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INVESTIGACIÓN

The contents of sesamol and related lignans in sesame, tahina and halva as determined by a newly developed polarographic and stripping voltametric analysis

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RESUMEN

Contenido de sesamol en sésamo, tahina, y halva por un nuevo método de análisis polarográfico y voltamétrico.

Se determinó el contenido del lignano antioxidante (Sesamol) en sésamo, formulaciones comerciales de tahina y halva, que es el producto procesado de tahina, por polarografía de pulsos diferencial (DPP) con un capilar conteniendo un electrodo de gota de mercurio (HMDE). Un hilo de platino fué usado como el electrodo contador y Ag/AgCl como electrodo de referencia. Las muestras fueron analizadas por procedimientos de adición de patrones y se encontró que wl procedimiento era cuantitativo (p< 0.01). Debido a que el sesamol es un compuesto fenólico soluble en aceite, se estudiaron los porcentajes de aceite en sésamo, tahina y halva. Las muestras de sésamo contenían un 51.05-56.46 % de aceite mientras que la muestras de tahina tenían un 52.12-53.79 % de aceite. Se encontró que el porcentaje de aceite de halva fue un 28.79-30.13 % (p< 0.01). El sesamol encontrado en el sésamo fue de 0.26-0.32 mg/100 g de aceite, mientras que el sesamol en tahina fue de 10.98-12.33 mg/100 g de aceite. El sesamol de muestra de halva de procedencia comercial diferente varió entre 8.24-9.12 mg/100 g de aceite y 4.97 mg sesamol/100 g de aceite, respectivamente (R2= 0.9999) (p< 0.01). El método polarográfico propuesto (DPP) es un método rápido y reproducible para la determinación simultánea de lignanos fenólicos en sésamo y otros productos alimentarios que contienen sésamo. Éste proporciona una detección cuantitativa adecuada y sensible de este compuesto nutraceútico en alimentos comerciales.

PALABRAS CLAVE: Halva – Lignanos – Polarografía – Sésamo – Tahina – Voltametría.

SUMMARY

The contents of sesamol and related lignans in sesame, tahina and halva as determined by a newly developed polarographic and stripping voltammetric analysis.

The contents of antioxidant lignans (Sesamol) in sesame, commercial formulations of tahina and halva, which are processed tahina foods, were determined by Differential Pulse Polarography (DPP) with a capillary hanging mercury drop electrode (HMDE). A platinum wire was used as the counter electrode and Ag/AgCl was the reference electrode. Samples have been analyzed by standard addition procedures and found to be quantitative (p<0.01). Due to the fact that sesamo is an oil-soluble phenolic compound , it was found in the oil levels of sesame, tahina and halvas. Sesame samples contained 51.05-56.46 % in oil whereas tahina samples contained 52.12-53.79 % in oil. The oil percentage of plain halva was found to be 28.79 - 30.13 % (p<0.01). Phenolic lignan sesamol in sesame was 0.26-0.32 mg/100g oil, whereas the sesamol in tahina was 10.98-12.33 mg/100g oil. The sesamol in commercial plain halva samples and the open marketed plain halva samples contained 8.24-9.12 mg/100g oil and 4.97 mg sesamol /100g oil, respectively (R^2=0.9999) (p<0.01). The proposed Differential Pulse Polarographic (DPP) method is a rapid, reproducible procedure for the simultaneous determination of phenolic lignans in sesame and food products with sesame. It provides an adequate, sensitive, quantitative detection of these nutraceuticals in the commercial food industry.

KEY-WORDS: Halva – Lignan compounds – Polarography – Sesame – Tahina – Voltammetry.

