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EFFECT OF GRAPE SEED EXTRACT ON LIPID OXIDITION AND HYDROPEROXIDE FORMATION IN SOYBEAN OIL

Abdalla .E. El-Hadary [a], Nawal .A. Tahoon [b]

[a] *Biochemistry Dept., Fac. Agric., Benha University, Egypt*
[b] *Home economics Dept., Fac. Specific education., Benha University, Egypt*

ABSTRACT

Efficiency of grape seed extract (GSE) on inhibition of lipid oxidation of soybean oil in comparison to butylated hydroxytoluene (BHT) was studied. Grape seed was extracted with ethanol 80% and total phenolic content was (90.26 mg/100gs dry weigh). 2, 2-diphenyl-picrylhydrazyl (DPPH) radical scavenging activity was used to assess the antioxidant efficacy of GSE. HPLC analysis of GSE sample showed that the extract contained phenolic acid such as gallic acid, caffeic acid, *m*-hydroxy benzoic acid and coumaric acid. GSE was examined for antioxidant activity under accelerated oxidation condition using soybean oil as oxidation substrates at different concentrations (200, 400, 600, 800 and 1000 ppm) for 10 days at 70˚C. Inverse relationships between peroxide values and oxidative stabilities were noted and also between secondary oxidation products. Conjugated dienes and conjugated trienes were increased gradually with the increase of storage time. Results indicated that GSE exhibit stronger antioxidant activity than butylated hydroxytolune (BHT).The obtained result inducted that GSE has great potential to be used as a natural antioxidant.

Keywords: Antioxidant activity; Grape seed extract; phenolic acid; Lipid oxidation

INTRODUCTION

In the last few years, an increased attention has been focused on the industrial wastes, especially those containing residual phenols from raw materials. Grape (*Vitis vinifera*) is one of the world's largest

fruit crops, which approximates an annual production of 58 million metric tons **(FAO, 1997)**. Grape is phenol – rich plant, and these phenolics are mainly distributed in the skin, stem, leaf and seeds of grape. Total concentration of phenolic compounds was about217.8 mg/g GAE (gallic acid equivalent) **(Pastrana-Bonilla *et al.*, 2003)**.Antiradical and antioxidant activities of plant extracts have been confirmed by 2, 2-diphenylpicrylhydrazyl (DPPH) and phosphomolybdenum complex methods **(Jayaprakasha *et al.*, 2003). Negro *et al.* (2003)** found that red grape a rich source of polyphenolic compounds with a clear antioxidant activity for ethanolic extract from red grape marc and its component (peel and seeds). The antioxidant activity has manifested itself in the protection of β- carotene in emulsion with linoleic acid against oxidation. Polyphenolic compounds such as gallic acid, *m*-hydroxy benzoic acid , syringic acid, ellagic acid, *p*-coumaric acid, caffeic acid, sinapic acid and catechins are found in grape peel and seed extracts **(Ghafoor *et al.*, 2011) .**Lipid peroxidation is one of the major reasons for deterioration of food products during processing and storage. The addition of antioxidants is considered as one of the methods of increasing shelf life, especially of lipids and lipid-containing foods. Synthetic antioxidant, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have restricted use in foods as these synthetic antioxidant are suspected to be carcinogenic **(Jayaprakasha *et al.*, 2001)**.Grape seed extract (GSE) was used to stabilize sunflower oil **(Shaker, 2006)**. **Rababh *et al.*, (2011)** compared grape seed extract with synthetic antioxidants on lipid oxidation of corn chips during storage. GSE could be very effective in inhibiting lipid oxidation of cooked turkey meat during chill-storage **(Mielnik *et al.*, 2006) .**On the other hand, **Brannan (2009)** studied the effect of GSE in precooked chicken breast systems. The objective of this study was to investigate the effects of adding the GSE on lipid oxidation of soybean oil. Consequently, the comparison of the effectiveness between synthetic and natural antioxidants on lipid oxidation of soybean oil were carried out.

MATERIALS AND METHODS

MATERIAL

1. Grape (*Vitis vinifera*) seeds were obtained from local market

2. Chemicals:-

2, 2-diphenylpicrylhydrazyl (DPPH) was purchased from Aldrich. Folin-ciocalteu reagent was purchased from Sigma.

