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Research Article

Sterol Profile of Some Medicinal and Aromatic Plant Oils: Effect of Silyl Derivatization Process

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Abstract

In this study, sterol composition (brassicasterol, campesterol, stigmasterol, β-Sitosterol and β-sitostanol) of the crude oils extracted from different parts (seed or fruit) of some medicinal and aromatic plants (fenugreek, fennel, coriander, black cumin and anise) was determined and two different methods used in sample preparation process were compared. In the first method, silyl derivatization process was applied and in the other method derivatizing agents were not used. It was determined that the oil levels of the samples ranged between 4.27 - 4.63% for fenugreek, 8.60 - 9.10% for fennel, 9.57 - 10.70% for coriander, 22.77 - 23.50% for black cumin and 11.10 - 11.50% for anise. In both methods, β- sitosterol was the major sterol compound for all oils. It was observed that lower sterol concentrations were recorded for the method performed using derivatizing agents. Brassicasterol was identified only in anise and fennel oils in the method performed without the derivatizing agents, while it was detected in only black cumin oil in the other method. According to literature, it was concluded that the derivatization process was more compatible.

Keywords: Medicinal and aromatic plants, crude oil, sterols, silyl derivatization

Bazı Tıbbi Ve Aromatik Bitki Yağlarının Sterol Profili Üzerine Silyl Türevlendirmenin Etkisi

Öz

Bu çalışmada, bazı tıbbi ve aromatik bitkilerin (çemen, rezene, kişniş, çörek otu ve anason) sterol kompozisyonu (brassicasterol, campesterol, stigmasterol, β-Sitosterol and β-sitostanol) belirlenmiş ve numune hazırlama işleminde kullanılan iki farklı yöntem karşılaştırılmıştır. İlk yöntemde silyl türevlendirme işlemi uygulanmış ve diğer yöntemde türevlendirme maddeleri kullanılmamıştır. Numunelerin ham yağ oranlarının çemen için % 4,27 - 4,63, rezene için % 8,60 - 9,10, kişniş için % 9,57 - 10,70, çörek otu için % 22,77 - 23,50 ve anason için % 11,10 - 11,50 arasında değiştiği tespit edilmiştir. Her iki yöntemde de, sitosterol, tüm yağlar için major sterol bileşiği olarak tespit edilmiştir. Türevlendirme maddeleri kullanılarak gerçekleştirilen yöntem için daha düşük sterol konsantrasyonlarının kaydedildiği görülmüştür. Brassicasterol, türevlendirme ajanları olmadan gerçekleştirilen yöntemde sadece anason ve rezene yağlarında tanımlanırken, diğer yöntemde sadece çörek otu yağında tespit edilmiştir. Literatüre göre, türevlendirme işleminin daha uyumlu olduğu sonucuna varılmıştır.

Anahtar Kelimeler: Tıbbi ve aromatik bitki, ham yağ, steroller, silyl türevlendirme

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1. Introduction

Plant sterols, also known as phytosterols include over 250 different sterols and related compounds in various plants. The most common members are sitosterol, stigmasterol and campesterol (Piironen et al., 2000). Phytosterols are stable components of the plant cell membranes, having stabilizing effects on phospholipids bilayer, just like cholesterol in animal cell membranes. (Choudhary and Tran, 2011) and they are able to compete with cholesterols due to their structural similarity, which in turn causes a reduced serum cholesterol level (Gao et al., 2017). Recently, a great deal of interest has been given to the importance of phytosterols in the protection from cancer and cardiovascular diseases (Awad and Fink, 2000, Jones and AbuMweis, 2009, Orem et al., 2017; Rideout et al., 2016; Alvarez Sala et al., 2018; Huang et al., 2017). The average daily intake of plant sterols by foods is about 78–500 mg while the recommended daily ingestion was 2 g (Orem et al., 2017). Since foods contain phytosterols in minor levels, their consumption on daily basis would not be enough to supply the dietary requirement. Therefore, its extraction and isolation from oilseeds for fortification of foods would be the best way to gain phtytosterols at appropriate dose (Sujith Kumar et al., 2017).

Plant materials contain free and esterified steryls, and steryl glycosides which can be esterified to acylated steryl glycosides (Wojciechowski, 1991). For the analysis of sterols by gas chromatography, derivatization is a prior step improves volatility, sensitivity, separation, peak shape and thermal stability of the analytes. Some authors informed that the injection of free sterols resulted in broader peaks or peak tailing and a lower detector response, accuracy, reproducibility and sensitivity (Poojary and Passamonti, 2016, Li et al., 2001). On the other hand, some authors tried to improve rapid methods for sterol analysis without transformation of sterols into silyl derivatives prior to analysis (Alonso et al., 1997).

Plants are the source for medicinal treatments for thousands of years. Traditional medicine utilizes plants for both their curative and preventive properties (Carović-Stanko et al., 2016). Except from the curative properties, phytosterol composition of the medicinal and aromatic plants is important for fortification of foods and there are limited literature sources about this topic. In this context, the sterol composition was mostly studied for edible seed oils. The main aim of the current study was to determine the sterol composition of the oils extracted from some medicinal and aromatic plants. And also, the effect of derivatization step on the levels of sterols was investigated and compared.

