J. Food and Dairy Sci., Mansoura Univ., Vol. 8 (7): 275 – 278, 2017

Utilization of *Faba Bean* Hulls (Seeds Coat)as a Source to Produce Antioxidants

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ABSTRACT

The current research investigates the opportunity of using *Faba bean* hulls (seeds coat) for producing some bioactive compounds such as natural antioxidants using several solvents, and to investigate the stability of edible oil with phenolic extract against oxidative rancidity compared with other containing (BHA) during heat at 180°C for one day. The results show that ethanol gave the highest yield extract and polyphenol content. Then it yields methanol, chloroform and water. The HPLC analysis is executed. It provides the total polyphenols extracted from *faba bean*. The hulls were indicated to the existence of high Pyrogallol, e-Vanillic, Ellagic and Catechin percentages. Also, the results showed that oils which treated by the extract at varied concentrations have PV and TBA values lower than control.

Keywords: *faba bean*; antioxidant activity; hulls; methanolic extract.

INTRODUCTION

It gradually becoming more recognized that oxidation stress by increasing oxygen and nitrogen reactive species production cause many diseases. Basically, it raises cancer and cardiovascular problems, which dramatically increase the worldwide mortality (Peuchant *et al.,* 2004). for the food manufacture, oxidation reactions are a main apprehension. The antioxidants are prerequisite on a large scope to protect against oxidative changes in food. However, they are requirement for antioxidants use in other oxidisable substances, such as cosmetics and drugs is in increment too (Shahidi and Naczk, 1995). In the food manufacture, artificial antioxidants are commonly used due to their efficiency and low cost than natural antioxidants. However, numerous investigators have revealed that, artificial antioxidants have mischievous effects because of their carcinogenicity and toxicity (Williams *et al.,* 1999). The mounting interest in substituting synthetic antioxidants by natural ones has bring up research on the screening of agricultural waste for finding new antioxidants. Researchers have focused on their extraction from in cheap sources such as agro-industrial wastes (Arai *et al.,* 2002). *Faba bean* is a versatile crop of *fabaceae* family. It is pulse crop commonly grown in mixed rain fed dry land or the agro pastoral systems in many parts of the world including North Africa. Egypt was the headmost biggest importer of *faba beans* in the world (FAO, 2011). The exfoliation process for faba bean produces 14.28% hulls (Güzel and Sayar 2012). They are disposed of as a junk or used for animal mash. The total hull produced is about 20% of the overall treated amount. Thus, this manufacture produces a great quantity of bio-waste, which is affluent in biological components. It could be prospect foundation of antioxidants such as polyphenols (Kanatt *et al.*, 2011). Various solvents have been applied for polyphenols extraction from materials (Chavan *et al.,* 2001). Extraction output is contingent on extraction procedure and solvent type (Goli *et al.,* 2005). The extraction manner necessitates authorize full extraction of the components of concern and should avert their alchemical modification (Zuo *et al.*, 2002).

The purpose of this research is to extract phenolic components from Faba bean hulls, then distinguish and utilize it as naturalistic antioxidants for maintenance refined oil (mixture of sunflower and soybean at 1:3 ratio) during storage at 180°C.

MATERIALS AND METHODS

1. Materials

Faba bean hulls (seeds coat) were obtained from feed market, Kafr El- Sheikh Governorate, Egypt. Antioxidants free oil extracted from mixture of sunflower and soybean oil about (1:3 ratio) was acquired from Tanta Company for oils and soaps, Tanta, Egypt. Synesthetic antioxidant (BHA) and 1,1- diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma chemical company (St. Louis, Mo., U.S.A.). All solvents and chemicals applied in this study were purchased from El- Gomhorea Company, Tanta, Egypt.

2. Sample preparation for antioxidant extracts

Hulls were soaked in distilled water at 2:1 (v/w) water / hulls for 6 hours after that they were oven dried (40oC) and crushed into fine powder. These fragments were put in polyethylene bags and stored at room temperature until used.

3. Approximate chemical composition

The methods described in the A. O.A. C. (2005) were utilized to determine moisture; crude protein; ether extract; ash and crude fiber contents. The total carbohydrates were calculated by difference.

4. Extraction of total phenolic components

Five grams of intended samples were homogenized in 50 mL of each solvent (absolute methanol, ethanol 70%, chloroform and water) and left for a day at room temperature. Then, they were filtrated through filter paper (What man No. 1). Finally, the supernatant were vaporized under vacuum in rotary evaporator at 45°C and weighted to set the yield (McGrath *et al.,* 1982).

5. Total Phenol content determination

Total phenol concentration was measured using Folin-Ciocalteau as reagent (Gutfinger, 1981).

6. Phenolic compounds identification

Implementing the method summed up by Evangelisti, *et al.* (1997), The Hulls Phenolic components were identified

7. Scavenging effect assay of *Faba bean* hulls extracts

Faba bean hulls extracts free radical scavenging activity was measured by 1, 1-diphenyl- 2- picryl-hydrazyl (DPPH) rendering to the procedure of Sousa *et al.* (2008).

