SESAME OIL EXTRACTION AND ANTIOXIDANT ACTIVITY OF LIGNANS FROM LOCALLY CULTIVATED SESAME SEEDS (*Sesamum indicum* L.)

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ABSTRACT

The present study was designed to evaluate oil extraction from locally cultivated sesame seeds using three extraction methods: soxhlet, cold extraction and batch hydraulic pressing. The soxhlet method was the most effective one. The highest performance value was obtained using n-hx as a solvent in the soxhlet at 60°C/ 6 h. The percentages of oil from unroasted dehulled sesame seeds was significantly higher than those extracted from roasted and unroasted undehulled sesame seeds. Lignans were isolated from roasted and unroasted sesame seeds oil, and the lignans purity was confirmed by analysis. The antioxidant activities of lignans were evaluated by DPPH and reducing power assays. The reducing power activity of lignans from roasted sesame seeds oil (LRSO) was higher than that for the lignans from unroasted sesame seed oil (LURSO), while reducing power activity of BHT as a control was higher than that of LRSO and LRSO. LRSO showed high scavenging radical activity compared to that for BHT and LRSO, due to the presence of sesamol in LRSO which it has strong antioxidant activity.

Key words: DPPH, reducing power, sesame oil.
Part of M.Sc. thesis of the first auther.

استخلاص زيت السمسم وقياس الفعالية المضادة للاكسدة للكنانات المستخلصة من زيت بذور السمسم المزروعة محلياً

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المستخلص

صممت هذه الدراسة لتقييم طرائق استخلاص زيت السمسم من بذور السمسم المزروعة محليا باستخدام : السوكسليت ، والاستخلاص البارد والضغط الهيدروليكي بالدفعات. تفوقت طريقة السوكسليت على الطريقتين الاخريتين في نسبة الزيت المستخلص. تم الحصول على أعلى قيمة للاستخلاص باستخدام n–هكسان عند 60 درجة مئوية كمذيب في الاستخلاص بطريقة السوكسليت لمدة 6 ساعات. وكان استخلاص الزيت من بذور السمسم منزوع القشور غير المحمصة أعلى بكثير من تلك المستخلصة من بذور السمسم غير المقشره المحمص وبذور السمسم غير المقشره غير المحمصة. تم عزل اللكنان من زيت بذور السمسم المحمصة وغير المحمصة وتم تأكيد نقاوتها بواسطة تقنية HPLC. قدر النشاط المضاد للأكسدة للكنان بأستعمال فحص DPPH والقوه الاختزالية. اشارت النتائج الى ان نشاط القوة الاختزالية للكنان المعزول من بذور السمسم المحمصة كانت اعلى من تلك المعزولة من بذور السمسم غير المحمصة ، في حين ان القوة الاختزالية لـBHT كانت اعلى من تللك للكنان المعزول من زيت بذور السمسم المحمصة وغير المحمصة. ان اللكنان المعزول من زيت بذور السمسم المحمصة يمتلك نشاط اعلى في كبح الجذور الحرة بالمقارنه بـ BHT واللكنان المعزول من زيت بذور السمسم غير المحمصة. ويعزى ذلك الى وجود السيسامول بتركيز اعلى في زيت بذور السمسم المحمصه والذي يمتلك نشاط عالي كمضاد للاكسدة .

الكلمات الافتتاحية: DPPH، قوة اختزالية،زيت السمسم.
البحث جزء من رسالة الماجستير للباحث الاول

