantly different (P> 0.05).

After 30 days of storage, there were no significant differences between all treatments (TBA value ranged between 0.515 and 0.788 mg malonaldehyde/Kg oil). At 45 days, there were significant differences between control from a side and blank, 0.20% acetone carrot leave extract from the other side. But at the end of storage period, there were significant differences between control and all treatments.

However, the highest TBA value was found in sample E (7.121 mg malonaldehyde/Kg oil), while the least value was found in sample H (5.491 mg malonaldehyde/Kg oil) at the end of storage period. So, C, D, F, G and H samples were the best treatment compared with control treatment.

Although the higher phenolic compounds (as gallic acid) of methanol extract, the best antioxidant effectiveness was found in D sample (0.15% acetone). But all methanol treatments showed the best TBA values after 45 days of storage period.

This observation indicated that single phenolic compound such as ferulic and salicylic (acetone extract) could be more effective than total phenolic compounds.

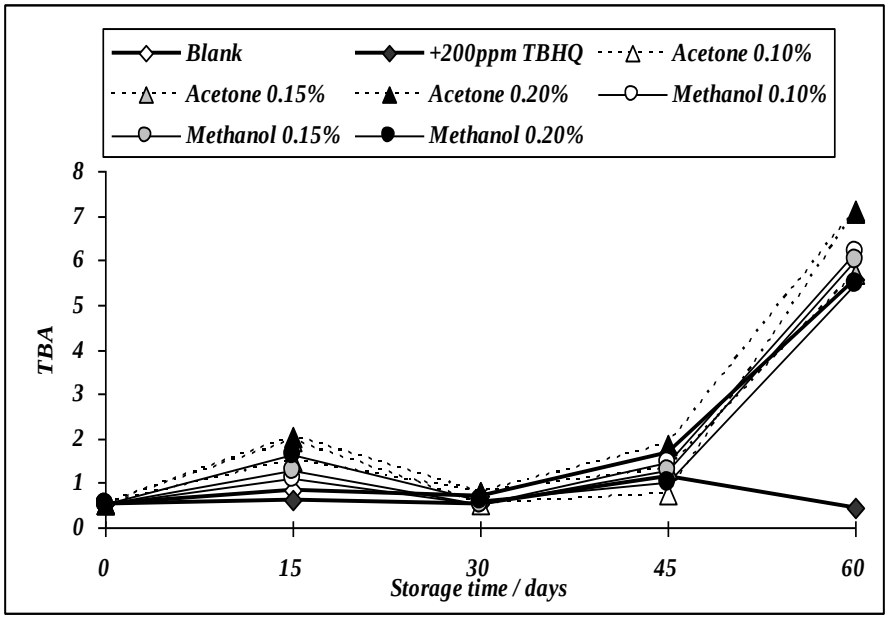

Fig. (4): TBA value (mg malonaldehyde/Kg oil) changes of sunflower oil samples treated with carrot leaves extracts during storage at 63°C for 60 days.

Conclusion

From all discussed results, it could be concluded that (1) carrot leaves had high protein, carbohydrate and potassium content. So it could be used in several bakery products such as cakes, (2) Addition of 0.10% acetone carrot leave extract as a natural antioxidant equal the action of 200ppm of TBHQ as an artificial antioxidant in delaying oxidative rancidity until 45 days at 63°C.

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أوراق الجزر: القيمة الغذائية و المضادة للأكسدة

جيهان علي غنيم ، فاتن يوسف إبراهيم و شادي محمد الشهاوي
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أجريت هذه الدراسة بهدف تقييم أوراق الجزر الأصفر كيميائياً و إمكانية إستخدام مستخلصات الميثانول و الأسيتون كمصدر جديد لمضادات الأكسدة الطبيعية بدلاً من مضادات الأكسدة الصناعية نظراً لأضرارها المتعددة و التي تصيب الإنسان.

لذا تم تقدير التركيب الكيماوي لأوراق الجزر و كذلك محتوى مستخلصات الميثانول و الأسيتون من المركبات الفينولية باستخدام جهاز الفصل الكروماتوجرافي كما تم دراسة النشاط المضاد للأكسدة لكلاً من المستخلصات السابقة على زيت دوار الشمس المخزن في زجاجات مفتوحة على درجة حرارة ٦٣°م كدرجة محفزة للأكسدة لمدة ٦٠ يوم باستخدام التغير في رقم الحمض و رقم البيروكسيد و فاعلية مضاد الأكسدة و قيمة حمض الثيوباربيتوريك.

هذا و قد أوضحت النتائج أن أوراق الجزر تعتبر مصدر للكربوهيدرات و البروتين حيث تمثل ٦١,٣٦% و ٢٠,٢٧% على الترتيب. و كذلك مصدر جيد للعناصر المعدنية مثل البوتاسيوم (٩٧٥,٠٠ جزء في المليون). لوحظ أن كفاءة الاستخلاص بالميثانول أعلى من الأسيتون حيث إحتوى مستخلص الميثانول على ٨٢,٠٧ مجم/مل فينولات كلية كحامض جاليك. و قد كان لكلى المستخلصين تأثيراً مضاد للأكسدة. حيث وجد أنه لا توجد فروق معنوية بين المعاملة الضابطة و جميع المعاملات حتى ٤٥ يوم تخزين و ظهرت الفاعلية الأعلى كمضاد أكسدة للمعاملة D و هي إضافة ٠,١٥% مستخلص أسيتون مقارنة بباقي المعاملات.

كما أظهرت النتائج أن قيمة حمض الثيوباربيتوريك لم تظهر فرق معنوي بين جميع المعاملات حتى ٣٠ يوم تخزين و تراوحت قيمته بين ٠,٥١٥ إلى ٠,٧٨٨ مجم مالونالهيد/كجم زيت. لذا نوصي باستخدام أوراق الجزر كمصدر جديد لمضادات الأكسدة الطبيعية و كذلك تدعيم بعض المنتجات الغذائية كمصدر للمعادن.

الكلمات الدالة: أوراق الجزر ، التركيب الكيماوي ، المركبات الفينولية ، مضادات الأكسدة الطبيعية.

قام بتحكيم البحث

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