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ANTIOXIDATIVE ACTIVITY OF PHENOLIC COMPOUNDS FROM WHITE AND BLACK BERRY LEAVES

El-Shehawy, Sh. M. M. ; Gehan A. A. Goneim ; Faten Y. Ibrahim and Rania E. El-Gamal.
Food Industries Dept., Fac. Agric., Mans. Univ., Egypt

ABSTRACT

Natural antioxidants are in great demand today due to both consumer preference and health concerns as allernative synthetic antioxidants. Therefore, this study was carried out to investigate the extraction, identification of antioxidant compounds from both white and black berry leaves. Phenolic compounds in the extracts were identified and determined by HPLC, then evaluated as natural antioxidants using sunflower oil during 60 days of storage at 63°C. Acid value, peroxide value and TBA value of the stored oil were periodically estimated (each 15 days). The results revealed that total phenolics were 100.54 mg/ml and 31.43 mg/ml (as gallic acid) for methanolic and acetonic extracts of black berry leaves, respectively. Both mentioned extracts exhibited antioxidant activity. Sunflower oil treated with white berry leaves 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. TBA value of 0.20% black berry leaves methanol extract treated sunflower oil had the highest value (2.512) after 15 days of storage. From an economical and environmental point of view, these fruit byproducts as natural source of antioxidants may act an important role in edible oil industry.

Keywords: Phenolic compounds, natural antioxidants, oxidative rancidity and accelerated oxidation.

INTRODUCTION

Oxidation of lipids occurs during treatment and storage is one of the basic process causing rancidity and deterioration of lipids and fatty foods *(Karpinska et al., 2001)*. Oxidative deterioration is responsible for lipids off-flavor and decreases nutritional value as well as decreases the safety of foods due to formation of secondary, potentially toxic compounds *(Moure et al., 2001)*. Recently interest has considerably increased in finding naturally occurring antioxidants to use in foods instead of synthetic ones which are being restricted due to the carcinogenicity effect *(Sasaki et al., 2002)*.

The plant kingdom offers a wide range of natural antioxidants. Such as herbal and plant infusions that frequently used in domestic medicine. *(Vinson et al., 1995)*. Fruit wastes and their by-products, generally, show a higher antioxidant activity than that of vegetable wastes *(Hilde et al., 2009)*.

Berries, wild or cultivated, are traditionally a part of the Finnish diet, since they contained many essential nutritional components and phenolic substances, which compose two large and heterogeneous groups of biologically active non-nutrients *(Shahidi and Naczk, 1995)* such as kaempferol, quercetin and myricetin ; p-coumaric, caffeic and ferulic acids (hydroxycinnamic acids) ; p-hydroxybenzoic, gallic and ellagic acids (hydroxybenzoic acids). These favonoids and phenolic acids have been

proposed to have beneficial effects on health as antioxidants *(Rice-Evans et al., 1996)*.

It was reported that high amounts of phenolics with high sensitivity antioxidant properties could be recovered from fruit and vegetable residues for both food and cosmetic applications *(Wieland et al., 2006)*. In addition, *Zeyada et al. (2008)* found that phenolic content in methanolic extracts in some fruits and vegetables could be summarized as follows: olive leaves > tomato peel > orange peel > cucumber peel > water melon peel > potato peel.

Ali et al. (2009) found that the maximum amounts of total phenolics were occurred in berry followed by turnip among chard, lettuce, berry, grape, mallow and turnip leaves, while, the lowest amount was found in chard leaves. The highest total phenolics content was found in raspberry leaves among the different type of berry plant leaves, while the lowest one was detected by blueberry leaves. Whereas, the main substances present in blueberry leaves extract was caffeic acid (60.4%) and ellagic acid (5.30%) compared with raspberry extract *(Skupien et al., 2006)*. Antioxidant activity of phenolic compounds present in plants is affected by their chemical structure, type and polarity of extracting solvent *(Moure et al., 2001)*. Since, the efficiency of solvents to extract phenolics from two peanut hulls was ranked in the descending order: methanol > acetone > chloroform > n-hexane *(Duh et al,.1992)*.

Therefore, this work was carried out to compare the ability of two organic solvents (methanol and acetone) to extract phenolics from white and black berry leaves which consider as a farm waste in Egypt. Antioxidant efficiency of these extracts was also determined comparing with the synthetic antioxidant TBHQ by using fresh sunflower oil stored at 63°C for 60 days.

