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Original article

Effect of Roasting Process on Sesamin and Sesamol Contents of Sesame (*Sesamum indicum* L.) from Different Parts of Iran

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Abstract
Background and objectives: Sesame (*Sesamum indicum* L.) seeds have been a well-known oil crop in the world for many centuries. Lignans are functionally important compartments of sesame. Sesamin and sesamol are the main sesame lignans, which have recently shown various activities with health benefits, like anti-oxidative, anti-proliferative, anti-atherosclerotic, anti-inflammatory and anticancer effects. The aim of this study was determination of sesamin and sesamol concentration in sesame seeds from three different regions of Iran with diverse climatic conditions (Dezful, Ardakan and Neka). In addition, the effect of roasting process on sesamin and sesamol content were investigated. **Methods:** Analysis of sesamin and sesamol was performed using the mobile phase water: methanol (70:30) on a reversed phase ACE C_{18} with flow rate of 0.6 mL/min and UV detection at 290 nm. **Results:** HPLC analysis revealed that the highest content of sesamin (1.156±0.002 mg/g of seeds) and sesamol (2.393±0.002 mg/g of seeds) were observed in roasted Dezful seeds samples and hulled roasted Dezful sesame seeds, respectively. The amount of sesamin and sesamol in roasted seeds was higher than unroasted samples. **Conclusion:** The present study showed that the roasting process and hot semi-arid climate increase the content of lignans in sesame seeds. Consuming sesame seeds with such characteristics will improve dietary lignan intake and has nutritive value. Moreover, sesame seed characterized by the highest amount of sesamin and sesamol is more strongly suggested for achieving biological properties of these components.

Keywords: climate; roasting process; sesamin; sesamol; *Sesamum indicum* L.

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Introduction
Sesame seed (*Sesamum indicum* L.) is an ancient plant originating from Africa and Middle East and is widely cultivated as an important oil seed crop in subtropical and tropical parts of the world [1]. As indicated in archeological reports, more than 5000 years ago in India and about 4000 years ago in Babylon and Assyria, sesame was used as a food crop [2]. Typically, it is cultivated by countries such as India, China, Tanzania, Nigeria, Ethiopia, Iran, Egypt, etc. and exported

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to different parts of the world [3]. Sesame seeds are used in different forms including whole/powdered and roasted/unroasted seeds. Its oil is also used worldwide and is available in homemade or manufactured forms [4]. For many decades, the oil content of sesame seeds has been of high interest for the health benefits along with its role in food and edible oil production [5]. Sesame seed components include lipid (43-50%), protein (15-20%), carbohydrate (10-15%) and fiber (4-5%) [6-8]. It has been mentioned in previous studies that sesame seed oil content is about 40-60% [9] and when it is extracted, the protein content increases up to about 41.5-49.5% in the remainings [10]. This fragment is a valuable source of essential amino acids, especially cystine and methionine. Therefore, it is suggested that it can be used in different parts of food industry as a protein source such as in dairy cattle protein supplement, in human nutritional balancing additives, etc. [11,12]. Nowadays, the attention of researchers about has been increased on lignans as di-phenolic compounds in sesame seeds; sesamin, sesamol and sesamolin have been reported as the main components of sesame lignans [13]. The molecular structures of sesamin and sesamol are shown in figure 1.

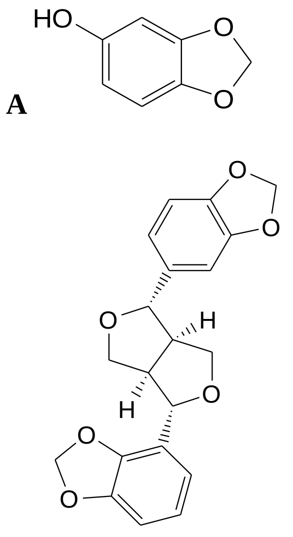

Figure 1. The molecular structures of sesamol (A) and sesamin (B)

Numerous biological studies have been conducted in humans and animals on the diverse effects of these lignans in health and treatment of diseases such as lipid lowering effects [14], anti-hypertensive [15], anti-atherosclerotic [16,17], anti-oxidative [14,18], anti-inflammatory [19], cancer-preventing [20,21], hepato-protective [22] and neuroprotective properties [23]. Also, previous studies have revealed that enough intake of sesamin can improve vitamin E efficacy in human body and has a modification role in fatty acid metabolism [24,25]. Sesamol prevents thermal decomposition of tocopherol and enhances free radical scavenging activity of sesame seeds [26,27]. Therefore, in a desirable dietary intake pattern, sesame is a valuable lignan resource that the amount and quality of which is important to improve health.

