

EVALUATION THE ANTIOXIDANT ACTIVITY OF SESAME COAT AND SESAME CAKE EXTRACTS

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ABSTRACT

This study was aimed to evaluate the anti-oxidant activity of methanol extracts from roasted and unroasted sesame seeds cake (RSC, URSC) and ethanol extracts from sesame coat (SC) using DPPH and reducing power activity (RPA) assay. The quantitative and qualitative analysis of obtained extracts were done using RP-HPLC technique, the results revealed that the extracts were contained anti-oxidants (lignans) sesamin, sesamol and sesamol in different amounts. Also the RPA of URSC extract at (10, 20, & 50 mg/ml) was similar to that of BHT, while that for RSC was similar to BHT at 30 mg/ml. Additionally, The RPA SC extract was comparable to that of BHT at (20, 30, and 50 mg/ml). It has been noticed that the radical scavenging ability (RSA) of BHT was greater than of the experimental samples that evaluated by DPPH assay. Mean time the RSA of the studied extracts was increased with increase of extracts concentrations from 10-50 mg/g. Based on the results of this study, sesame coat, sesame cake (from roasted and unroasted sesame seeds) extracts could be used as potential natural anti-oxidant to protect oil-rich food to avoid the possible risks resulted from using the synthetic antioxidants to prevent food.

Key words: sesame hulls, sesame residues, DPPH, reducing power

شامراد وآخرون

مجلة العلوم الزراعية العراقية - 2019: 50(3): 776-782

تقدير الفعالية المضادة للأكسدة لمستخلصات أغلفة السمسم ومخلفات زيت السمسم

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المستخلص

هدفت الدراسة الحالية الى تقييم الفعالية المضادة للاكسدة للمستخلصات الميثانولية لمخلفات زيت بذور السمسم (الكيك) من البذور المحلية غير المحمصّة والمحمصّة والمستخلصات الايثيلية لأغلفة بذور السمسم باستخدام فحص القوة الاختزالية و DPPH. تم إجراء التحليل النوعي والكمي لمضادات الأكسدة (lignan) الموجودة في مستخلص الكيك والأغلفة باستخدام RP-HPLC. أظهرت نتائج RP-HPLC أن مستخلص الكيك ومستخلص الأغلفة يحتوي على السيسامين والسيسامولين والسيسامول ولكن بكميات مختلفة، وكانت القوة الاختزالية للكيك من مستخلص بذور السمسم غير المحمصّة مماثلة لـ BHT عند التراكيز 10 و 20 و 50 ملغم/مل، في حين أن القوة الاختزالية للكيك من مستخلص بذور السمسم المحمص كان مشابهاً لـ BHT عند تركيز 30 ملغم/مل، كان مستخلص الأغلفة مماثل لـ BHT في 20، 30 ملغم/مل. فضلاً عن ذلك كان نشاط الكيك الجذري لـ BHT أعلى في جميع العينات قيد الدراسة المقدرّة باستخدام مادة DPPH، حيث زاد نشاط الكيك الجذري من كل عينة مع زيادة التركيز من 10 إلى 50 ملغم/مل. وفقاً لهذه النتائج، يمكن استخدام مستخلص اغلفة السمسم ومستخلص كيك السمسم (من بذور السمسم المحمصّة وغير المحمصّة) كمضاد للأكسدة الطبيعية لحماية المواد الغذائية الغنية بالزيت لتجنب المخاطر المحتملة الناجمة عن استخدام مضادات الأكسدة الاصطناعية.