8. Antioxidative efficiency of *Faba bean* hulls extract

Faba bean hulls extracts antioxidative activities were assayed according to Lee *et al.* (2004).

1. Peroxide values (PV)

Stored oils peroxide values were measured as outlined by Leonard *et al.,* (1987). They are stated as the sample's mill equivalents of peroxide per kilogram.

2. Thiobarbituric acid number (TBA)

Thiobarbituric acid values were measured according to the procedure explained by Sidwell *et al.* (1990). Expressed as mg malondialdhyde per 1000gm oils.

9. Statistical analysis

Results were analyzed of variance (ANOVA). The SAS ANOVA program (Statistical analysis system, 1988) was implemented. Duncan's multiple range test (Duncan 1955) was used to compare the differences among individual means.

Approximate chemical composition of *faba bean* hulls:

Table (1) shows gross chemical composition of *faba bean* hulls. Data show that moisture, crude protein, ether extract, ash, crude fiber and available carbohydrate of *faba bean* hulls were 14.86, 2.30, 1.16, 1.86, 12.59 and 67.23%, respectively. Those results are in the same line with those reported by Nigus *et al.* (2015)

Table 1. Gross chemical composition of *faba bean* hulls (%on dry weight basis).

Component (%)	Moisture	Crude protein	Ether extract	Ash	Crude fibers	Total carbohydrate*
Faba bean hulls	14.86	2.30	1.16	1.86	12.59	67.23

***Total carbohydrates were calculated by difference**

Effect of using various solvents on extraction yield and total polyphenol content of *faba bean* hulls

Extraction yield and total polyphenol contents of *faba bean* hulls are tabulated in Table 2. The data revealed that using water gave the highest value of extracted yield reaching 18.43%, followed by ethanol 15.82%.

Table 2. Various solvents effect on extraction

Extraction solvent	Extraction yield (%)	Total polyphenols (mg GAE/g)
Ethanol	15. 82^{b}	50.65^{a}
Chloroform	15.69^{b}	50.05^{a}
Methanol	13.95^{c}	44.24^{b}
Water	18.43^{a}	2.94^{c}

Values followed by the same letter in the same column are not significantly different P≤ 0.05.

On the other side, the results refer that ethanol was the bestead solvent for excavate polyphenolic components from faba bean hulls. High amounts of polyphenolic compounds were given with ethanol (50.65mg GAE/g), in comparison with others. These results are in accordance with those achieved by Amarowicz *et al.* (1996).

Table 3. Phenolic compounds in ethanolic extract of *faba bean* hulls

Compounds	% of the Total	Compounds	% of the Total
Gallic acid	1.27	Ferulic	0.35
4-Amino benzoic acid	0.87	Isoferulic	0.23
Protocatchuic	1.67	Reversetrol	0.05
Catechin	1.74	Ellagic	2.79
Chlorogenic	0.36	Alpha-Coumaric	0.21
Catechol	1.41	e-Vanillic	7.31
Epicatechin	0.44	Benzoic	0.94
Caffeine	0.50	3,4,5 Methoxycinnamic	0.05
Caffeic	0.12	Coumarin	0.12
Vanillic	0.54	Salycilic	0.29
β-coumaric acid	0.43	Cinnamic	0.09
Pyrogallol	78.13		

Identified phenolic compounds extracted from *faba bean* hulls

HPLC procedure was utilized to fractionate and identificate the polyphenolic components extracted from the tested sample. The results were tabulated in Table (3).

The data obtained revealed that, *faba bean* hulls ethanolic extract have 23 phenolic components. Pyrogallol, e-Vanillic, Ellagic and Catechin were the main phenolic components given and determined in the ethanolic extract. These results are in the same line with others obtained by Amarowicz *et al.* (1996).

Antioxidant activity

Results given in Table (4) revealed that ethanolic extract has the highest activity followed by methanol, chloroform and water extract at the minimal concentrations (100 and 200 ppm).

Table 4. Antioxidant activities of various solvent extracts from *faba bean* hulls in DPPH assay

Extract type	DPPH reduction %	
	100 ppm	200 ppm
BHA	83.76^{a}	94.55^{a}
Ethanol extract	56.47^{b}	73.92^{b}
Methanol extract	44.28^{c}	61.05^{c}
Chloroform extract	31.12^{d}	39.81^{d}
Water extract	5.71^{e}	11.20^{e}

BHA = butylated hydroxyl anisole, DPPH = 1,1-diphenyl-2-picrylhydrazyl.

Values followed by the same letter in the same column are not significantly different P≤ 0.05.