METHODS

1. Ethanolic extraction of grape seeds:

Grape seeds were dried in drying oven at 50ºC for 3 days. Grape seeds were ground to fine powder. About100g powder was extracted with 500 ml of 80% ethanol and left for one week at room temperature in dark bottle. The extract was then filtered through Büchner funnel followed by removal of ethanol using vacuum rotary evaporator (N-N series, EYELA, Japan) at 40˚C.The obtained residue was kept at 4 ˚C until further use according to **Ahn *et al.* (2002).**

2. Determination of phenolic compounds:

Total phenolics in the ethanolic extracts of grape seeds were determined according to the Folin-Ciocalteu procedure **(Singleton and Rossi, 1965).**

3. Determination of antiradical activity:

The free radical activity of the GSE was determined using 2, 2-diphenylpicrylhydrazyl (DPPH) according to **(Lee *et al.*, 2002).**

4. Analysis of bioactives using high performance liquid chromatography:

The HPLC system consisted of a GBC system with GBC pump LC1110, UV/Vis detector, outsampler and degasser. Data processing was performed by using the software Win Chrome Chromatography Ver. 1.3. Separation was performed on HYOPERCARB5U C18 Column (100* 4.6 mm) at room temperature. Injection volume was 20µL, flow rate was set at 1 mL/min and UV detection was carried out at 254 nm. Solvent consisted of 2% acetic acid and 50% acetonitrile.

5. Preparation of Soybean oil with antioxidant:

Antioxidants were added at five concentration levels for GSE (200,400,600,800 and 1000 ppm) beside two levels of BHT (200 and 400ppm) in soybean oil. Samples were stored in brown glass jars (50ml) in dark at 70˚C for ten days. Three replicate oil samples with

and without antioxidants were carried out for each treatment and each time for analysis

6. Determination of peroxide value (PV):

Peroxide value of samples was measured according to **Kirk and Sawyer (1991).**

7. *p-Anisidine value (AV).*

The *p*-Anisidine value (AV) was determined according to Cd 18-90 method **(AOCS, 1995)**, by using a spectrophotometer (UV-1800 spectrophotometer, TOMOS, Italy).

8. Conjugated dienes (CD) and conjugated trienes (CT).

Specific extinctions at 232 nm and 270 nm (i.e., conjugated dienes and conjugated trienes) were determined using a spectrophotometer (UV-1800 spectrophotometer, TOMOS, Italy). Oil samples were diluted with iso-octane to bring the absorbance within limits following the standard method of **IUPAC method II. D.23 (IUPAC, 1979).**

9. Statistical analysis of data:

Data were analyzed using SAS program **(SAS, 1996)**. Differences between means were tested ($P < 0.05$) based on Duncon's method using PROC MEANS **(SAS, 1996).**

RESULTS AND DISCUSSION

1. Phenolic compounds and DPPH scavenging activity of grape seeds extract.

From the data presented in Table (1) it is clear that values of the ethanolic extract of GSE was 15.98% and total phenolic contents of GSE was 90.26 mg/100g. These results are in good agreement with those reported by **(Ghafoor *et al.*, 2011).** $DPPH^{\bullet}$ is a stable radical showing a maximum absorbance at 515 nm. In $DPPH^{\bullet}$ assay, the antioxidants were able to reduce the stable radical DPPH to the yellow colored diphenyl picrylhydrazone. The method based on the reduction of $DPPH^{\bullet}$ in alcoholic solution in the presence of a hydrogen-donating antioxidant due to formation of the non-radical from DPPH-H in the reaction. The disappearance of the DPPH radical absorption at 515 nm by action of antioxidant is taken as a measure of antioxidant activity. Grape seed extracts showed strong scavenging activity against $DPPH^{\bullet}$ radicals **(Table, 1).** Samples which contain low

phenolic compound had also lower antioxidant activity. The antioxidant activities of phenolic compounds may be due to the number of hydroxyl groups on the aromatic ring. The higher number of hydroxyl groups, the greater expected antioxidant activity.

Table (1): Extract yield, phenolic content and antiradical activities of grape seeds extracts.

Material / Parameter	Grape seed
% Total ethanolic extracts	15.98
*Total phenolic content mg/100g	90.26
%Antiradical activity	61.39

*Calculated on the dry weight basis

2. Identification of phenolic compounds from grape seed extract.

Phenolic acids, i.e. gallic acid, caffeic acid, *m*-hydroxy benzoic acid and coumaric acid contents were evaluated for sample of GSE by using high performance liquid chromatography. Figure (1) showed the Chromatogram of GSE sample. Benzoic acid derivatives including gallic acid, caffeic acid, *m*-hydroxy benzoic acid and coumaric acid were detected in grape seed extract. **(Shi *et al.*, 2003)** showed that polyphenol compounds in GSE were mainly flavonoid, including gallic acid, the monomeric flavan-3- ols catechin, epicatechin, gallocatechin, epigallocatechin and procyanidin dimmers were observed.