الكلمات المفتاحية: غلاف السمسم، مخلفات السمسم (كيك)، DPPH، قوة اختزالي

*Received:22/9/2018, Accepted:17/1/2019

INTRODUCTION

One of the well known procedures to extend the shelf life of lipids and lipid rich food is the addition of antioxidants. Synthetic antioxidants have undesirable effects which lead to many health risks such as heart disease, carcinogenic effects and others (21). Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have limited use in food (5), while the use of tertiary butyl hydroquinone (TBHQ) is not permitted in many countries (15). Sesame seeds (*Sesamum indicum* L.) is one of the important sources for edible oil, it contain about 48-55% oil. It is also rich in protein (20-25%), the residual of oil extraction process is known as sesame cake which contains around 50 % protein depending on the extraction method. Sesame cake extracts contain various lignans (sesamin, sesamol and sesamol) that are phenolic compounds with antioxidant activity (14, 18) Sesame seeds coat contain phenolic components and other components which has antioxidant activity. The presence of natural antioxidants (γ -tocopherols, sesamin and sesamol) in sesame seeds coat enhances the ant-oxidative stability of whole sesame seeds oil (1). Hence, many researchers focused on investigating for natural antioxidant to replace synthetic antioxidant, the natural antioxidant of plant origin (flavonoids, tannins, coumarins, curcumanoids, xanthon, phenolics, lignans ...etc) which presences in fruits, leave, seeds and oils are known to protect lipids and lipid containing foods from oxidation (6). In recent years there is a greet tendency toward conversion of bio-waste to value added compounds, Shui and Leong (17) stated that the antioxidant components of agriculture industrial waste are not only useful for increasing food stability by preventing lipid per-oxidation but also protect bio-molecules and supramolecular structures such as membranes and ribosomes from oxidative damage. Seed wastes from fruit processing and those derived from oil seeds are possess a variety of bioactive substance with many medical properties (12). Chang et al. (2) reported that the sesame coat ethanol extracts analysis using HPLC technique was showed the presence of the lignan (sesamin and

sesamol). Accordingly, the present study carried out to extract the antioxidant compounds from locally cultivated sesame seeds coat and sesame cake and evaluate their antioxidant activities using DPPH and reducing power assay.

MATERIALS AND METHODS

Sample collection

Locally cultivated sesame (*S. indicum* L.) seeds were purchased from local market in Baghdad-Iraq.

Particle impurities such as dust, sands, stones, spoiled seeds, small weed seeds and other extra materials were separated by sieves, the sesame seeds coat were removed and stored at 4°C until used. Sesame cake was obtained from roasted and unroasted sesame seeds by extracting the oil as described by Hadeel and Khalid (4).

Extraction and purification of lignans from sesame coat

The extraction method was adapted from Chang *et al.* (2). Ten grams of sesame coat blended with 100 ml of ethanol using magnetic stirrer at room temperature for 12-16 hour. The ethanol extract was separated and the residual from the first extraction was subjected to a second extraction with a fresh impulse of ethanol(ETH). The ethanol extracts were concentrated using rotary evaporator at 40°C. The obtained extract was subjected to qualitative and quantitative analysis by RP-HPLC.

Extraction and purification of lignans from sesame cake

Fifty grams sesame cake from each roasted and unroasted sesame seeds (SCR, SCUR) were defatted with 400 ml n-hexane in soxhlet apparatus at 60°C for 6 hour. Forty grams of defatted sesame cake was treated with 222 ml of NaCL 10% (w/v) and soaked for 1 hour with occasionally stirring. This process was repeated two more times at 1:3 (v/v) ratios. Then followed by washing with water for three times also at 1:3 ratios, the residue obtained after these washings was dried at 50°C. Ten grams of the dried residue was extracted with methanol (200 ml) in soxhlet apparatus for 12 hour. The methanol extracts were concentrated using rotary evaporator at 60°C, according to method described by Lieu

and Dang (10). The final extract was identified by HPLC.

RP-HPLC analysis

Sesamol, sesamin, and sesamolin content of sesame coat and sesame cake extracts were analyzed by (HPLC). Standard sesamin, sesamolin and sesamol) were dissolved in methanol (1mg/ml). Standards and sample were filtered through a polytetrafluoroethylene membrane filter (0.45 mm _ 13 mm; National Scientific Co. Lawrenceville,GA, USA). The filtrates were injected into a shimadzo HPLC system model LC-2010 A HT equipped with a C18 column (4.6 mm _ 150 mm; id, 5 mm; Waters Co., Milford,MA, USA). The mobile phase was a mixture of methanol and water (70:30, v/v) at a flow rate of 0.5 ml/min, The injection volume 0.2 µl, a UV detector was set at 290 nm and column temperatures were set at 30C°, according to the method described by (9), extracts sesame coat and sesame cake and standard were injected into HPLC to determine their retention time.

Reducing Power

The reducing power(RP) of sesame coat and sesame cake extracts was determined according to the method described by (13) with some modification. Aliquot of 1.5 ml of various concentrations (10, 20, 30, 40, 50 mg/ml) from sesame coat and sesame cake extracts and BHT (as a control) were mixed separately with 1.5 milliliter phosphate buffer (0.2M, pH 6.6) and 1.5 milliliter potassium ferricyanide 10 mg/ml. The mixture was incubated at 50°C/20min. then 1.5 milliliter of trichloroacetic acid (TCA)

was added to the mixture, which was then centrifuged at 650 g for 10 min. Equal volumes of supernatant (1.5 ml) and distilled water (1.5ml) was mixed with ferric chloride (0.3 ml, 1.0%), and then the absorbance of the mixture was measured at 700 nm using a spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power.

