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Original Article

The Contents of Sesamol in Iranian Sesame Seeds

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Abstract

Sesamol is a sesame lignan. Sesame lignans have multiple functions, including antioxidant activity and also seem to have potential as a source of phytostrogens. This study was condncted to evaluate sesamol contents of 7 brands of Iranian sesame seeds (*Sesamum indicume* L.). The brands were named *Karaj 29, Darab 14, Ultan, Dezful, Varamin, Branching Naz,* and *Nonbranching Naz.* After method validation, the methanolic extracts of seeds were investigated by HPLC. Their mean of total sesamol was found to be 4.67±0.92 mg/g (ranging between 2.75 and 6.13 mg/g). The brands *Karaj 29* with 5.84±0.25 mg/g, *Dezful* with 5.48±0.08 mg/g, and *Varamin* with 5.4±0.1mg/g had the highest content, and *Darab 14* with the content of 3.30±0.57mg/g had the lowest ($p<0.05$). Iranian sesame can be considered to be a good source of natural antioxidants for medicinal and commercial uses.

Keywords: Sesamol; Lignan; Antioxidant activity; Sesame seed; Analyze; HPLC.

Introduction

Sesame (*Sesamum indicume* L.) seed is one of the most important oil seed crops in the world (1) and is also known as sesamum, gingelly, beniseed, sim-sim, and till. It has been cultivated for centuries, particularly in Asia and Africa, for its high edible oil and protein content (2). It is also considered to be a beneficial food to health (3).

Sesame oil compounds have multiple physiological functions, such as estrogenic activity (4), providing anti-inflammatory functions (5), decreasing blood lipids (6) and arachidonic acid levels (7), and increasing antioxidative ability and γ-tocopherol bioavailability (8).

Recently the development of novel lipid-containing processed foods has increased rancidity caused by lipid autoxidation, posing a serious problem for the keeping quality of such systems (9). However, the oxidative stability of sesame oil is superior to that of other vegetable oils even though it contains nearly 85% unsaturated fatty acids (10). Sesame oil is especially stable because of the presence of unusual compounds known as lignans, comprised of sesamin, sesamolin, sesamol, and γ-tocopherol (11). Some chemicals that occur naturally in plants have begun to receive much attention as safe antioxidants, as they have been consumed by people and animals for years (12).

The present study was undertaken to determine the levels of sesamol lignan in some Iranian sesame seed cultivars and to find the richest one containing this chemical antioxidant.

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Experimental

Reagents

The sample of seven different brands of sesame seeds (*Sesamum indicume* L.) were donated by Seed and Plant Improvement Institute, Karaj, Iran.

The brands were named *Karaj 29, Darab 14, Ultan, Dezful, Varamin, Branching Naz* and *Nonbranching Naz.*

The standard of sesamol was obtained from Sigma Chemical Co. (St. Louis, MO, USA), and all solvents were of HPLC or analytical grade (Merck).

Standard preparation

A stock solution of sesamol (2 µg/mL) was prepared in methanol, which was stable for weeks in the dark and at 0 °C. The stock was used for the preparation of working standards (0.05, 0.1, 0.2, 0.3, 0.4, 0.5 µg/mL) and calibration curve.

Sample preparation

Sesame seeds (0.1 g), after grinding in a commercial blender, were extracted with 2 mL of methanol in a shaking incubator at a room temperature. The resulting slurries were centrifuged for 5 min (1000 rpm). The supernatant was kept and the residue was re-extracted under the same condition for three more times. The combined extracts (0.1 g/2500 mL) was injected to HPLC column (100 µL) after filtering through a 0.22 µm filter (Satorius, Goetting, Germany).

Liquid chromatography

The HPLC system consisted of a manual injector (Rheodyne, California, USA), a HPLC pump (Maxi-Star k-1000, Knauer, Berlin, Germany), a C_8 reversed-phase-column [Nova Pak C_8 (4 µm, 250×4.6 mm), Waters, Milford, USA], a spectrophotometer UV-Visible detector (k 2500, Knauer, Berlin, Germany) controlled by a computer software (Eurochrom 2000 ver. 106, Knauer, Berlin, Germany). The mobile phase consisted of methanol. The flow rate was 1 mL/min and the analysis was carried out at room temperature. The UV detector was set at 294 nm and 0.001 AUFS.