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INTRODUCTION

Sesame (*Sesamum indicum* L.), commonly as sesamum or benniseed, belonging to the family *Pedaliaceae* and genus Sesamum, is one of the oldest oilseeds crop known to mankind. Sesame plays an important role in nutritious food beneficial for human health. Sesame seeds containing crude oil, moisture, crude proteins, carbohydrate, crude fiber, (48.4%, 5.6%, 20.2%, 7.69%, 9.3%) respectively (6,1). Oil from sesame is markedly different from all other vegetable oil because of its high nutritional and therapeutic values. The antioxidant and antihypertensive properties of sesame oil is related to unusual compound known as lignans substance which is responsible for chemical and physiological properties of oil (22, 24). Although sesame oil is rich in USFA where the fatty acids composition is 14% saturated, 39% mono-unsaturated, and 46% poly-unsaturated fatty acids (27), but it is very resistant to rancidity because of the presence of natural antioxidants like sesamin, sesamolin and sesamol. Raw Sesame oil containing 0.5–1.1 % sesamin, trace amounts of sesamol and 0.2–0.6 % sesamolin (9). It can be used to increase the shelf life of margarine and other vegetable oil products .It has been propositioned that sesame seeds and oil could have a positive effect on cholesterol levels because of its antioxidant function (13, 21). Sesame lignans like sesamin, sesamolin have shown general interest, with other lignans like sesaminol and sesamol which produced during bleaching and roasting, those compounds could improve food quality and antioxidative stability. Roasting is the most important step in processing of different seeds, that causes important changes physical, chemical, structural and sensorial making the oil extracted from roasted sesame seeds considered more antioxidative and with better than unroasted sesame oil in sensory and nutritional properties (8,4). Sesame seed lignans have good effects on health, especiallyssesamol compound which provides protection against harmful free-radicals, it is proven to have strong antioxidant properties (19, 9). Phenolic antioxidants can be react directly with free radicals and convert them into stable products by donating a hydrogen atom this mechanism for primary antioxidant. As for Secondary antioxidants lower the rate of oxidation by several mechanisms. They may act by binding metal ions which enhancing oxidative processes or by inactivating enzymes, or by scavenging free radicals. Natural phenolic compounds can act as primary and secondary antioxidants (5).Since there is no local or regional studies about sesame seeds lignans present study was amid to investigate the effect of extraction methods on oil recovery from local sesame seed, isolation of oil soluble lignans and determination of the antioxidant activity using DPPH and reducing power assay.

MATERIALS AND METHODS

Sample collection

Locally cultivated sesame (*S. indicum L.*) seeds were obtained from local market in Baghdad-Iraq. Impurities such as stones spoiled seeds and other undesired materials were removed by sieve, then washed and dried in an oven at 40 C.

Oil extraction

1-Sesame seed oil was obtained from unroasted and roasted seeds using the batch hydraulic pressing

2-Unroasted and roasted sesame seeds (50gm each) milled in mechanical grinder and then refluxed in a Soxhlet apparatus using 400 ml n-hexane (1:4 w/v) at 60°C for 6 h. according to method described by (18).

3-Unroasted and roasted sesame seeds (50gm) milled in mechanical grinder was extracted with 300 ml n-hx (1:6 w/v) by stirring for 24 h at room temperature according to method described by (6). The solvents then were evaporated under vacuum at 40 C. The obtained oil stored in a freezer (-20°C) until time for use. The percentage of oil in sesame seeds were calculated as shown below. Percentage (%) of oil in sesame seeds = (Weight of oil (gm)/ Weight of the sample (gm)) χ 100

Extraction of lignans

A sample of (100gm) sesame seeds were roasted at 200°C / 30 min until the seeds color becomes dark brown. After the roasted sesame seeds were cooled down, (100gm) from each roasted and unroasted sesame seeds were taken individually for oil extraction using the batch hydraulic pressing. The oils sample were

blended with Eth 95% (oil: Ethanol (Eth), 1: 2 v: v) with stirring and placed on shaking incubator at 50C/ 4 h. After cooling, oil extract was stored at -50°C/ 20-24 h to be separate in two layers. The upper Eth layer was drawn and filtered using Whatman No. 2 filter paper. The filtered layer was concentrated under vacuum evaporator at 50°C. The resulted extract was dried in oven at 50C/6-8 h, according to the method described by (3). Extrected lignans were identified by HPLC.

RP-HHPLC analysis

Contents of sesamol, sesamin and sesamolin (samples) in lignans samples were analyzed by (HPLC). Standards (sesamin, sesamolin and sesamol) and the samples were dissolved in methanol and 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 at2290 nm and column temperatures were set at 30C°, according to the method described by (13),lignans (samples) and standard were injected into HPLC to determine their retention time.