DPPH Free Radical Scavenging Assay

DPPH assay was performed according to Kitts *et al* (8). One milliliter of 0.1mM DPPH solution was mixed with one milliliter of various concentrations (10, 20, 30, 40, 50 mg/ml) from ethanol extracts of sesame coat and methanol extracts of sesame cake and BHT (as a control) and vortexed thoroughly. The solutions were kept at room temperature (in dark) for 30 min and absorbance was measured at 517 nm against a blank using spectrophotometer. The radical scavenging activities (%) were calculated according to the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \left[\frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \right] \times 100$$

Abs_{control} is the absorbance of DPPH radical
Abs_{sample} is the absorbance of DPPH radical + extracts from sesame coat and sesame cake and the standard antioxidant (BHT).

RESULTS AND DISCUSSION

The RP-HPLC method was used for the quantification of sesamol, sesamin and sesamolin in sesame cake and sesame coat extracts. HPLC analysis of standard sesamol, sesamin and sesamolin were run separately and gave single peak with retention times (RTs) of 4.08, 4.41 and 2.51 min respectively (Fig.1).

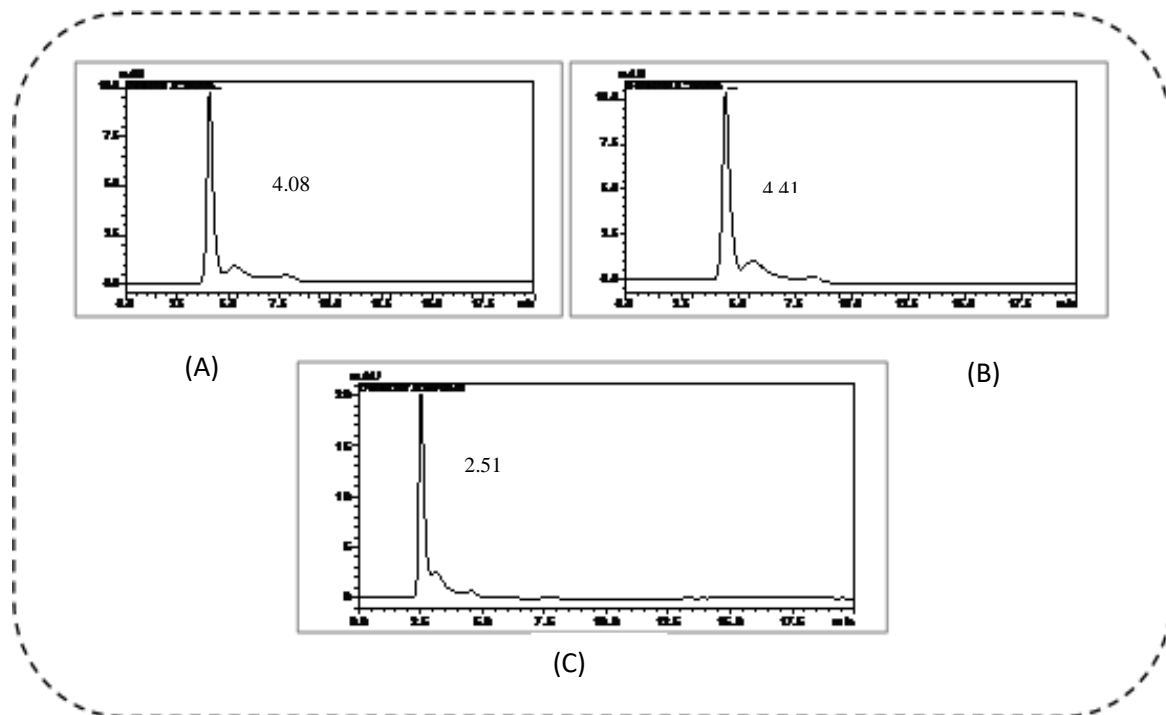


Figure 1. RP-HPLC Chromatograms of Sesamin(A), Sesamol (B), Sesamol(C) Standard

Figure (2) illustrates the RP-HPLC profile of sesame coat extract in ethanol, sesamol, sesamin and sesamol were appeared at 2.68 (peak 1), 3.98 (peak 3) and 4.34 (peak 4) min, respectively, the amount of the above mentioned components were 120.46, 235.82 and 175.29 mg /100g sesame coat . Identification of sesamol, sesamin and sesamol based on comparing their RTs with those of the standard compounds (Fig.1), obviously, sesamol, sesamin and sesamol are