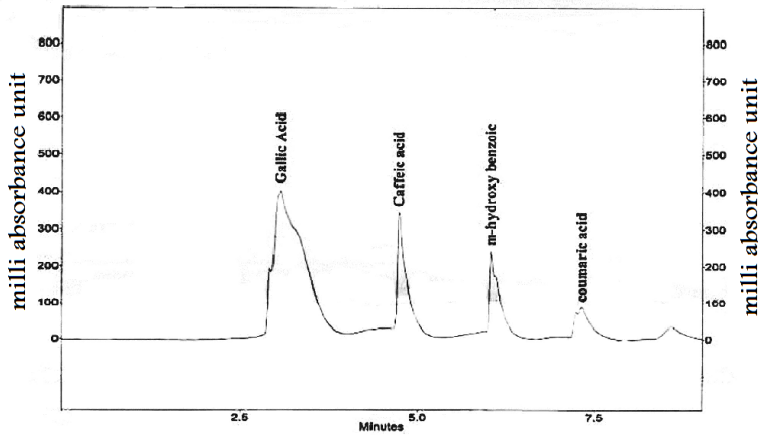

Fig (1): HPLC profile of grape seed extraction by 80% aqueous solution of ethanol at room temperature.

3. Effect of grape seed extract on the stability of soybean oil.

Oil stability is usually determined under accelerated oxidation conditions (60°C or more) because ambient conditions demand an excessively long period. To evaluate the antioxidant efficacies of the extracts in soybean oil, peroxide value (PV), Anisidine value (AV), conjugated diene (CD) and conjugated triene (CT) were determined as indicators of lipid oxidation. The oxidative stability studies were carried out at 70◦C in an oven. This temperature was ideal, because at higher temperatures the peroxides will be decomposed very fast **(Duh and Yen, 1997 and Mariod *et al.*, 2010).**

3.1. Effect of grape seed extract on peroxide value of soybean oil.

Peroxide value (PV) is one of the most widely- used tests for the measurement of oxidative rancidity in oils and fats. Fig (2) showed the PV developments during the storage of soybean oil at 70°C for ten days using various concentration of GSE, additional treatments included BHT at 200ppm, 400ppm and control without additives. The PV of soybean oil containing GSE or BHT was lower than the control. GSE, which contained a large amount of polyphenolic and phenolic compounds **(Rababh *et al.*, 2011),** and BHT minimized lipid oxidation in soybean oil. The lower PV could be due to the inhibition of free radical formations during the initiation step, interruption of the propagation of the free radical chain reaction by acting an electron donor, or scavenger of free radicals in soybean oil samples. The PV increased significantly during storage(2.53- 35.07 mequiv / kg) in the control and treated samples with GSE at 200 , 400 and 600 ppm level (2.44-15.12 mequiv / kg) , (2.4-11.35 mequiv / kg) and (2.35- 8.31 mequiv / kg) respectively . Whereas, no significant changes in PV were observed due to the addition of GSE at the 800 and 1000 ppm levels. These results agreed with the results reported by **(Rababh *et al.*, 2011)** who found GSE has great potential for use as a natural antioxidant to preserve the extruded corn chips.