Reducing power

The reducing power of LRSO and LURSO were determined according to the method described by (17). Aliquot 1.5 ml of various concentrations (0.5, 5 and 10 mg/ml Dimethyl sulfoxide (DMD)) from both LRSO and LURSO were mixed separately with 1.5 milliliter phosphate buffer (0.2mM, pH 6.6), and 1.5 milliliter potassium ferricyanide (10mg/ml). The mixture was incubated at 50 C°/20min. then 1.5 milliliter of trichloroacetic acid (TCA) was added to the mixture, then was centrifuged at 650 g for 10 min. Equal volumes of supernatant (1.5 ml) and distilled water (1.5ml) was mixed with ferric chloride[4] (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.

RSA assay:

The dissolving of DPPH in Eth gives a purple colored solution. Free radical scavenging capacity is determined by the decrease in color intensity of the solution, which was determined by measuring the absorbance at 517nm. The method was adapted from (11), one ml of 0.1mM DPPH (in Eth) was mixed with one ml of various concentrations (0.5, 5, 10mg/ml DMS) of LRSO and LURSO and vortexed thoroughly the solutions were kept at (RT) for 30 minute and absorbance was measured at 517 nm. The radical scavenging activity (RSA) was calculated according equation bellow to the following:

Percentage (%) of oil in sesame seeds = (Weight of oil (gm)/ Weight of the sample (gm)) χ 100

Statistical analysis

SAS (2012) program was depend on–LSD to compared between mean in this work test was used to significant compare between means in this study (26).

RESULTS AND DISSECTION

The effect of different extraction methods (soxhlet, cold extraction, batch hydraulic pressing) on oil recovery from sesame seeds were illustrated in Table 1. The results indicated that the yield of oil extracted from sesame seeds was significantly affected by the extraction method. The soxhlet method was more effective than cold extraction and batch hydraulic pressing, since it was gave in higher percentage of oil yield being (53, 44 and 38.6%), (48, 40 and 34.4%), (39.89, 36.25 and 28%) from unroasted dehulled sesame, roasted sesame seed and unroasted sesame seed, respectively. The highest performance value was obtained using n-hx as a solvent in the soxhlet at 60°C/ 6 hours. Currently n-hx is the preferred solvent throughout the globe because its extraction efficiency and easiness of availability (more nonpolar than other solvent) (23). The soxhlet method more effective than cold extraction due to the extraction temperature which gives higher extraction rates (20). (12) Stated that the averaged oil content of sesame seeds from 25ssesame genotypes was higher (average 52.2%) by soxhlet method as compared to batch hydraulic pressing (average 37.8%). Table 1 also explains that the cold extraction by using n-hx

technique was more effective than batch hydraulic pressing. Extraction of sesame oil (SO) has developed significantly over the years. (24) Reported that the batch hydraulic pressing was an early means of separation which was physical pressure to (compression the oil out). Such techniques are no longer prevailing currently due to the higher cake oil content. Solvent extraction, providing higher yields (98- 99%) is nowadays the dominant technique applied in most extraction processes. Table 1. illustrates the yield (oil mass/raw material mass) of the extracted oil from unroasted dehulled sesame seeds were significantly higher than those extracted from roasted sesame seed and unroasted sesame seed (whole seed). The yields of the extracted oil from roasted sesame seeds were higher than those extracted from unroasted sesame seeds. (15) Stated that dehulling not only increased oil content but also gave oil of better color quality compared with the whole seed. (28) Mentioned that roasting process increases the efficiency of total lipids extraction, and his findings suggested that roasting caused denaturation of protein which could have appositive impact on lipid extractability.