proven to present in sesame coat extracts. These results agree with Chang et al.,(2) who found sesamin, sesamol and very small amounts of sesamol in the sesame coats extract, using RP-HPLC analysis. He also mentioned that ethanol is safer than methanol and acetone from the toxicological point of view. Additionally, using ethanol as a solvent reduces the sesame coat oxalic acid content; therefore it decreases the risk of Ca-oxalate kidney stone (11)

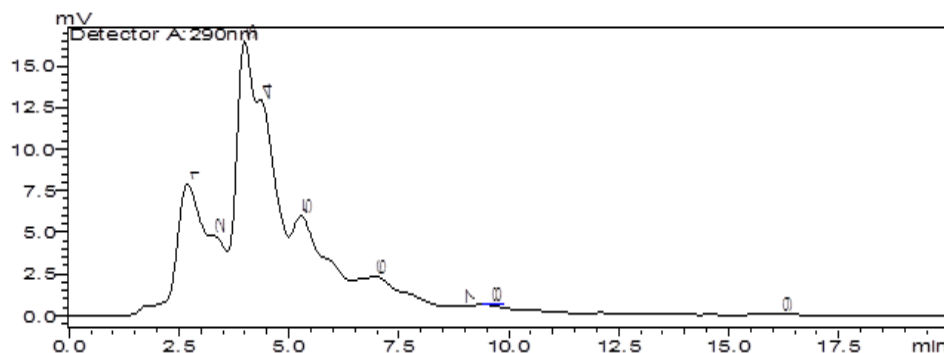


Figure 2. RP-HPLC Chromatograms of sesame coat extracts in ethanol

According to Fig.(3) RP-HPLC analysis of the extracts of sesame cake from roasted and unroasted sesame seeds, sesamol, sesamin and sesamol were appeared at 2.60 (peak 2), 3.93 (peak 3) and 4.48 (peak 4) min, respectively, sesame cake from roasted sesame seeds contained 642.61, 199.55 and 68.96 mg/100g sesame cake of sesamol, sesamin and sesamol, respectively, while sesame cake from unroasted sesame seeds contained

707.81, 441.80 and 212.94 mg/ 100 g sesame cake of sesamol, sesamin and sesamol, respectively. Therefore, sesamol, sesamin and sesamol are proven to present in extracts of sesame cake from roasted and unroasted sesame seeds These results is in agreement with Suja *et al.*,(18) findings ,who mentioned that the compounds present in sesame cake extract included sesamin, sesmolin and sesamol as identified by RP-HPLC

analysis. Shamurad,(16) found that oil from roasted sesame seeds contained higher amount of sesamol as compared to oil from unroasted

seeds. The latter compound is known to be a strong antioxidant agent (7)

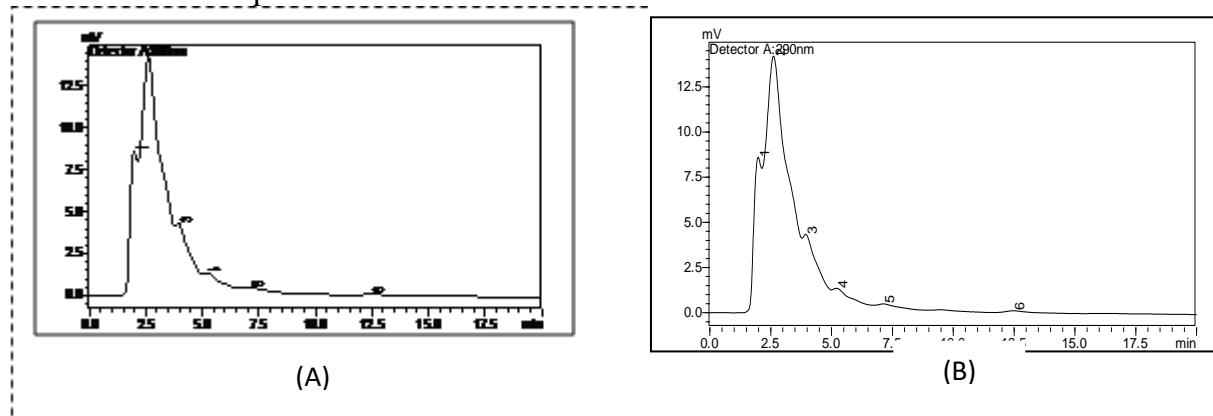


Figure 3. RP-HPLC Chromatograms of sesame cake from unroasted sesame seeds (A) and sesame cake from roasted sesame seeds(B)