1. INTRODUCTION

The sesame seed (*Sesamum Indicum* L.) is a valuable oil crop in Turkey. In 1998, Turkey's annual production was 33.500 metric tons and it was the fifth oilseed in the agricultural field after cottonseed, sunflower, soy, and peanut (Tokusoglu *et al.*, 2004a; 2003a,b; Anonymous, 1999). Sesame seeds are mainly cultivated in the Aegean ("Ege"), Mediterranean ("Akdeniz") area and the GAP area in the eastern part t of Turkey. 5 varieties of sesame seeds are cultivated: 59% brown, 30% yellow, 13% white, 7% dark-brown and 1% black (Tokusoglu *et. al*, 2004). Sesame seeds are used in the baking industry, for the production of tahina (sesame paste) , in the manufacturing of a sweet meat called "halva", as a spice mixture and specialty gourmet oil in addition to its application in soap, cosmetics,

pharmaceuticals and by-product industries (Tokusoglu *et. al*, 2003 a,b).

Sesame seed oil is unique due to its unusually high oxidative stability as compared to other edible oils (Budowski, 1962; Kamal-Eldin *et al*.,1994). This strong antioxidant activity has been attributed mainly to the presence of γ-tocopherol and antioxidative sesame lignans such as sesamin, sesamolin and sesamol (Tokusoglu *et. al*, 2003 a,b; Yoshida *et al*.,1995; Yoshida and Takagi,1998).

Sesame oil contains important antioxidants, sesamolin and sesamol, that are believed to promote the integrity of body tissues in the presence of oxidizing compounds. Sesame oil is rich in polyunsaturated fatty acids (PUFAs) which are known to reduce harmful (LDL) cholesterol and prevent the oxidation of fats in the blood vessels. Sesame seed oil contains calcium, an important nutrient for the entire vascular system. *In vitro* studies showed that sesame oil also protects the body against skin and colon cancer and produces anti inflammatory effects (Sahelian, 2007). Sesame seed oil is naturally antimicrobial for common skin pathogens, such as *Staphylococcus* spp. and *Streptococcus* spp. as well as common skin fungi, such as athlete's foot fungus. It has a unique ability to penetrate the skin easily, nourishing and detoxifying even the deepest tissue layers (Sahelian, 2007).

Tocopherols and lignans as sesame seed oil constituents have been reported to be *unsaponifiable matters* (Tokusoglu *et. al*, 2003 a; Yoshida and Takagi,1998; Mohamed and Awatif, 1998). Tokusoglu *et. al* (2003 a) reported tocopherols such as γ-tocopherol and the minor homologues α-and δ-tocopherol in 14 species of sesame seed oils in Turkey. The γ-tocopherol content in sesame seeds varied from 793.0 to 1330.0 mg kg^{-1} (p<0.01) whereas seeds contained 1.9-5.1 mg kg^{-1} of α-tocopherol and 0.5-0.6 mg kg^{-1} of δ- tocopherol (p<0.01) and according to that study which examined 3 different roasting conditions, the optimal roasting conditions were 5 min at 220°C (p<0.01) and the levels of gamma (γ-) tocopherol remained in over 95% of the original amounts in Turkish sesame seeds (Tokusoglu *et. al*, 2003 a).

Sesamol (3, 4-Methylenedioxyphenol) ($C_7H_6O_3$) (Figure 1) has been generally regarded as the main antioxidative component in sesame seeds (Fukuda *et al*.,1981). Sesame oil is characterized by the presence of a number of compounds from the furofuran family, mainly sesamin and sesamolin (Kamal-Eldin and Appelqvist, 1994; Kamal-Eldin *et al*., 1994).

Lignan compounds and lignan glycosides present in sesame are important functional components. The main sesame lignans, sesamin and sesamolin in sesame oil, possess no antioxidative activity but sesamol, sesaminol and sesamol dimmers are reported as possibly strong antioxidants and as playing an important role in the oxidative stability of sesame oil. During sesame oil manufacturing sesamolin can be converted to other lignans containing sesamol, sesaminol and sesamol dimmers (Kamal-Eldin and Appelqvist, 1994; Tokusoglu, 2005). Sesamol exhibits antimutagenic activity against oxygen species mediated mutagenicity (Kaur and Saini, 2000).