MATERIALS AND METHODS

Materials:

White (*Morus alba*) and black (*Morus nigra*) berry leaves were collected from Bosat village farms, Mansoura, Egypt.

Sunflower oil free from synthetic antioxidants and TBHQ were kindly obtained from Misr Oil and Soap Company, Mansoura, Egypt. Solvents and other reagents were purchased from El-Gomhoria for Chemicals Company, Mansoura, Egypt.

Methods:

Extraction of antioxidant compounds: The antioxidant compounds were extracted according to *Adegoke and Gopala (1998)* with some modification as follows: Both of white and black berry leaves firstly were dried at 45-50°C for 8-10 hours using an air drying oven (Officine specializzate, GARBUIO, essiccatioi, TREVISO, ITALY). then, were extracted by using 100 g dried leaves/500 ml solvent with methanol and acetone (*Duh et al., 1992*) 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) to reduce the total volume to the minimum. 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 , white and black berry leaves extracts were added as described in Table (1). After that all treatments (14 samples) were stored in accelerated oxidative 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.

Table (1): Sunflower oil treatments with white and black berry extracts.

Sunflower oil treatments												
Blank*	Control**	Extract of white berry leaves *(w/w)*						Extract of black berry leaves *(w/w)*					
		Acetone			Methanol			Acetone			Methanol		
		0.10%	0.15%	0.20%	0.10%	0.15%	0.20%	0.10%	0.15%	0.20%	0.10%	0.15%	0.20%

*** Sunflower oil without any additives. ** Blank + 200ppm TBHQ.**

Chemical analysis:

Total phenolic compounds were analyzed at Bio-technology Lab., Plant Pathology Institute, Agricultural Research Center, Giza, Egypt. Analysis was performed with a high pressure liquid chromatography HPLC "HP1050" equipped with a 4.6 mm x 150 mm ODS C18 column with UV detector and the injection volume was 5µl. Isocratic mobile phase was 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) were determined as described in *AOAC (2000)* while, 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 x O.D.

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

Antioxidant effectiveness (AE) was calculated from peroxide values as mentioned by *Adegoke and Gopala (1998)* using the following equation:

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

Statistical analysis (ANOVA) was done using SPSS 12 program for windows.

RESULTS AND DISCUSSION

Phenolic compounds content in white and black berry leaves determined in acetone and methanol extracts:

Figure (1) shows phenolic compounds in acetonic and methanolic extracts of white and black berry leaves. Whereas, total phenolics in methanolic extract of black berry leaves was 100.54 mg/ml (as gallic acid) and decreased to 31.43 mg/ml for acetonic extract. The same trend was also observed for methanolic and acetonic extracts for white berry leaves (76.66

and 37.40 mg/ml, respectively). This observation stated that methanol was more effective than that acetone to extract total phenolics.

These previous data point that total phenolic compounds in black berry leaves extract was higher than that of the white one. It is obvious that acetone could not extract salicylic from both black and white berry leaves. Also, acetone was not able to extract ferulic, salicylic and qumarin from black berry leaves. The acetonic extract of white berry leaves had not fractioned querecetine and phenol. While, methanol had the ability to extract gallic and qumarin.

White berry leaves methanolic and acetonic extracts contained ferulic in quantity of 2.94 and 9.13 mg/ml, respectively. Gallic acid represented 50.31 and 44.66 mg/ml in black and white berry leaves methanolic extracts, respectively. As for salicylic, it was 4.81 and 3.11 mg/ml in black and white berry leaves methanolic extracts, respectively. White berry leaves acetonic extract contained the highest value of qumarin which was 91.12 mg/ml. Finally, querecetine and phenol were found only in black berry leaves acetonic extracts and represented 106.5 and 58.95 mg/ml, respectively.