Table 1. Percentages sesame oil extracted from roasted and unroasted sesame seeds by three different methods (Soxhlet extracted, Cold extraction and batch hydraulic pressing)

Sample	Soxhlet (%)	Cold extraction (%)	Batch hydraulic pressing (%)	LSD value
Unroasted dehulled sesame seed	53.20 a A	48.00 b A	39.86 c A	5.028 *
Roasted sesame seed	44.00 a B	40.00 a B	28.00 b C	4.633 *
Unroasted sesame seed	38.60 a C	36.25 ab B	34.40 b B	4.166 *
LSD value	6.18 *	5.27 *	5.19 *	---

*** (P<0.05).**
Means having with the different small letters in same row and big letters in same column differed significantly

The HPLC method was used for the quantification of sesamol, sesamin and sesamolin which it was found in sesame oil. HPLC analysis of standard sesamol, sesamin and sesamolin when run separately gave single peak with retention times (RTs) of 2.51, 4.08 and 4.41 min respectively (Fig.1).

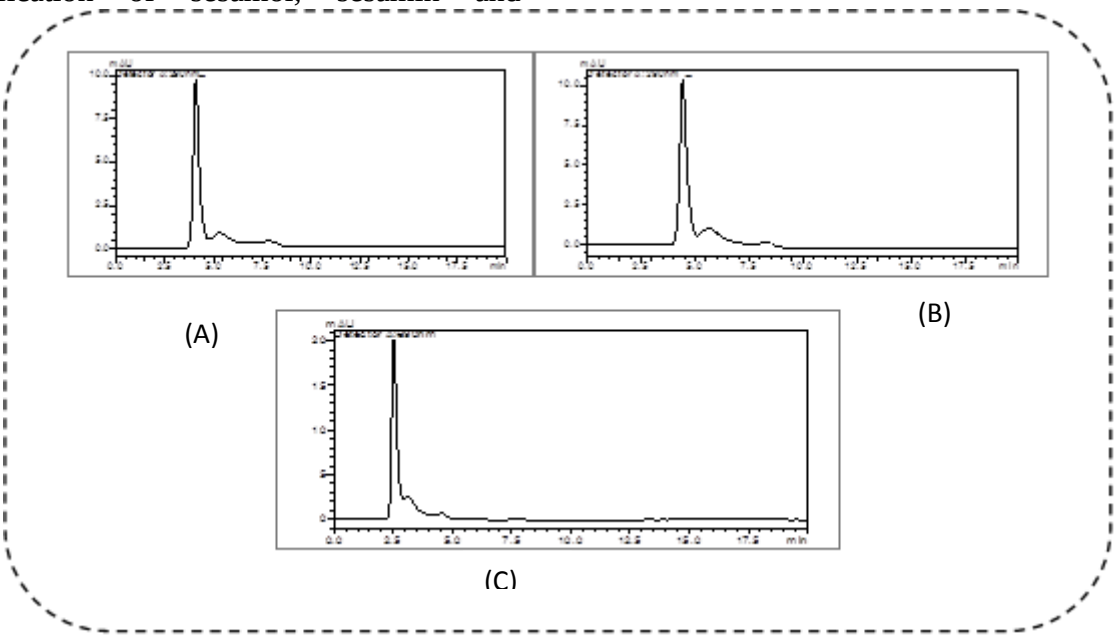

Figure 1. RP-HHPLC Chromatograms of Sesamin(A), Sesamolin (B), Sesamol(C) Standards

According to, Figure (2) RP-HHPLC analysis of the experimental LRSO gave three well resolved peaks for sesamin, sesamolin and sesamol. The major peak showed retention

times (RT) at 3.93min conform with identity of standard sesamin (Fig. 1). The second minor peak had a RT of 4.55min this RT matched with that of standard sesamolin (Fig. 1). The first minor peak had a RT of 2.63min as it is shown in Fig. 1 this RT coincident with that of standard sesamol. The yield of total lignan was 0.2 gm/ 100 gm of roasted sesame seed oils.