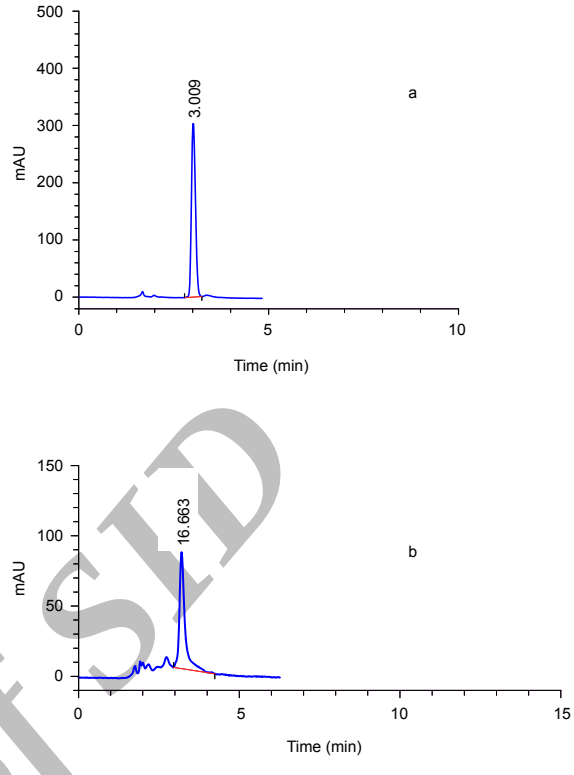

Figure 1. HPLC chromatograms of sesamol in: a) Calibration standard solution, b) *karaj 29* cultivar sample

Statistical analysis

All experiments were carried out in triplicate. Mean ± SD was reported for each case and the significance of differences among means were determined at $p<0.05$ using one way ANOVA followed by Tukey's multiple range test.

Results

We noticed that determination at 294 nm was much more suitable. The chromatograms of sesamol are shown in Figure 1.

Linearity and analytical range

The correlation between the peak area ratios and the sesamol concentrations was evaluated over the range 0-0.5 µg/mL and was found to be linear ($y=76.063x+0.5316$; $r^2=0.999$; $n=6$).

Accuracy

The accuracy of the method was verified by

Table 1. precision, sensitivity of method.

Precision (CV%)

(µg/ml)	Repeatability (n=3)	Internal reproducibility (n=3)
Standards:		
0.1 (µg/ml)	1.4	2.3
0.2 (µg/ml)	3.5	3.2
0.4 (µg/ml)	2.8	5.9
Sample (darab 14)	3.0	16.4

Sensitivity and linearity range

Calibration (n=6)	Range R^2	Slope (RAUC/µg/ml)	Intercep (RAUC)
0-0.5 µg/ml	0.999	76.06	0.5316

means of recovery assay.

This was accomplished by analyzing standard solution and spiked (enriched) sample. The analytical recovery was 100.33% for total sesamol.

Precision

The repeatability of the method was calculated by using the measured data from 3 successive days. Both values were expressed by coefficient variation (CV %). Some method validation (13), (14) data, including precision and linearity, are shown in Table 1. The data of validation method (Table 1) showed that the HPLC is a suitable method for sesamol analysis.

Concentration of sesamol in samples

Sesamol content (mg/g) of Iranian sesame seed brands are shown in Table 2.

Discussion

The mean content of sesamol lignan in 7 brands of Iranian sesame seeds (n=63) was 4.67±0.92 mg/g, ranging between 2.75 and 6.13 mg/g (Table 2). Among the 7 brands of Iranian sesame seeds, the samples of *Karaj 29* (5.84±0.25 mg/g), *Dezful* (5.48±0.08 mg/g) and *Varamin* (5.4±0.1 mg/g) had higher concentrations of sesamol, while the sample of *Darab 14*. (3.30±0.57 mg/g) had the lowest ($p<0.05$).