Recently, there has been increasing interest in nutraceuticals in dietary foods due to their protective biochemical functions and antioxidant effects. Amarowicz *et. al*.(2001) reported the application of semipreparative RP-18 HPLC for the purification of the furofuran lignans sesamin and sesamolin in sesame seed oil by rechromatography using an analytical HPLC and GC-MS. The antioxidant activity of the crude extract of lignan glycosides from the unroasted Burma black sesame meal has also been reported (Shyu and Hwang, 2002). Sesame lignan aglycons (sesaminol, sesamol, sesamolin and sesamin) (Figure 1.) in tahina (sesame paste) were determined using a rapid GC procedure by Tokusoglu *et al*. (2004c).

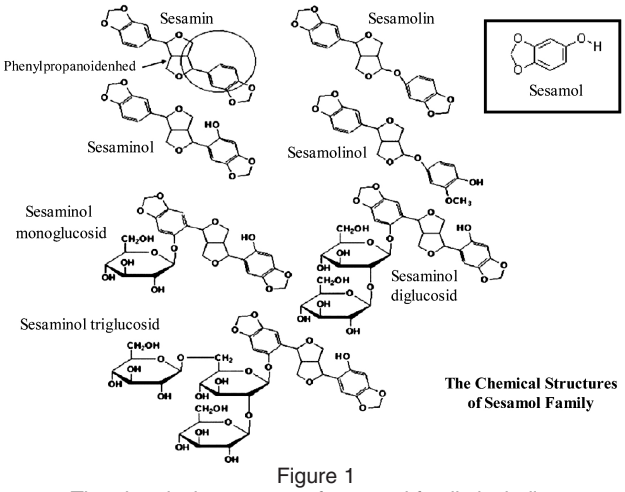

Figure 1
The chemical structures of sesamol family including sesame lignan

The need for regular monitoring of lignan nutraceuticals in sesame-based food products and relevant foods has led to an increasing demand for adequately sensitive and selective analytical techniques with low-cost determination capabilities. Differential Pulse Polarography (DPP), which is a voltammetric technique, has become accepted as one the most powerful electroanalytical tools for the microelement analysis of food and biological matrices owing to its extreme sensitivity and selectivity (Koçak *et al.,* 2005; Tokusoglu *et al*, 2004b; Aycan *et al*., 2003; Li-Qiong *et al*., 1999; Rodrigues *et al*., 1999; Aycan, 1994).

No study could be found concerning effective Differential Pulse Polarographic (DPP) methods for the determination of lignan compounds in sesame and sesame-based products.

The objective of this study was to determine the sesamol and related lignan compounds in sesame, tahina and halva by DPP and to propose new methods concerning the determination of sesamol

and related lignans by Differential Pulse Polarography (DPP).

2. MATERIALS AND METHODS

2.1. Research Material

3 brown sesame seed genotypes [*Gölmarmara (Menemen), Muganl› (Menemen), Özberk (Ege Univ.)*] were obtained from the Ege Agricultural Research Institute, Menemen, Izmir, Turkey and the Dept. of Field Crops of Ege University Bornova, Izmir,Turkey. Tahina (sesame paste) and plain halva materials were obtained from "*Koska A.fi.*",*Istanbul,Turkey, "Ömer Befle A.fi., Izmir, Turkey*"and "*Manisali G› da A.fi.",* in April, 2003. 3 jars of the same brand of tahina samples and 3 different homogeneous parts of the same kind of tahina were used for the analysis (*n*=9). 3 packaged halva samples and 3 different homogeneous parts of the same kind of halva were analyzed (*n*=9). Each was analyzed in duplicate.

2.2. Sample Preparation

Proximate Analysis

The moisture content of 5.0 g samples was determined at 110°C for 24 h in an oven using the procedure described by AOAC (1999).