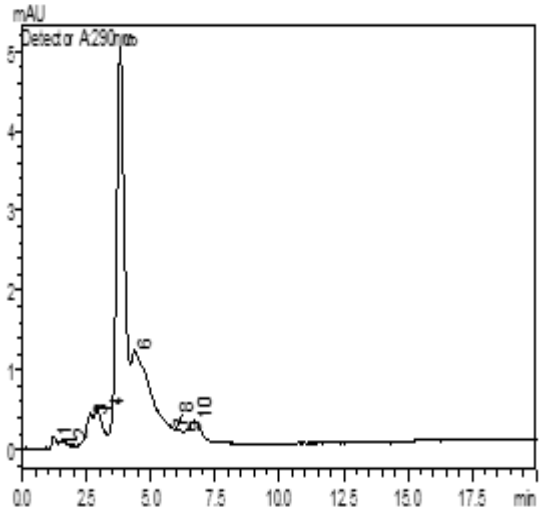

Figure 2. RP-HHPLC chromatograms of lignans from roasted sesame seeds

Figure (3) reveals that the HPLC analysis of the LURSO gave two well resolved peaks for sesamin and sesamolin, the major peak showed retention times (RT) at 3.93min conform with identity of standard sesamin (Fig. 1). The second minor peak had a RT of 4.55min which was identical to identity of standard sesamolin (Fig. 1). Total lignan yield was 0.18 gm/ 100 gm oil

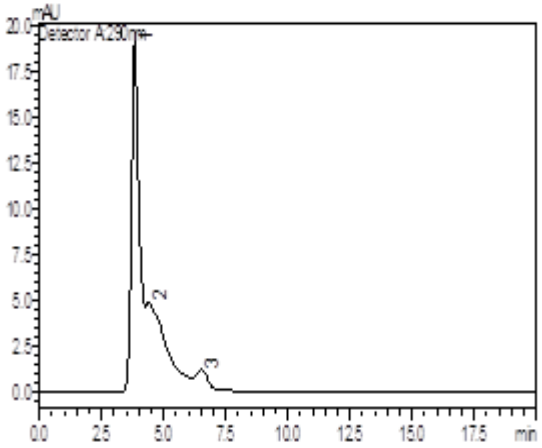

Figure 3.RP-HHPLC chromatograms of lignans from unroasted sesame seed

(3) Stated that the yield of lignan extracted from roasted sesame seeds produced was 0.22gm/100gm oils. Sesame lignan compounds consisted mainly of sesamin, sesamolin and sesamol. (2) Reported that roasted sesame oils had 0.67g/100g of sesamin, 0.52g/l00g of sesamolin and 0.012 g/100 g of sesamol which were relatively higher than those obtained in the present study. (10) Stated that the distribution of antioxidant components contents was varied in studied varieties of sesame (sesamin, 0.4-1.0% and sesamolin, 0.2-0.7%). The reducing power activity of the experimental samples illustrated in (Fig. 4), it has been noticed that the antioxidant activity was lignan concentration dependent. The reducing power activity of LRSO was higher than that of LURSO. The absorbance reading of LRSO and LURSO were (0.07, 0.21 and 0.32 nm) and (0.05, 0.18 and 0.26 nm) at 0.5, 5 and 10mg/ml, respectively. The higher antioxidant activity of LRSO attributed to the presence of sesamol in higher amount, it is noteworthy that the antioxidant activity of BHT (as a control) was higher than that for LRSO and LURSO, since the absorbance reading for BHT was (0.68, 0.84 and 0.95 nm) at 0.5, 5 and 10mg/ml, respectively.