A few studies in other countries investigated the contents of lignans in sesame seeds or sesame oil. The sesamol content of 14 brands of roasted commercial sesame oils in Taiwan is reported as 0.30±0.11 mg/g by the total lignans of 11.51±2.81 mg/g, and in Japanese sesame oil, sesamol content was 0.03±0.03 mg/g by the total lignans of 8.02±2.93 mg/g (15). As reported, Indian sesame oil content of the total lignans is 10-20 mg/g. Sesamol was the lowest among the sesame lignans (16).

Sesamol is also produced from the degradation of sesamolin during the roasting process of sesame seeds (17). Namiki reported that the mean sesamolin content (1.7 mg/g) was lower than that of Japanese sesame oil (3 mg/g) and the level of sesamol was 10 times higher than that reported in Japan (15). The results were expected because Japanese sesame seeds were not roasted prior to expelling the oil, so sesamol could not be detected in the unroasted seed oil (17). Sesamol presents in crude sesame oil in small amounts (11).

In a study, crude sesame seeds were examined, and determination of sesamol was performed on extract of sesame, rather than on sesame oil. There are two basic strains of sesame seeds: black and white. It was observed that samples were apparently darker, had higher contents of sesamol, so they postulated that the colour of the oils reflected the content of sesamol (18). Nagashima also reported that the water extract (black materials) of black sesame seed coats possessed strong antioxidant activity (19). The total antioxidant status as determined by Trolox equivalent antioxidant capacity assay and expressed as Trolox equivalents was highest for black sesame (65.9±1.7 mg/g), while white seeds showed the lowest (4.4±0.6),

Table 2. Sesamol content of Iranian sesame seeds.

Cultivar	N	Mean (mg/g)	SD	Minimum (mg/g)	Maximum (mg/g)
Branching Naz	9	4.4	0.79	3.51	5.24
Darab 14	9	3.3	0.57	2.75	4.08
Ultan	9	4.07	0.48	3.4	4.65
Non branchig Naz	9	4.2	0.54	3.76	5.05
Karaj 29	9	5.84	0.25	5.3	6.06
Varamin	9	5.4	0.1	4.66	6.13
Dezful	9	5.48	0.08	4.42	6.01
Total	63	4.67	0.92	2.75	6.13

concentrated mainly in the hull fraction (20). Otherwise, Mohamed believes that the brown variety contained higher amounts of total sterols and tocopherols but lower amounts of sesamin, sesamolin, and total hydrocarbons than the white variety (21). A conflicting report is that higher values of sesamin and sesamolin were found in the oil sample from the white sesame seed variety (22). But, in our study, there was no difference between the amount of sesamol content among the seeds of different colours, as the highest content was for *Karaj 29* (white), *Dezful* (dark brown), and *Varamin* (brown). This may indicate that the colour is related to the total antioxidant content, not just sesamol.

Sesamol is a potent phenolic antioxidant (15). Fukuda reported (11) that the addition of 0.5 g/kg sesamol was found to enhance the antioxidative action of γ-tocopherol at all concentrations. Kajimoto found (23) that sesamol had a great preventive effect on the thermal decomposition of tocopherol in oil. But, it seems that this level is insufficient to explain the high stability of the oil, suggesting the presence of other antioxidants. It is important that no single compound can be considered responsible for this antioxidant activity. Combinations of a number of minor constituents such as tocopherols, sesamol, squalene, and antipolymerization sterols in the sesame seed have a synergistic role, increasing the oxidation stability (21, 24-28).

From ancient times to today, sesame has been considered to be a valuable oil-seed, not only because of its medicinal uses, but also because of its medical effects. Some valuable components in sesame contribute to a nutritional and functional food for humans. Our results indicated that Iranian sesame seeds possess antioxidant components such as sesamol, so the seeds can be easily incorporated into a normal diet at a level that might benefit health as a natural antioxidant with wide food-related applications.

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