The total lipids of sesame, tahina, and halva were determined by a reported method regarding sesame lipids from Tokusoglu *et al.* (2003a). 15 g of sample were homogenized in a homemade homogenizer with 45 mL of chloroform/ methanol (2:1 v/v) at 0°C and then mixed using a vortex for 30s. Homogenized samples were centrifuged at 4000 rpm for 15 min. The chloroform phase including the extracted lipids was transferred and the residue was extracted three more times using the same procedure and then filtered through lipid-free filter paper. The combined filtrates were concentrated in a rotary evaporator at 30°C under reduced pressure and evaporated using an N_2 flow to dryness. After drying over anhydrous sodium sulphate (Na_2SO_4), final extracts were stored in chloroform/methanol (2:1 v/v) solutions kept in screw cap containers, in the dark and at –28°C until needed.

Electroanalytical determination for Sesamol Analysis

For Differential Pulse Polarographic Analysis; A polarographic analyzer (METROHM 746 VA Trace Analyzer) together with a capillary hanging mercury drop electrode (HMDE) and Lenseis LY 1600 Model recorder was used for all electroanalytical measurements.A platinum wire was used as the counter electrode and Ag/AgCl was used as the reference electrode.

The Sesamol analysis was confirmed by the official method of AOCS concerning the phenolic antioxidants of sesame oil (AOCS,1998). DPP method validation was also performed with the standard reference method AOCS Method No: Cb 2-40 (1998).

2.3. DPP Analysis

For the quantitative determination of the lignan sesamol, the polarogram of the sesamol in the standard mix solution was obtained using the standard addition procedure. For the sample analysis, 100 ppm of sesamol standard solution were prepared daily. A trial electrode system was used including HMDE (Hanging Mercury Drop Electrode) as the working electrode, Ag/AgCl (saturated KCl) as the reference electrode and Pt electrode as the support electrode. For sesamol analysis, 1.00 ml (0.1M) of tetramethylamoniumhydroxide was used as the support electrolide solution in the Pyrex-polarographic cell. 1 ml of sample was added to 10 mL of ethyl alcohol including 1 mL (0.1 M) of tetramethylamoniumhydroxide (Me_4NH_4OH) electrolide solution.The cell contents were de-aerated by passing pure nitrogen (N_2) gas (99.999%) for about 300s at a flow rate of 100 ml/min to obtain the inert media. For 60 s, pre-concentration was performed and a measurement was obtained. E_{Meas} : -1650 mV and $E_{meas\ time}$: 60 s ; E_{Start} : -1650 mV, E_{End} : -2250 mV, E_{Peak} : -1257 mV and the scan velocity was 50 mV/s for the sesamol analysis. Then 1000 ppm 100 μL sesamol were added to the cell and cell contents were de-aerated for 30 s with N_2 gas. At the same time, 1000 ppm 100 μL sesamol standard was added to the cell and cell contents were de-aerated for30 s with N_2 gas and a measurement was obtained.Total analysis time was about 10 min.

For sesame, tahin, halva samples

5 g of sesame, tahina and halva samples were homogenized with 20 ml of ethyl alcohol for 1 h in a magnetic stirrer. The homogenized solution was centrifuged at 3500 rpm and filled to 50 mL with ethyl alcohol.

2.4. Cyclic Voltammetry and Stripping Analysis

1mL (10^{-2} M) sesamol standard including 1 mL (0,1 M) of tetramethylamoniumhydroxide was added to 10 mL of ethyl alcohol. HMDE (Hanging Mercury Drop Electrode) was used as the working electrode. The scanning velocity was 50 mV/s, current height was 1.4 μA, scanning potential was from-1600 mV to -2250 mV. Scanning was performed at this scale and anodic and cathodic peaks were obtained. If there is a positive current for the peak it is called cathodic peak, if there is a negative current for the peak it is called anodic peak. This is shown in the CV voltammogram (Figure 2).