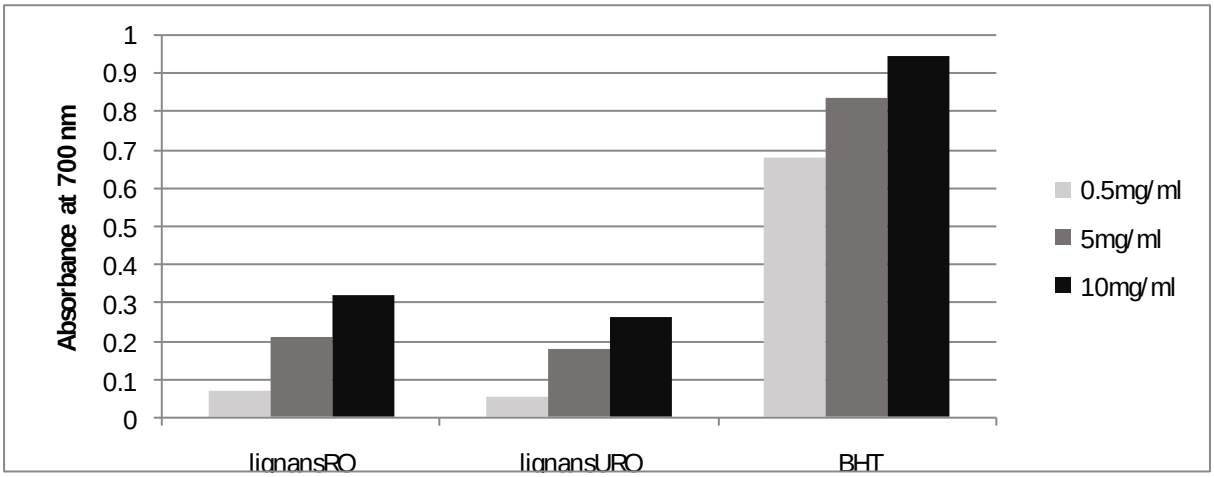

Figure 4. Reducing power of lignans from roasted sesame seed oil (LRSO), lignan from unroasted sesame oil (LURSO) and BHT

Figure 5 represents the antioxidant activity of lignan samples and BHT (as a control) using DPPH. The percentages of radical scavenging activities for different varies (0.5 , 5, 10 mg/ml) of LRSO, LURO and BHT were (36.04, 63.85, 67.70%), (17.08, 22.81, 23.33%) and (51.97, 55.52, 61.14%) respectively. The radical scavenging activity of LRSO at (5, 10 mg/milliliter) was higher than of BBHT at the same concentrations, while the radical scavenging activity of LRSO at all concentrations (0.5, 5, 10 mg/ml) was higher than that of LURSO. This is because LRSO contains sesamol where usually produced during roasting process Sesamol is proven to have strong antioxidant activity because of the existence of (OH) group along withmmethylendioxy (9). (25) Reported that the antioxidant activity of lignans may be attributed to the presence of a methylendioxy group beside the stereochemistry of the furan-phenyl bond. The results of present work show that the isolated lignan samples possess RSA to-ward DDPPH and reducing power activity but to different degrees.

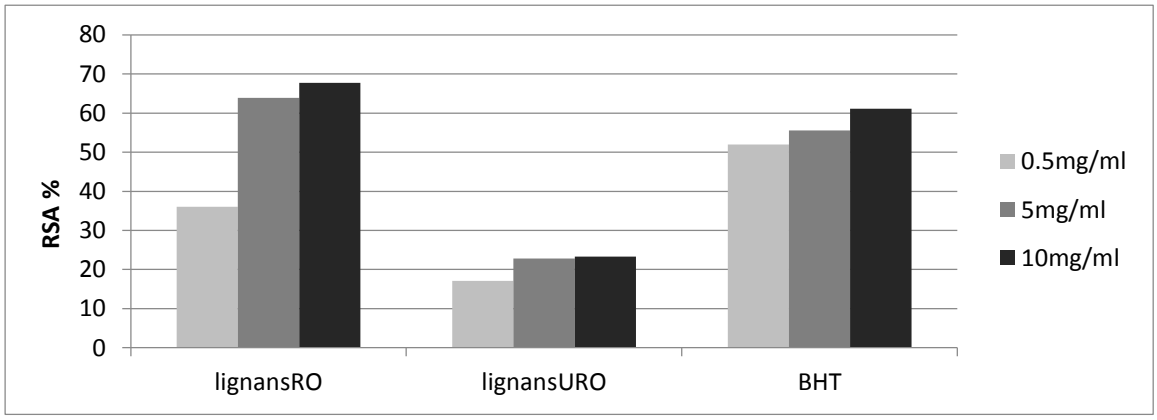

Figure 5. Free radical scavenging activities (RSA%) of different concentration of lignan from roasted sesame seed oil, lignan from unroasted sesame seed oil and BHT using DPPH assay

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