2. Material and Method

2.1. Material

In the present study, the aromatic and medicinal plants namely fenugreek, fennel, coriander, black cumin and anise seeds and fruits were provided from Field Crops Department in Erciyes University. Five analytical sterol standards (Brassicasterol, Campesterol, Stigmasterol, β-Sitosterol and β-Sitostanol, Matreya LLC.) were purchased.

2.2. Methods

2.1.1. Crude oil extraction

The samples were ground using laboratory type mill and then dried. Crude oils of five different medicinal plants (fenugreek, fennel, anise, coriander, black cumin) were extracted by n-hexane according to the method described in AOAC (1990) using Soxhlet extraction system (VELP, SER 148, Italy). Crude oil content of the samples was calculated according to the mass balance of the samples after extraction.

2.1.2. Crude oil extraction

In the sample preparation step, sterols were conversed with and without silyl derivatives, and the results were compared. For this purpose, the cholesterol standard (5 mg) was mixed with the oil samples (0.5 g) and then, 5 ml of 1 M methanolic KOH solution was added into the oil samples. The samples were left at 70 °C for 30 min, then 1 ml of distilled water and 1 ml of n-hexane were incorporated into the tubes. After centrifugation, the upper phase was collected, and then the same procedures were repeated three times. In this method having no derivatization process, the supernatant was injected directly into the GC device. For silyl derivatization method, 0.2 ml of pyridine and 0.3 ml of BSTFA (N,O-Bis(trimethylsilyl) trifluoroacetamide) were added to the residue after evaporation of the supernatant and then completed by 1 ml of chloroform. The derivatized sterol extracts were then injected into the GC device. The carrier gas was helium (0.5 ml / min) and the injection volume was 0.5 ml. The detector (FID) temperature was 360 ° C. Oven schedule: 285 ° C - 35 minutes, with a heating rate of 285-310 ° C-10 ° C / min, 310 ° C-10 minutes (Alonso et al.,1997; Kozlowska et al., 2016).

2.1.3. Statistical analysis

Statistical analysis was conducted using SAS (2000) software. Duncan multiple range test was used for the determination of the significant differences for the samples at the confidence level of 95**%.**

3. Results and Discussion

The crude oil levels of the samples were given in Table 1. The highest crude oil content (23.2%) was determined for black cumin while the lowest content (4.48%) was measured in fenugreek seeds.

Table 1. Crude oil content of the samples

Samples	Plan part	Oil content (%)
Fenugreek	Seed	4.48±0.17^{e}
Fennel	Fruit	8.80±0.21^{d}
Coriander	Fruit	9.94±0.51^{c}
Black cumin	Seed	23.20±0.32^{a}
Anise	Fruit	11.35±0.18^{b}

The superscript small letters show statistically difference ($p<0.05$)

Two different methods were compared in the sample preparation step for the sterol analysis and the sterol levels determined by the method including silylation agents were shown in Table 2. β-sitosterol was found to be the highest component of sterol in oils. The highest β-sitosterol value was obtained from the coriander seed oil (1284.95 mg / 100 g). Brassicasterol was detected only in anise and fennel seed oils as 71.68 and 55 mg/100 g, respectively while it was not observed for the other oils. The total sterol composition was calculated as 1958.64 mg / 100 g in fenugreek oil, while it was 1156.64 in anise, 810.06 in black cumin, 1442.66 in coriander and 950.91mg / 100g in fennel.

Table 2. Sterol composition (mg / 100g) of the samples analysed by the method having no silylation agents

Sterols	Fenugreek	Anise	Black cumin	Coriander	Fennel
Brassicasterol	-	71.68±0.59^{a}	-	-	55.00±0.99^{b}
Campesterol	331.76±8.02^{a}	145.20±0.39^{d}	139.15±3.91^{e}	240.60±9.67^{b}	190.41±2.95^{c}
Stigmasterol	48.03±0.22^{d}	158.63±3.97^{c}	31.28±0.27^{e}	264.32±16.96^{a}	259.04±7.27^{b}
β-Sitosterol	1284.95±42.01^{a}	509.51±14.20^{c}	358.04±12.27^{e}	651.44±29.91^{b}	446.46±13.54^{d}
β-Sitostanol	293.91±0.81^{a}	271.52±1.97^{a}	281.59±19.68^{a}	286.31±1.41^{a}	-
Total	1958.64	1156.54	810.06	1442.66	950.91

The superscript small letters in each row show statistically difference ($p<0.05$)

The sample chromatograms of sterol standards analysed using two different methods were given in Figure 1. A minor change was observed for the retention times of the standards.