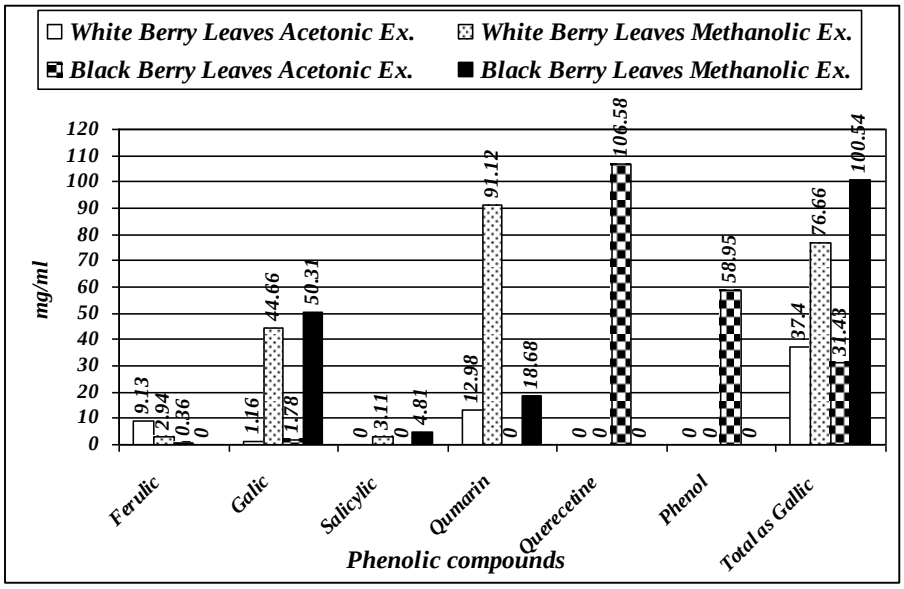

Fig.(1): Phenolic compounds content (mg/ml) in methanolic and acetonic extracts of white and black berry leaves.

The polyphenols extraction yield was higher in both of black berry extract than white berry extract, this may be due to plant cell walls breakdown caused by methanol which reduced the cellulose and increased the antioxidants activity *(Marinova and Yansihtevia, 1996 and Weinberg et al., 1999)*.

Regarding to the same figure, it could be noticed that quercetine had the highest level of phenolic compounds followed by qumarin while, salicylic

and gallic recorded the least concentrate in compare with the major extracted phenolics.

Determination of sunflower oil oxidation stability contained white and black berry leaves extracts comparing with synthetic TBHQ during storage:

1- Acid value:

Acid value of sunflower oil containing different concentrations of white and black berry leaves extracts comparing with sunflower oil involved 200 ppm TBHQ as an artificial antioxidant was present in figures (2 and 3). It is obvious that acid value of sunflower oil was 0.10 mg KOH / g oil at zero time which increased due to stress condition of storage (63°C) to reach the maximum value after 60 days of storage. The increment had no regular trend where the acid value of oil fluctuated between 0.15 to 0.75 mg KOH / g oil. However, acid value of the oil containing different levels of black berry leave extracts had the same trend of white one. So, it could be concluded that no clear effect was found between acid value and concentration of TBHQ or white and black berry leaves extracts. It is well known that hydrolytic or the acid rancidity of oils and fats is brought by the action of lipase enzymes which by hydrolysis split the fat into glycerol and fatty acids or free fatty acids may also be liberated by a relatively high hydrogen-ion concentration in contact with the fat especially at the high temperature of storage.

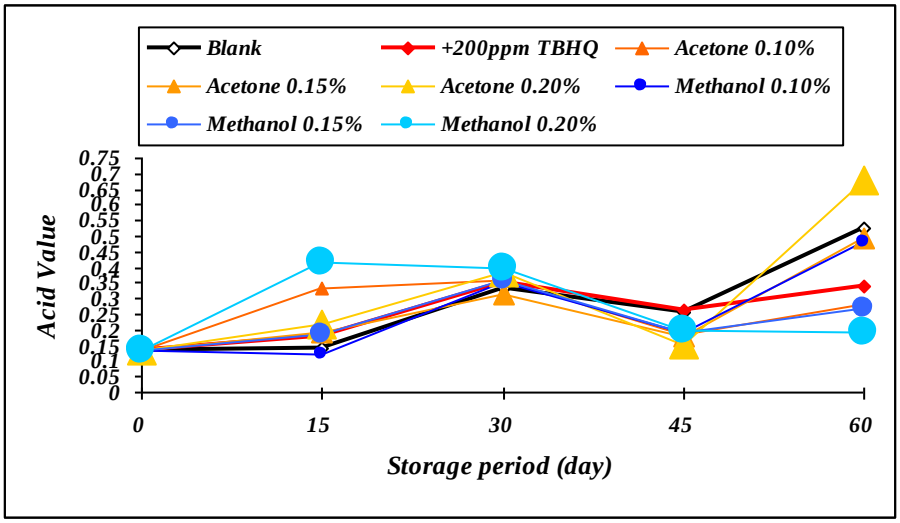

Fig.(2): Acid value (mg KOH/g oil) changes during storage at 63°C of treated sunflower oil samples. (White Berry Leaves extract).