Table (1) shows the anti-oxidative-ability of sesame coat and sesame cake from roasted and unroasted sesame seeds extracts and compared to that of BHT. The reducing ability of all sample under study showed a concentrations dependent increases, as the concentration increased the reducing ability increased. It has been noticed that the reducing power activity of sesame coat and RSC at all concentrations (10, 20, 30 40, 50 mg/ml) were significantly lower than BHT, while there were no significant difference among reducing power activities of URSC and BHT at all concentration except at (30, 40 mg /ml), where URSC was significantly higher at (30 mg/ml) and the BHT were significantly higher (at 40 mg/ml) in reducing power activities. URSC reducing power activities were significantly greater than RSC and sesame coat at (30, 40

and 50) mg/ml. The superiority of URSC in reducing power could be attributed to the presence of sesamol in higher amount as compared to the RSC and seeds coat extracts. Chang et al.,(2) reported that the reducing power of the sesame coats extract increased with increasing amount of extract, these results were agreed with Xie *et al.*(20) who reported that the extract concentrations an important factor in enhancing anti- oxidant activity. The anti-oxidant functionality mechanism of sesame coat extract could be due to its ability as scavenger of hydroxyl radical besides its ability in donating-hydrogen. Esmaeilzadeh Kenari *et al.*,(3) found that the reducing power of sesame cake extract in methanol and in ethanol were lower than BHT

Table 1. Antioxidant activity of sesame coats extract, cake extract from roasted and unroasted sesame seeds and BHT by reducing power

Concentration (mg/ml)	Reducing power(Abs at 700 nm)				
	Seeds coat	Cake from roasted sesame seeds	Cake from unroasted sesame seeds	BHT	LSD
10	0.047	0.041	0.082	0.099	
20	0.079	0.056	0.092	0.105	
30	0.105	0.090	0.164	0.109	0.046*
40	0.116	0.114	0.172	0.225	
50	0.126	0.154	0.235	0.242	

*P<0.05

The antioxidant activity of sesame coat, RSC and URSC were evaluated by DPPH assay, and the results are listed in Table (2). It has been noticed that antioxidant activities of all sample under study were increased with the increase in their concentration from 10 to 50

mg/ml. The radical scavenging activity (RSA) of BHT was significantly higher than the experimental samples at all concentration. The antioxidant activities of sesame coat extract at 10mg/ml was significantly lower than that of RSC and URSC at the same concentration. Whereas, there were no significant differences

among their radicals scavenging activities at (20, 30 and 40 mg/ml), at 50 mg/ml the seeds coat extract RSA was significantly greater than that of RSC. In this regard, Chang *et al.*,(2) found that the radicals scavenging activities(RSA) of sesame coats extract(SCE) was less than BHT. Esmailzadeh Kenari *et al.*,(3) found the scavenging effect of sesame cake methanol extract on DPPH radical was less than BHT. Suja *et al.* (18) stated that anti-

oxidant activity of sesame cake extract was concentration-dependent, and the purified extract was more effective than BHT, while the crude extract activity was comparable to that of BHT. Moreover, Suja *et al.* (19) proposed that SCE could be a good alternative for synthetic anti-oxidant to protect the vegetable oils which contain different levels of unsaturated fatty acids.

Table 2. Radical scavenging activity of sesame coats extract, cake extract from roasted and unroasted sesame seeds and BHT by DPPH method

Concentration (mg/ml)	DPPH %				
	coat	Cake from roasted sesame seeds	Cake from unroasted sesame seeds	BHT	LSD
10	32.50	50.07	54.80	71.18	
20	50.58	50.11	57.16	73.89	
30	58.52	54.48	61.10	75.12	8.937*
40	67.50	56.22	62.83	78.07	
50	70.29	57.48	63.93	81.00	

*P<0.05

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