Effect of using various concentrations of natural extracts and synthetic antioxidants on PV

Table (5) presents the change in PV of mixture of sunflower and soybean oil at 1:3 ratios without antioxidants and others contained artificial and natural antioxidants. Generally, the presence of these components leads to reduction the peroxide value increment in comparison with oil without antioxidants. However, the data showed that there was gradually increment in peroxide value during heating and the increment was higher in case of untreated more than treated samples. The level of the untreated oils increase was greater than all samples of treated oils. Results are in agreement with those of Habib and Shah

(2004) who revealed that PV was significantly decreased by using antioxidants in processed oils and foods.

Table 5. peroxide value per milli-equivalent O_2/ kg oils

Time (hr)	Control	BHA	Methanol	
		200 ppm	200 ppm	300 ppm
0	1.2[a]	1.2[a]	1.2[a]	1.2[a]
4	11.3[a]	10.3[d]	10.9[b]	10.3[c]
8	29.3[a]	19.7[d]	25.3[b]	22.8[c]
12	33.6[a]	22.8[d]	30.9[b]	27.6[c]
16	39.4[a]	26.5[d]	35.3[b]	32.1[c]
20	44.2[a]	32.8[d]	41.5[b]	37.9[c]
24	53.8[a]	36.3[d]	46.3[b]	41.3[c]

Sunflower and soybean mixture oils (1:3 ratio) treated by faba bean hulls phenolic extract during storage at 180°C. Values followed by the same letter in the same column are not significantly different P≤ 0.05.

Effect of using various concentrations of natural extracts and BHA on Thiobarbituric acid (TBA) of oils

Fresh and heated sunflower and soybean oil mixture (1:3 ratios) TBA value was measured and the data are tabulated in Table (6). There was highly increment in TBA values with increasing the storage period. Control sample TBA value was (2.530 mg malonaldhyde/kg oil). Meanwhile, TBA values were 1.620, 2.022 and 1.870 mg malonaldhyde/kg oils for BHA, Methanol 200ppm and 300 ppm, respectively. Those results were in the same line with Farag *et al.* (2007).

Table 6. Thiobarbituric acid number mg malondialdhyde / kg oils.

Time (hr)	Control	BHA	Methanol	
		200 ppm	200 ppm	300 ppm
0	0.040[a]	0.04[a]	0.04[a]	0.04[a]
4	0.330[a]	0.142[d]	0.293[b]	0.234[c]
8	0.825[a]	0.376[d]	0.649[b]	0.530[c]
12	1.191[a]	0.549[d]	0.772[b]	0.882[c]
16	1.645[a]	0.998[d]	1.400[b]	1.194[c]
20	2.103[a]	1.334[d]	1.752[b]	1.502[c]
24	2.530[a]	1.620[d]	2.022[b]	1.870[c]

Sunflower and soybean mixture oils (1:3 ratio) treated by faba bean hulls phenolic extract during storage at 180°C. Values followed by the same letter in the same column are not significantly different P≤ 0.05.

CONCLUSION

In conclusion, it was found that ethanol give the highest polyphenols extraction from *faba bean* hulls. Additionally, several extracts of faba bean hulls appeared powerful antioxidant activity less than the BHA. The antioxidative efficiency can be due to the existence of phenolic components. Faba bean hulls extract identification referred to existence high levels of Pyrogallol, e-Vanillic, Catechin, and Ellagic. Sunflower and Soybean oil mixture (1:3 ratios)peroxide and TBA values that treated by different levels of methanolic extract were lower than control and the bespoke natural antioxidants level is 300ppm.

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الاستفادة من قشور الفول(غلاف البذرة) كمصدر لإنتاج مضادات الأكسدة

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تهدف هذه الدراسة لتعظيم الاستفادة من قشور الفول (قشر البذور) باستخدامها في استخلاص بعض المركبات الحيوية كمضادات للأكسدة الطبيعية باستخدام المذيبات المختلفة وكذلك دراسة تأثير هذه المركبات المستخلصة علي سلامة الزيوت الصالحة للاستهلاك ضد التزنخ التأكسدي الحادث لها أثناء التخزين علي ١٨٠ درجة مئوية لمدة يوم واحد مقارنة بمضادالأكسدة الصناعي(BHA). وقد أوضحت النتائج أن أفضل مذيب كان الإيثانول متبوعا بالميثانول يليه الكلوروفورم ثم الماء. وقد تم تفريد البولي فينولات الكلية المستخلصة من قشور بذور الفول باستخدام جهاز (HpLC) وأوضحت النتائج أن البيروجالول يليه إ- فانيليك،إيلاجيك والكاتيشين هي أكثر المركبات تواجدا في المستخلص الإيثانولي. وتم اختبار قدرة الاحماض البولي فينولية المستخلصة علي الحفاظ علي جودة الزيوت الصالحة للاستهلاك. وقد أظهرت النتائج انخفاض رقم البيروكسيد ورقم حمض الثايوباربيتيوريك للزيت المعامل بمستويات مختلفة من المستخلص عنه في حالة الكنترول.