However, acid values of all samples treated with black berry leaves extracts were less than those treated with white berry leaves extracts. There were unclear trend in acid values in white berry leaves extracts treated samples especially, after 15 days of storage. Acid value of sunflower oil sample E was the highest after 60 days of storage which was 0.682. On the other hand, acid values in black berry leaves extract treated samples were in

ascending order during storage period, where it reached the maximum value at the end of storage (60 days) for most samples.

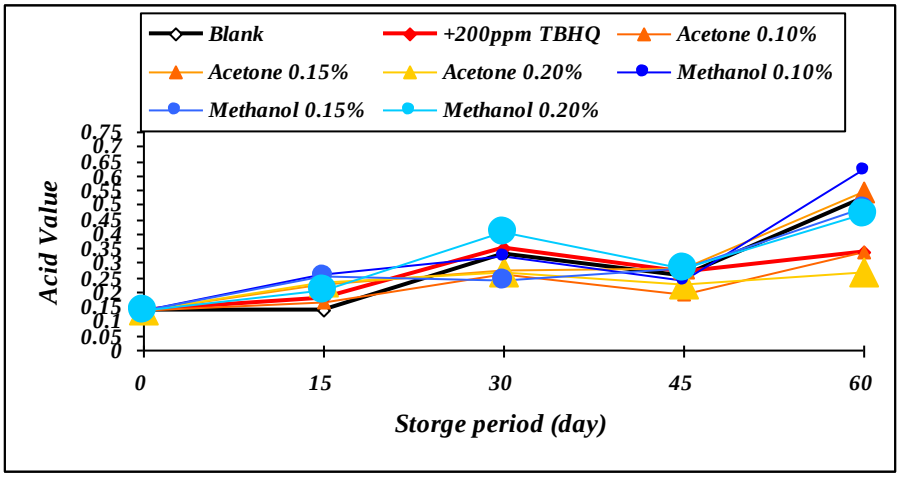

Fig.(3): Acid value (mg KOH/g oil) changes during storage at 63°C of treated sunflower oil samples. (Black Berry Leaves extract).

2- Peroxide value:

As shown in Table (2), peroxide value of sunflower oil treated with TBHQ and berry leaves extracts gradually increased during storage at 63°C for 60 days (Figures 4 and 5). The increment rate in peroxide of oil was raised with berry leaves extracts than that of oil contained TBHQ. This means that the efficiency of such extracts were lower than that the artificial one. However, no significant differences between peroxide value of control oil and sunflower oil sample E at the end of storage course.

Table (2): Peroxide value (PV) "meq. O_2/Kg oil" changes during storage at 63°C of treated sunflower oil samples.

Code	Treatments			Storage period/day				
				0	15	30	45	60
A	Blank (Sun flower oil)			5.05	8.09^{d}	40.95^{l}	101.96^{l}	207.44^{i}
B	Control (Blank+200ppm TBHQ)			5.05	5.66^{a}	28.17^{h}	32.01^{c}	33.69^{a}
C	White Berry Leaves	Acetone	0.10%	5.05	9.75^{e}	22.52^{d}	62.21^{j}	138.36^{e}
D			0.15%	5.05	6.41^{b}	27.83^{g}	29.41^{a}	149.83^{f}
E			0.20%	5.05	7.52^{c}	25.53^{e}	33.92^{d}	36.06^{a}
F		Methanol	0.10%	5.05	6.57^{b}	21.14^{c}	30.91^{b}	42.54^{b}
G			0.15%	5.05	7.97c^{d}	30.85^{j}	41.99^{h}	170.74^{h}
H			0.20%	5.05	5.34^{a}	20.04^{b}	30.35^{b}	118.24^{d}
I	Black Berry Leaves	Acetone	0.10%	5.05	7.79c^{d}	18.90^{a}	71.00^{k}	171.68^{h}
J			0.15%	5.05	6.96^{b}	25.08^{e}	52.43^{i}	185.43^{h}
K			0.20%	5.05	13.71^{f}	29.87^{i}	37.61^{e}	164.54^{g}
L		Methanol	0.10%	5.05	18.77^{h}	26.73^{f}	38.63^{f}	110.57^{c}
M			0.15%	5.05	23.86^{i}	29.07^{h}	41.10^{gh}	113.72^{cd}
N			0.20%	5.05	16.42^{g}	34.15^{k}	70.66^{k}	153.43^{f}