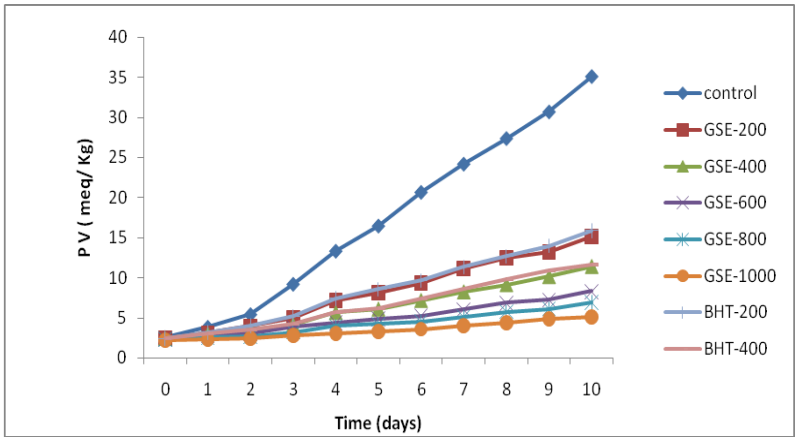

Fig (2): Relative increase in peroxide value (PV) of treated soybean oil samples

3.2. Effect of grape seed extract on - *p*-Anisidine value (AV) of soybean oil.

The- *p*-Anisidine value (AV), which measures the secondary oxidation products produced during the oxidative degradation of oil, was determined by reacting - *p*-Anisidine with the oil in iso-octane and the resultant colour was measured at 350 nm. *p*-Anisidine value (AV) plays an important role in the oxidation process of edible oil and fats. Calculating AV is one of the oldest methods for evaluating secondary lipid oxidation.

Fig (3) depicts the *p*-Anisidine values for soybean oil samples stabilized with ethanolic extracts, BHT and control. The - *p*-Anisidine value of control reached its maximum of 20.12± 0.15 after ten days of storage. While the values after treatments with GSE extracts at 200,400, 600, 800, 1000 ppm and BHT at 200, 400 ppm were 6.13± 0.12, 4.96±0.12, 4.05±0.12, 3.68±0.12, 2.96±0.12, 6.68±0.12 and 5.61±0.12, respectively. Significant differentiations were noted between the values for control and experimental samples. The results demonstrated that GSE had higher antioxidant activity than BHT.

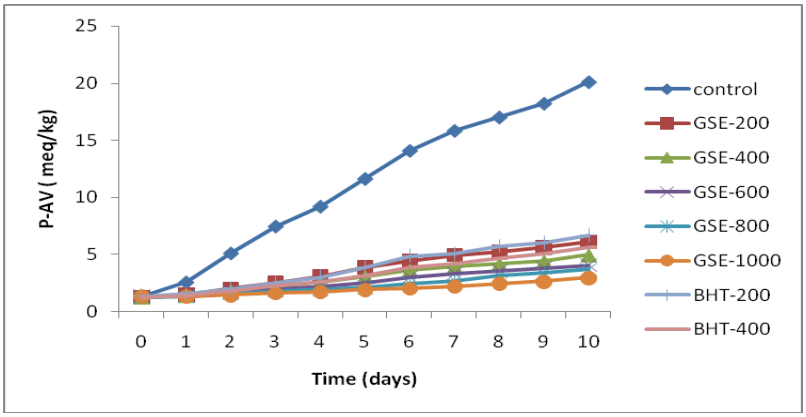

Fig (3): Effect of grape seed extracts - *p*-Anisidine values (AV) of soybean oil.

3.3. Effect of grape seed extract on conjugated diene (CD) and conjugated triene (CT) of soybean oil.

The assessment of CD and CT is a good parameter for measurement of oxidative deterioration of oils, hence indicates the effectiveness of antioxidants in oils **(Shahidi &Wanasundara, 1997)**. Conjugated dienes (CD) and conjugated trienes (CT) contents of soybean oil samples stabilized with GSE, BHT and control are shown in fig 4 (A) & (B) .CD and CT contents increased parallel to increase of storage time with greater rate for control. The oil samples stabilized with GSE showed lower levels of CD and CT comparing to control, indicating antioxidant potential of GSE components.