Iran has been a sesame producing and consuming region for thousands of years and sesame seeds have been known as one of the main ingredients in Persian cuisine, whether intact or in different processed forms [9]. There are different varieties which are cultivated in various parts of the country with diverse climate and geographical characteristics, which therefore cause the diversity of the components of the seeds. On the other hand, a few studies have investigated the roasting effect on the sesame seed total poly phenols. Thus in this study, we investigated the differences of sesame's main lignans (i.e. sesamin and sesamol) in three sesame samples from three different geographical zones, considering, also the effect of the roasting process.

Materials and Methods

Ethical considerations

This research was approved by the ethical committee of Tehran University of Meical Sciences (IR-TUMS-REC.1395.2509)

Chemicals

The standards of sesamol and sesamin for HPLC analysis were obtained from Sigma Chemical Co. (USA). All solvents such as n-hexane, acetone, ethyl acetate, hydrochloric acid, and ethanol were of HPLC or analytical grade (Merck, Germany).

Plant material

Sesame seeds were collected from three different regions of Iran located in southwest (Dezful), center (Ardakan) and north (Neka) of the country. The collected samples were divided into two groups: roasted (200 ºC for 15 min) and unroasted, based on the characteristics of the collection region summarized in table 1. All samples were identified by a botanist and were given a voucher number by the Herbarium Center of Al-Zahra University, Tehran, Iran. The seeds were used in the same status they are used by the people of the region: unroasted seeds used after separation of bran and the germplasm layer (hulled), roasted seeds used with bran (whole seed), coated (without bran but unhulled), and hulled seeds. The first and second forms are commonly used in southwest area.

Preparation of the standard solutions

The stock standard solutions were prepared by dissolving 10 mg of sesamol or sesamolin, reference standards, in 2 mL methanol. Daily prepared working standards in the range were obtained from the stock solution. The stock was used for the preparation of working standards (0.25, 0.55 and 0.95 µg/mL) and calibration curve.

Sample preparation

The sesame seeds (0.01 g) were ground by a commercial blender. Methanol (2 mL) was added to the ground samples and extraction was performed using a shaking incubator at room temperature. Afterwards, they were centrifuged at 1000 rpm for 5 min. The obtained solution was filtered via a membrane filter with a pore size of 0.45 µm prior to injection

HPLC analysis

The extracted sesamin and sesamol were analyzed using high performance liquid chromatography (HPLC) with Watter smart line 1050 (Germany) pump. The applied wavelength for chromatograms (290 nm) was extracted from UV spectra collected across the range of 200-900 nm. Empower software was used for programming instrument. A C_{18} reverse phase column (5 µm, 250×46 mm) was eluted with a flow rate of 0.6 mL/min by the mobile phase water: methanol (70:30) and the analysis was carried out at room temperature.

Method validation

The method for analysis of sesamin and sesamol were evaluated regarding the validation parameters including linearity, limit of detection (LOD) and limit of quantization (LOQ) [28].

Results and Discussion

The linearity, limit of detection (LOD) and limit of quantization (LOQ) of analyts were validated. The results have been summarized in table 1. All calibration curves showed good linearity with high correlation coefficient ($R^2 \geq 0.9972$) over the tested range. The LOD and LOQ values were calculated as 0.003 and 0.011 for the sesamin. These parameters determined as 0.037 and 0.123 for sesamol respectively.

Table 1. Regression equations, LOD and LOQ of sesamol and sesamin

Analyte	Linear range*	Equation	r square	LOD*	LOQ*
Sesamol	1.5-85	Y=27.5x-24.717	0.9997	0.037	0.123
Sesamin	1.5-110	Y=35.907x-31.586	0.9972	0.003	0.011

*Represented as (µg/mL)

Table 2. Region characteristics of Sesame seed samples

Geographical Region	Province	Location	Climate	Latitude	Longitude	Altitude (m a.s.l /meters)	Seed Type
North	Mazandaran	Neka	Mediterean	36° 39′ 3 N	53° 17′ 57 E	47	Roasted Unroasted
Center	Yazd	Ardakan	Tropical and subtropical desert	32° 18′ 36 N	54° 1′ 3 E	1039	Roasted Unroasted
South West	Khuzestan	Dezful	Mid-latitude steppe and desert	32° 22′ 57 N	48° 24′ 7 E	142	Roasted (coated) Unroasted Roasted whole seed Roasted

Based on the results obtained from the HPLC peaks, it became clear that sesamin was one of the major lignans in the sesame seeds. The highest amount of sesamin belonged to the roasted Dezful seed samples (1.156±0.002 mg/g of seeds). In fact, all roasted sesame seeds collected from the three regions of Iran revealed a higher level of sesamin compared to unroasted groups.