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

It could be observed gradual increases in peroxide values from zero time up to the end of storage at 63°C. This ascending increase could be clearly observed in Fig. (4 and 5). Also, it could be noticed that there were no clear differences between all treated, blank and control oil samples until 30 days then, peroxide values gradually increased and reached the maximum value of 207.44 (meq. O_2/Kg oil) in blank and the minimum value of 33.69 in control. Concerning our treatments, peroxide values of all experimented samples were found between the minimum and maximum values, this observation indicated to antioxidant effectiveness of white and black berry leaves extracts. Sunflower oil sample E had the best peroxide value of 36.06 at the end of storage period, followed by oil sample F which was 42.54.

Data also, revealed that the stability of sunflower oil is dependant on solvent used for the solubility and removal of polyphenolic compounds. Both of acetone and methanol extract treated sunflower oil samples were stable up to 30 days at 63°C. Increasing of storage temperature above 60°C significantly lowered the phenolic compounds quantity and treated sunflower oil samples were less stable against oxidation process *(Azizah et al., 1999)*.

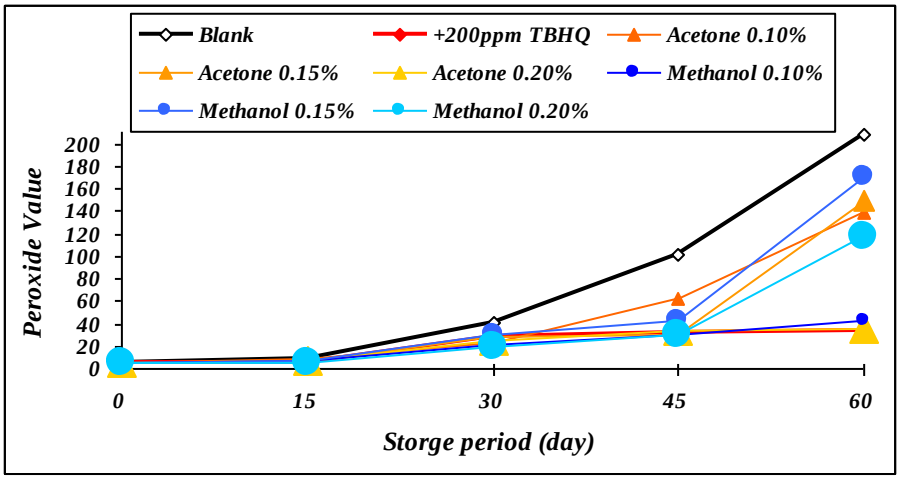

Fig.(4): Peroxide value (meq. O_2/Kg oil) changes during storage at 63°C of treated sunflower oil samples. (White Berry Leaves extract)

Selective extraction (methanol) was reported to enhance the antioxidant activity followed by those sample in acetone extracts which was less efficient. However, less polar solvents such as acetone provided slightly some active compounds at different concentration (0.10, 0.15, 0.20%). The antioxidant activity depends on the extract concentrates. In general, the more of extract concentration, the more antioxidant activity. The high concentration of white berry leaves (0.20%) leading to the maximum antioxidant activity was closely depended on the solvent type.

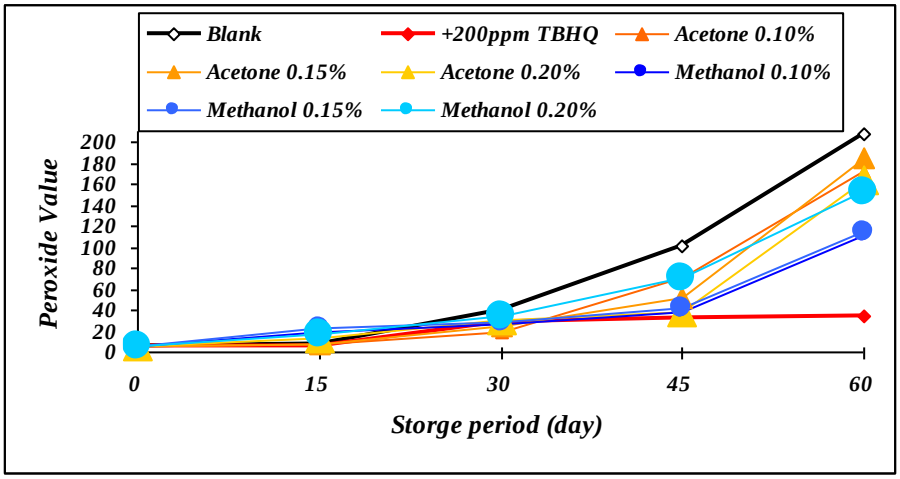

Fig.(5): Peroxide value (meq. O_2/Kg oil) changes during storage at 63°C of treated sunflower oil samples. (Black Berry Leaves extract)