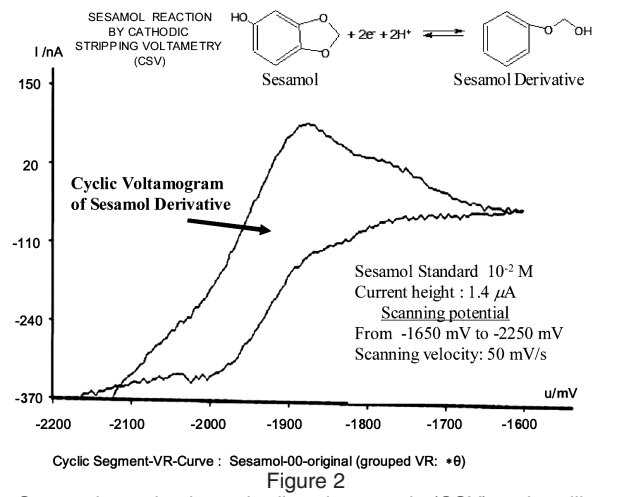

Figure 2
Sesamol reaction by cathodic voltammetric (CSV) and cycilic voltamogram of sesamol derivative

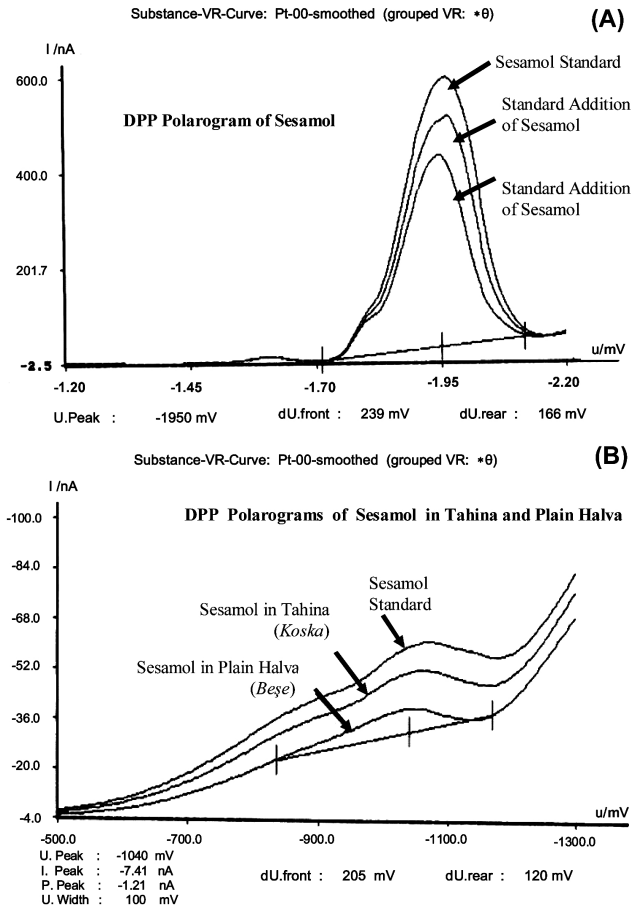

Figure 3
A. DPP polarograms of sesamol calibration
B. DPP polarograms of sesamol standard and samples

3. RESULTS AND DISCUSSION

A rapid electroanalytical method was developed for the identification of the nutraceutical lignan sesamol in sesame (Sesamicum Indicum *L.*), tahina and halva. Figure 3 A and B shows DPP polarograms of sesamol calibration and DPP polarograms of sesamol standard and samples [sesamol in tahina (*Koska*), in plain halva (*Befle*), respectively (Figure 3 A-B).

With the stripping analysis, sesamol was also perfectly separated (Figure 2). This electrochemical procedure provided for good separation of sesamol. Figure 2 shows the sesamol reaction by cathodic stripping voltammetry (CSV) and cyclic voltamogram of the sesamol derivative. With this oxidation-reduction reaction, a sesamol derivative has been formed. This reaction in cathodic stripping voltammetry included two steps. In the first step, called preconcentration, sesamol was accumulated in a solution to electrode-surface at -1650 mV for 60 s; whereas in the second step, called scanning, stripping was performed from -1650 mV to -2250 mV of potential (Figure 2).