Minor changes in sesamin content of coated sesame oil after processing (roasting and/or steaming) were reported by Shahidi et al. in 1997. They also demonstrated that sesamin content of hulled seed oil decreased more markedly compared to coated seed oil [33]. A Chinese study showed a broad variation of in 2016 sesamin content in a collection of 215 sesame samples (2.49-18.01 mg/g of seeds) [26]. Chellamuthu et al. reported an average of 2.03 to 6.45 mg/g of sesamin content for different samples of sesame from various parts of India. The highest and lowest levels of sesamin were reported as 18.6 (g/kg of oil) and 3.1 (g/kg of oil), respectively. They explained that the geographical differences of the studied Indian states such as the soil composition and climate were the main causes of sesamin content variation [32].

One of the main reported lignans in sesame seeds is sesamol. Based on the results obtained from the HPLC method, it became clear that the highest concentration of sesamol was observed in coated roasted Dezful sesame seeds (2.393±0.002 mg/g of seeds) (figure 2). The mean±SD of sesamol concentration in roasted seeds was higher than unroasted groups.

In a previous study by Sadeghi et al. in 2009, researchers reported the highest level of sesamol in Karaj variety among the seven samples of Iranian sesame seeds, although they did not mention the condition of roasting of seeds in their study. In addition, they reported the mean amount of sesamol in Dezful variety as 5.48±0.08 mg/g [24]. Such differences may be due to the yield season and/or the amount of water the plant had received. The content of sesamol in a germplasm collection of Tamil Nadu sesame seeds, analyzed by Chellamuthu, has been reported to be 0.03 to 0.55 mg sesamol/g of seeds which is in contrast with this study (2.393±0.002 mg sesamol/g of seeds) [32].

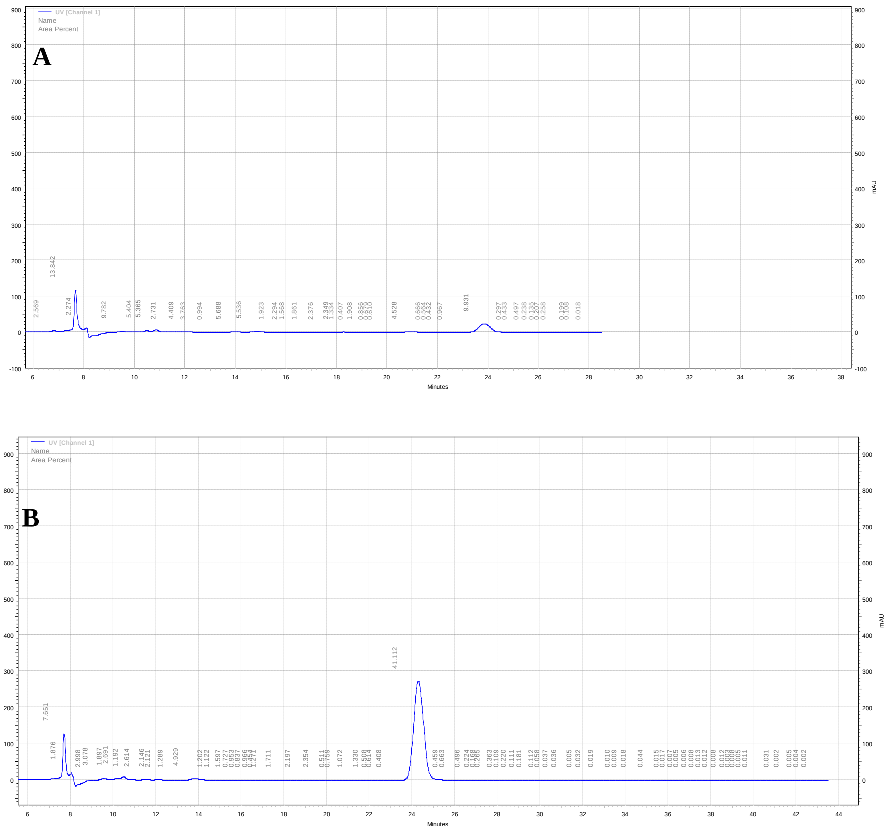

Figure 2. HPLC chromatographs of : a) The highest content of sesamol in coated roasted Dezful seeds, b) The highest content of sesamin in roasted Dezful sesame seeds