Generally, extracts from vegetable materials showed antioxidant activity in some cases were similar to that of synthetic antioxidants and their extraction could be alternative for obtaining natural extracts *(Bonitta et al., 1999)*.

As statistical analysis for peroxide value data, significant differences between all oil treatments could be observed in certain time periods (15, 30, 45 and 60days) at probability 5%. While, no significant differences were noticed between the control sample and sample E at the end of storage period.

3- Antioxidant effectiveness (AE) during storage of sunflower oil at 63°C:

Data in Table (3) illustrated the percentage of antioxidant effectiveness of sunflower oil treated with various extracts of white and black berry leaves by different solvent (acetone and methanol) compared with synthetic antioxidant (TBHQ) during storage for 60 days at 63°C. Data revealed that the percentage of antioxidant effectiveness (AE) ranged from 30.04 to 83.76% for 200 ppm TBHQ during storage.

While, the percentage of (AE) ranged from 7.05 to 82.62% for sample E during storage period. The same percentage varied from 18.79 to 79.49% for sample F after 60 days of storage period.

The same date indicated that the average of antioxidant effectiveness percentage of acetonic extracts were slightly higher than those of methanolic extracts. Similarly, the average of antioxidant effectiveness percentage of white berry leaves extracts were higher than those of black berry leaves extracts. These observations were found despite of differences in some cases.

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

Code	Treatments			Storage period/day			
				15	30	45	60
A	Blank (Sun flower oil)			-	-	-	-
B	Control (Blank+200ppm TBHQ)			30.04	31.21	68.61	83.76
C	White Berry Leaves	Acetone	0.10%	-20.52	45.01	38.99	33.30
D			0.15%	20.77	32.04	71.16	27.77
E			0.20%	7.05	37.66	66.73	82.62
F		Methanol	0.10%	18.79	48.38	69.68	79.49
G			0.15%	1.48	24.66	58.82	17.69
H			0.20%	33.99	51.06	70.23	43.00
I	Black Berry Leaves	Acetone	0.10%	3.71	53.85	30.36	17.24
J			0.15%	13.97	38.75	48.58	10.61
K			0.20%	-69.47	27.06	63.11	20.68
L		Methanol	0.10%	-132.01	34.73	62.11	46.70
M			0.15%	-194.93	29.01	59.69	45.18
N			0.20%	-102.97	16.61	30.70	26.04

4- TBA value:

Data in Table (4) and Figures (6 and 7) illustrated the effect of adding white and black berry leaves extracts with different solvents on thiobarbituric acid (TBA) value in sunflower oil during storage period at 63°C for 60 days. Results revealed that TBA values in control oil sample (200ppm TBHQ) ranged from 0.515 mg malonaldehyde/kg oil at zero time to 1.170 after 45 days of storage. The same data indicated that TBA values were clearly affected by various concentrations in both of oil samples treated with white or black berry leaves extracts.

Table (4): Thiobarbituric acid value (TBA) "mg malonaldehyde/Kg oil" changes during storage at 63°C of treated sunflower oil samples.