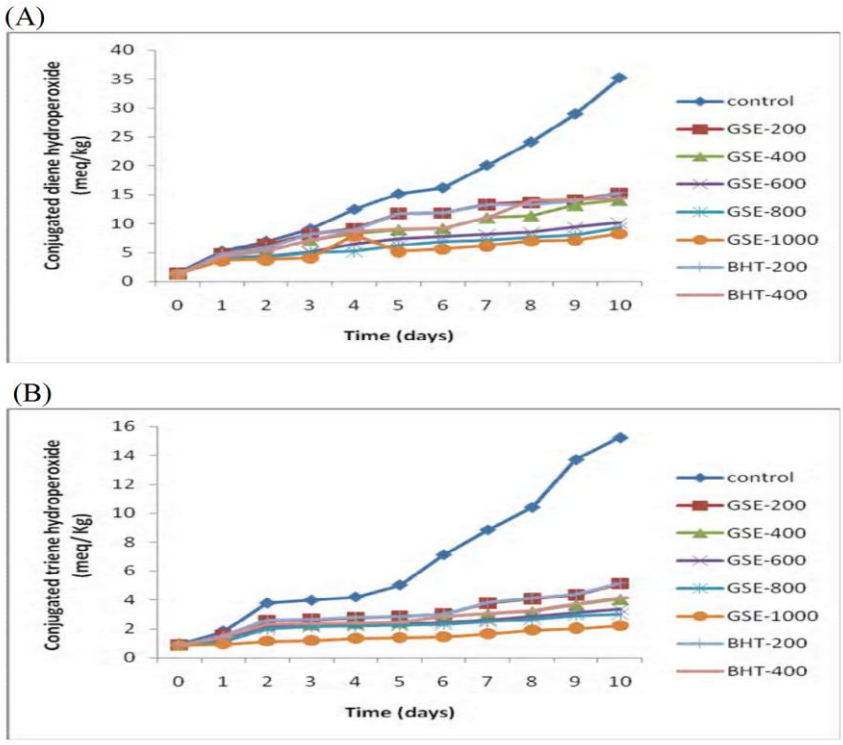

Fig (4 A, B): Relative increase in conjugated dienes of treated soybean oil samples (A) and conjugated trienes (B)

CONCLUSION

From the present study, it can be concluded that GSE may stabilize soybean oil and very effectively at all concentrations. They inhibit thermal deterioration of oil by improving its hydrolytic stability, inhibiting double bond conjugation and reducing the loss of polyunsaturated fatty acids. Grape seed extract at concentration of 200,400, 600, 800 and 1000 ppm have potential stabilization efficiency comparing to commonly- employed synthetic antioxidant BHT at their legal limit. Grape seed extract has a strong antioxidative effect during initial and final steps of oxidation in dark in an oven at 70 ˚C for 10 days. Therefore, GSE can be recommended as a potent source of antioxidants for the stabilization of food systems, especially unsaturated vegetable oils. The phenolic compounds appeared to be responsible for the antioxidant activity of GSE, although further

studies are required to reveal whether they contain other antioxidative constituents. In addition, in vivo evidence and isolation of antioxidant components in grape seed need to further investigation.

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تأثير مستخلص بذور العنب على أكسدة الليبيدات والهيدروبيروكسيدات المتكونة فى زيت فول الصويا

عبدالله السيد الحضرى [*] ، نوال عباس طاحون [**]

[*] قسم الكيمياء الحيوية الزراعية – كلية الزراعة – جامعة بنها
[**] قسم الاقتصاد المنزلى - كلية التربية النوعية – جامعة بنها

- يهدف هذا البحث الى دراسة تأثير المستخلص الكحولى لبذور العنب كمضاد للاكسدة لزيت فول الصويا وذلك بالمقارنة بأستخدام مضادات أكسدة صناعية مثل (BHT) . تم عمل مستخلص كحولى لبذور العنب وذلك بأستخدام كحول ايثايل ٨٠% وتم تقدير الفينولات الكلية فى هذا المستخلص وكانت نسبتها ٩٢,٦٢ ميلليجرام/ ١٠٠ جرام بذرة جافة .كما تم تقدير نشاط المستخلص كمضاد للاكسدة معمليا بأستخدام (DPPH) .

- تم التعرف على مكونات المستخلص الكحولى وذلك بأستخدام جهاز التحليل الكروماتوجرافى السائل وأظهرت النتائج الى ان المستخلص يحتوى على المركبات التالية حمض الجاليك ، حمض الكافيك ، ميتا هيدروكسيى حمض البنزويك ، حمض الكيوماريك.

- تم عمل تجربة تطبيقية وذلك بأضافة المستخلص الكحولى لبذور العنب بعد تركيزة وتجفيفة تحت ضغط وذلك بتركيزات مختلفة (٢٠٠، ٤٠٠، ٦٠٠، ٨٠٠، ١٠٠٠ جزء فى المليون) الى زيت فول الصويا لمدة عشرة أيام على درجة حرارة ٧٠ م° وذلك بجانب المقارنة بعمل تركيزين من مضادات الاكسدة الصناعية (BHT) (٢٠٠ ، ٤٠٠ جزء فى المليون) وتم تقدير رقم البيروكسيد ، رقم الانيزيندين ، الروابط الزوجية المتبادلة الثنائية ، الروابط الزوجية المتبادلة الثلاثية.

- أظهرت النتائج ان المستخلص الكحولى لبذور العنب له نشاط قوى كمضاد للاكسدة بالمقارنة بمضادات الاكسدة الصناعية ويوصى باستخدامة كمضاد أكسدة طبيعى لمنع تزنخ الزيوت النباتية .