Using these electrochemical methods, sesamol was detected at 0.26-0.32 mg/100g oil in three varieties of sesame (p<0.01); whereas 10.98-12.33 mg/100g oil in tahina samples (p<0.01) and 4.97-9.12 mg/100g oil in halva samples (p<0.01)were detected. Table 1 shows the moisture and crude oil composition of sesame, tahina and halva and Table 2 illustrates the sesamol levels of sesame, tahina and halva samples consumed in Turkey as determined by our suggested procedure.

Table 1
The moisture and crude oil composition of sesame, tahina and halva samples (p<0.01)

Sesame, Tahina and Halva Samples	Moisture (%)	Crude Oil (%)
Sesame Gölm. (Menem.)	4.142 ± 0.025	56.460 ± 0.022
Sesame Muganli (Menem.)	4.264 ± 0.043	51.053 ± 0.041
Sesame Özberk (Ege Univ.)	4.513 ± 0.053	54.554 ± 0.023
Tahina (Koska A.fi.)	0.403 ± 0.022	52.120 ± 0.017
Tahina (Befle A.fi.)	0.349 ± 0.011	53.792 ± 0.020
Tahina (Manisali A.fi.)	0.388 ± 0.015	52.123 ± 0.024
Plain Halva (Koska A.fi.)	0.502 ± 0.029	29.235 ± 0.010
Plain Halva (Befle A.fi.)	0.553 ± 0.032	30.137 ± 0.026
PlainHalva (ManisaliA.fi)	0.561 ± 0.014	28.792 ± 0.021
Plain Halva (Market)	3.746 ± 0.030	28.378 ± 0.018

Sesamol in sesame varieties occurred in the order : Muganli (Menemen) > Gölmarmara (Menemen) > Özberk (Ege University). The sesamol concentrations of tahina *(Koska A.fi.)* (12.33±0.05 mg/100g oil) (p<0.01) and that of plain

halva *(Befle A.fi.) (*9.12 ±0.02 mg/100g oil) were significantly higher (p<0.01) (Table 2).

Table 2
Sesamol concentrations in samples (p<0.01)

Sesame, Tahina and Halva Samples	Sesamol (mg/100g oil)
Sesame Gölm.(Menem.)	0.28 ± 0.02
Sesame Mug. (Menem.)	0.32 ± 0.05
Sesame Özb. (Ege Univ.)	0.26 ± 0.03
Tahina (Koska A.fi.)	12.33 ± 0.05
Tahina (Befle A.fi.)	10.98 ± 0.09
Tahina (Manisali A.fi.)	11.87 ± 0.02
Plain Halva (Koska A.fi.)	8.24 ± 0.01
Plain Halva (Befle A.fi.)	9.12 ± 0.02
PlainHalva (ManisaliA.fi)	9.05 ± 0.06
Plain Halva (Market)	4.97 ± 0.01

Tahini is 100 % composed of ground sesame seeds and constitutes 50% of the composition of halva by weight (Sahelian, 2007). Wen-Huey (2007) reported that the mean of total lignan contents of 14 brands of Taiwan sesame oils was 11.5 mg/g: 82% and 15% of lignans were sesamin and sesamolin, respectively. Additional data on the limits of the identification and quantification of sesamol in oils is needed. Therefore, a recent study gives a data set of sesamol in sesame, tahina and halva products (Table 2 and Figure 3 A-B).

With the proposed DPP method, sesamol and related lignans were effectively determined in sesame, tahina and halva samples. These above-mentioned procedures provided a sufficient, reproducible and sensitive quantitative detection for lignans. A sensitive detection of these compounds in related foodstuffs can be useful for quality control analyses.

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