Code	Treatments			Storage period/day				
				0	15	30	45	60
A	Blank (Sun flower oil)			0.515	0.835^{abc}	0.694^{a}	1.661^{ghi}	5.585^{de}
B	Control (Blank+200ppm TBHQ)			0.515	0.601^{ab}	0.530^{a}	1.170^{bcdefg}	0.445^{a}
C	White Berry Leaves	Acetone	0.10%	0.515	2.480^{h}	0.710^{a}	1.466^{efghi}	6.419^{de}
D			0.15%	0.515	1.872^{efg}	0.593^{a}	1.061^{bcdefg}	2.800^{b}
E			0.20%	0.515	1.661^{def}	0.406^{a}	0.913^{abcd}	5.444^{cd}
F		Methanol	0.10%	0.515	0.780^{ab}	0.460^{a}	1.427^{fghi}	4.196^{c}
G			0.15%	0.515	1.349^{cdef}	0.764^{a}	1.076^{bcdefg}	6.131^{de}
H			0.20%	0.515	1.427^{def}	0.515^{a}	1.303^{cdefgh}	5.663^{de}
I	Black Berry Leaves	Acetone	0.10%	0.515	1.092^{abcd}	0.437^{a}	0.905^{bcde}	6.443^{de}
J			0.15%	0.515	1.349^{cdef}	0.655^{a}	0.811^{abcd}	8.237^{f}
K			0.20%	0.515	1.069^{abcd}	0.484^{a}	0.772^{ab}	6.053^{de}
L		Methanol	0.10%	0.515	1.069^{abcd}	0.530^{a}	0.733^{ab}	6.466^{de}
M			0.15%	0.515	1.131^{bcde}	0.499^{a}	1.303^{defgh}	6.209^{de}
N			0.20%	0.515	2.512^{h}	0.562^{a}	1.981^{i}	6.107^{de}

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

TBA values of all treated oils ranged from 0.515 to 2.512. Although TBA values of all treatments were below 2.512, addition of both berry leaves extracts lead some times to raise TBA values. From the same results, TBA values of sunflower oil treated with white and black berry leaves extracts using acetone or methanol often showed lower values than that of blank sample after 30 and 45 days of storage. However, the highest antioxidant effect was clearly observed in 200ppm TBHQ except after 45 days where, TBA value reached 1.170 mg malonaldehyde/kg oil. The antioxidant effectiveness of white and black berry leaves extracts was slightly observed.

Phenolic compounds are also related to inhibiting the activity of conjugated rings and hydroxyl groups *(Decker, 1995)*. Generally, addition of 0.10, 0.15 or 0.20% from both acetonic and methanolic extracts improved the stability of sunflower oil and had special effect in preventing oxidation steps during storage at 63°C up to 45 days.

As statistical analysis for TBA value data, it could be observed that there were significant differences between all oil treatments in most time periods (15, 45 and 60days) at probability 5%. There was no significant differences between them after 30 days. In conclusion, there was slightly significant differences between the control sample and sunflower oil containing 0.15% acetonic white berry leaves extract (D) at the end of storage period.

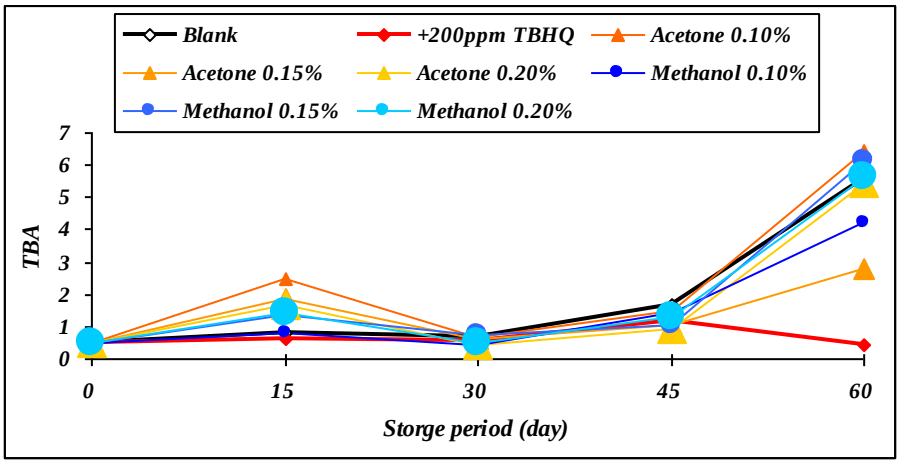

Fig.(6): TBA value (mg malonaldehyde/Kg oil) changes during storage at 63°C of treated sunflower oil samples. (White Berry Leaves extract)