Table 3. Sesamin and sesamol contents in the sesame samples

Sesame sample	Sesamin (mg/100 g seed)	Sesamol (mg/100 g seed)
Roasted Dezful (RD)	1.156 ± 0.002	1.402 ± 0.004
Coated Roasted Dezful (coated RD)	1.090 ± 0.010	2.393 ± 0.002
Whole sesame Roasted Dezful (whole RD)	1.142 ± 0.040	1.622 ± 0.004
Unroasted Dezful (unRD)	1.027 ± 0.023	1.555 ± 0.004
Roasted Ardakan (RA)	1.091 ± 0.012	1.451 ± 0.002
Unroasted Ardakan (unRA)	1.028 ± 0.024	1.238 ± 0.001
Roasted Neka (RN)	1.119 ± 0.021	1.604 ± 0.002
Unroasted Neka (unRN)	1.080 ± 0.040	1.435 ± 0.002

The roasting condition (200 ºC for 20 min) which was utilized in the present study was similar to what is usually used in traditional sesame processing in Iran [29]. In a study by Jannat et al. in 2010 which was conducted on 8 samples of sesame from Iran, the effect of 9 different roasting processes on total phenolic compounds was investigated. They reported that the highest amount of total phenolic compounds of Dezful samples were observed when the same roasting method as the present study was applied [30]. In another previous research, it was shown that appropriate roasting temperature and time to increase antioxidant stability of sesame seeds was 200 °C for 15 min [31], which is close to our method in the present study.

There are various studies which have shown that the roasting process would increase the total phenolic compounds of sesame seeds, especially sesamol [25,34,35]. This may be related to sesamolin (another sesame lignan) degradation during the heating and roasting process [31,36]. Increasing the amount of sesamol and other polyphenols after pretreatment procedure (roasting) also resulted in higher oxidative resistant of sesame oil [37].

As previously mentioned, the sesamin content of Ardakan and Neka landraces were lower than Dezful sample, which may be due to the agro-climatic conditions of the regions. Dezful is a plain area with mid-latitude subtropical steppe which is nearly similar to other regions cultivating sesame throughout the world. Neka has a Mediterranean climate and Ardakan is a subtropical region. Therefore, the humidity and the average temperature differ in these areas which cause the diversity in yielding crops such as sesame.

To our knowledge, this is the first study which evaluated the sesamin content of some sesame seed samples in Iran. Also, this paper was the first study conducted on the various climatic regions. Here we studied the landraces of Ardakan and Neka samples for the first time and because Mazandaran province (Neka) is one of the new sites of sesame cultivation, more research may be needed to introduce it as a sesame cropping site. Indeed, Khuzestan province (Dezful) is one of the most ancient sites of sesame in Iran due to its historical background which goes back to Assyrian and Babylonian times. On the other hand, Yazd province (Ardakan) is a well-known area for its "Halve-Ardeh" (Iranian tahini halva) which is made from sesame seeds and is used across the country.

Today, treatment of diseases is attributed to herbal medicines because they have fewer side effects and are more easily available and safe. Indeed, the so-called green medicine is getting more popular since many people (about 80%) around the world prefer to use traditional medicine which mainly consists of plant extracts [38]. It is now well-understood that all of the bioactive components of sesame seeds (i.e. different sesame lignans) have inter-molecular adaptation and transfiguration during roasting process and storage. Based on the present findings, roasting process and climate conditions have significant influences on lignans' contents in sesame seeds as the highest amount of lignans was observed in mid-latitude steppe and desert climate of Dezful. These findings indicated this type of sesame seeds is a more valuable resource of edible lignans in human diet.

It seems that using roasted sesame seeds, especially from Dezful region, in diet increases the intake of a functional food and yields more health benefits. Lignan extraction, especially sesamin, of this variety of sesame is suggested for pharmaceutical purposes and medicinal use.

Acknowledgments

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Author contributions

Marzieh Mokhber and Abbas Hadjiakhoondi participated in the study design and conducted the systematic literature review. Zohreh Eftekhari performed the data analysis and drafted the manuscript. Zahra Nazem Bokaee and Farzad Shidfar participated in experimental studies. All authors have read and approved final version of manuscript

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

HPLC: High Performance Liquid Chromatography