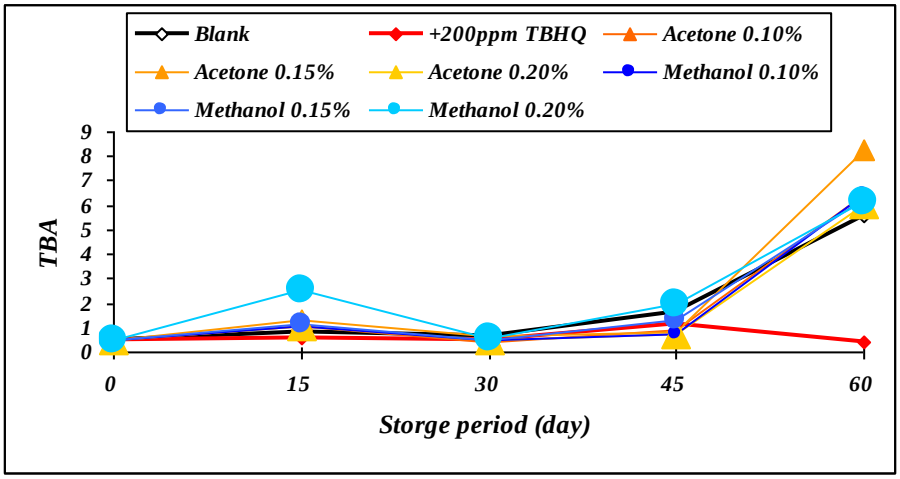

Fig.(7): TBA value (mg malonaldehyde/Kg oil) changes during storage at 63°C of treated sunflower oil samples. (Black Berry Leaves extract)

CONCLOSION

In this manuscript, it could be emphasized that many food wastes are natural antioxidant sources, such as seeds, peels, and leaves. Also, extract production is a key step for obtaining an acceptable antioxidants yield. Besides, conventional extraction with solvent as acetone and methanol offer a good yield and preserve the properties of extracted antioxidants.

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فاعلية المركبات الفينولية من أوراق التوت الأبيض و الأسود كمضادات أكسدة

شادي محمد محمود الشهاوي ، جيهان علي عوض غنيم ، فاتن يوسف إبراهيم و رانيا إبراهيم الجمال
قسم الصناعات الغذائية – كلية الزراعة– جامعة المنصورة – مصر

تزايد الطلب في الآونة الأخيرة على مضادات الأكسدة الطبيعية نظراً لتفضيل المستهلك لها كما أنها أكثر فائدة صحية من نظيرتها الصناعية. لذا كان الإستخلاص و التعرف على المركبات المضادة للأكسدة في كلاً من مستخلصات أوراق التوت الأبيض و الأسود ثم تقييم هذه المركبات كمضادات أكسدة هو الهدف الرئيسي من هذه الدراسة. تم تقدير المواد الفينولية في مستخلصات الأسيتون و الميثانول وتم التعرف على هذه المركبات باستخدام الفصل الكروماتوجرافي عالي الكفاءة. و تم قياس فاعلية هذه المركبات كمضادات أكسدة بتغير رقم الحامض ، رقم البيروكسيد و قيمة حمض الثيوباربيتوريك لزيت دوار الشمس خلال ٦٠ يوم من التخزين على درجة حرارة ٦٣°م لدرجة محفزة لعمليات الأكسدة. احتوى مستخلص الميثانول لأوراق التوت الأسود على ١٠٠,٥٤ مجم/مل كحامض جاليك ، بينما احتوى مستخلص الأسيتون لنفس الأوراق على ٣١,٤٣مجم/مل. و كان لكل المستخلصات تحت الدراسة تأثير كمضادات أكسدة. و قد كان زيت دوار الشمس المعامل بمستخلص الأسيتون لأوراق التوت الأبيض بنسبة ٠,٢٠% الأفضل من حيث رقم البيروكسيد حيث مثل ٣٦,٠٦ و كذلك تراوحت فاعليته كمضاد أكسدة من ٧,٠٥% في بداية فترة التخزين حتى ٨٢,٦٢% في نهاية الفترة. أما بالنسبة لحمض الثيوباربيتيوريك فكان زيت دوار الشمس المعامل بـ ٠,٢٠% مستخلص ميثانول لأوراق التوت الأسود هو الأعلى حيث مثل ٢,٥١٢ مجم/كجم بعد ١٥ يوم من التخزين. من وجهة نظر إقتصادية نستطيع أن نقول أن مخلفات الفاكهة كمصدر طبيعي لمضادات الأكسدة من الممكن أن تلعب دوراً مهماً في صناعة الزيوت الغذائية.

قام بتحكيم البحث

أ.د / أبو بكر محمد حسن	**كلية الزراعة – جامعة المنصورة**
أ.د / محمد بسيم عطا	**كلية الزراعة – جامعة طنطا**