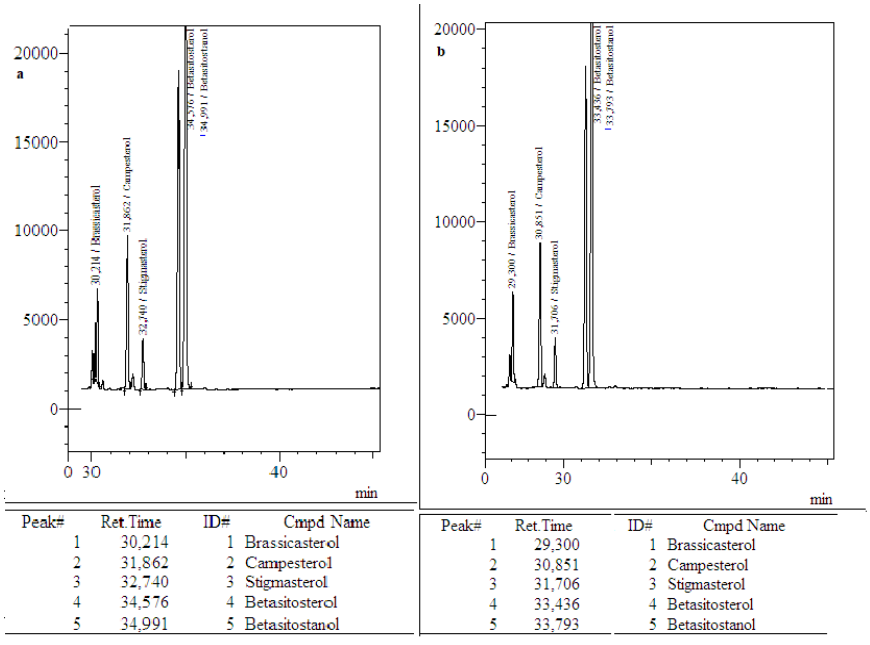

Figure 1. Sample chromatogram of the standards a) with silylation method b) without silylation method

Figure 2 shows the % composition of the sterols detected by the method having no silylation agents. The % composition results obtained for the fenugreek was in accordance with Kıralan et al. (2017), and the same authors reported the beta sitosterol content as in the range of 59.94 to 68.24%. Similarly to our results, β-sitosterol level was found to be in the range of 44.53 to 53.95% by Cheikh Rouhou et al.

(2008). Campesterol values in black cumin were calculated as in the range of 12.09 and 13.76% by same authors while the ratio of campesterol in our study was 17.18%. Jazia Sriti et al. (2011) reported that the β-sitosterol content in coriander was 21.33-21.97 mg/g and these values were higher than the results of our study.

Figure 2. Profile (%) of the sterols determined by the method having no silylation agents

The results of the sterol composition detected by the method having silylation agents were given in Table 3. It could be seen that the highest sterol component was detected as to be β-sitosterol similar to the other method. When compared to the all results, it could be concluded that the lower results were obtained from the method having the silylation agents. Additionally, brassicasterol could be detected in fenugreek in the method having the silylation agents. Kozlowska et al. (2016) did not detect brassicasterol in coriander similar to our results. However, in our study, brassicasterol was identified as 56.01 mg/100g in anise oil, while the same authors could not detect brassicasterol. In another study, the content of β-sitosterol in coriander was found to be 2.31 g/kg and it was found to be more consistent with the results of the sterol composition obtained from the method performed using the silylation agents (Uitterhaegen et al., 2016). Figure 3 shows the % composition of the sterol composition obtained from the method having silylation agents. Kıralan et al. (2017) found the stigmasterol as in the range of 1.08-2.49%, and these values were found to be lower than those of our study. In addition, it could be seen from Table 3, the highest stigmasterol percentage was recorded for fennel oils. In the current study, two different methods were applied to prepare sterol extraction, in one of the method applied, the samples were converted into the silyl derivative and in the other method, there was no silylation agent. According to the literature, it could be said that the silylation process is more compatible. On the other hand, the highest sterol component in the oil samples was determined as to be β-sitosterol. Yang et al. (2018) reviewed the chemical composition and nutritional properties of some edible oils and stated that corn oil and rice bran oil had higher contents of campesterol and total phytosterol with the values of 490.6 mg/100 g and 775.2 mg/100 g, respectively.

Table 3. Sterol composition (mg / 100g) of the samples analysed by the method having silylation agents

Sterols	Fenugreek	Anise	Black cumin	Coriander	Fennel
Brassicasterol	74.33±0.01[a]	56.01±0.02[c]	-	-	63.11±13.84[b]
Campesterol	198.14±1.53[a]	103.64±0.48[e]	108.90±0.29[d]	114.58±0.03[b]	111.03±0.01[c]
Stigmasterol	38.63±0.12[d]	96.56±0.35[b]	25.66±0.26[e]	72.77±0.03[c]	147.71±0.68[a]
β-Sitosterol	660.76±13.99[a]	304.43±3.50[b]	263.78±4.20[d]	266.66±1.03[d]	291.48±0.26[c]
β-Sitostanol	247.36±1.14[a]	252.23±7.31[a]	221.42±1.28[c]	229.66±1.51[b]	250.06±4.65[a]
Total	1219.22	812.86	619.75	683.67	863.39

The superscript small letters in each row show statistically difference ($p<0.05$)

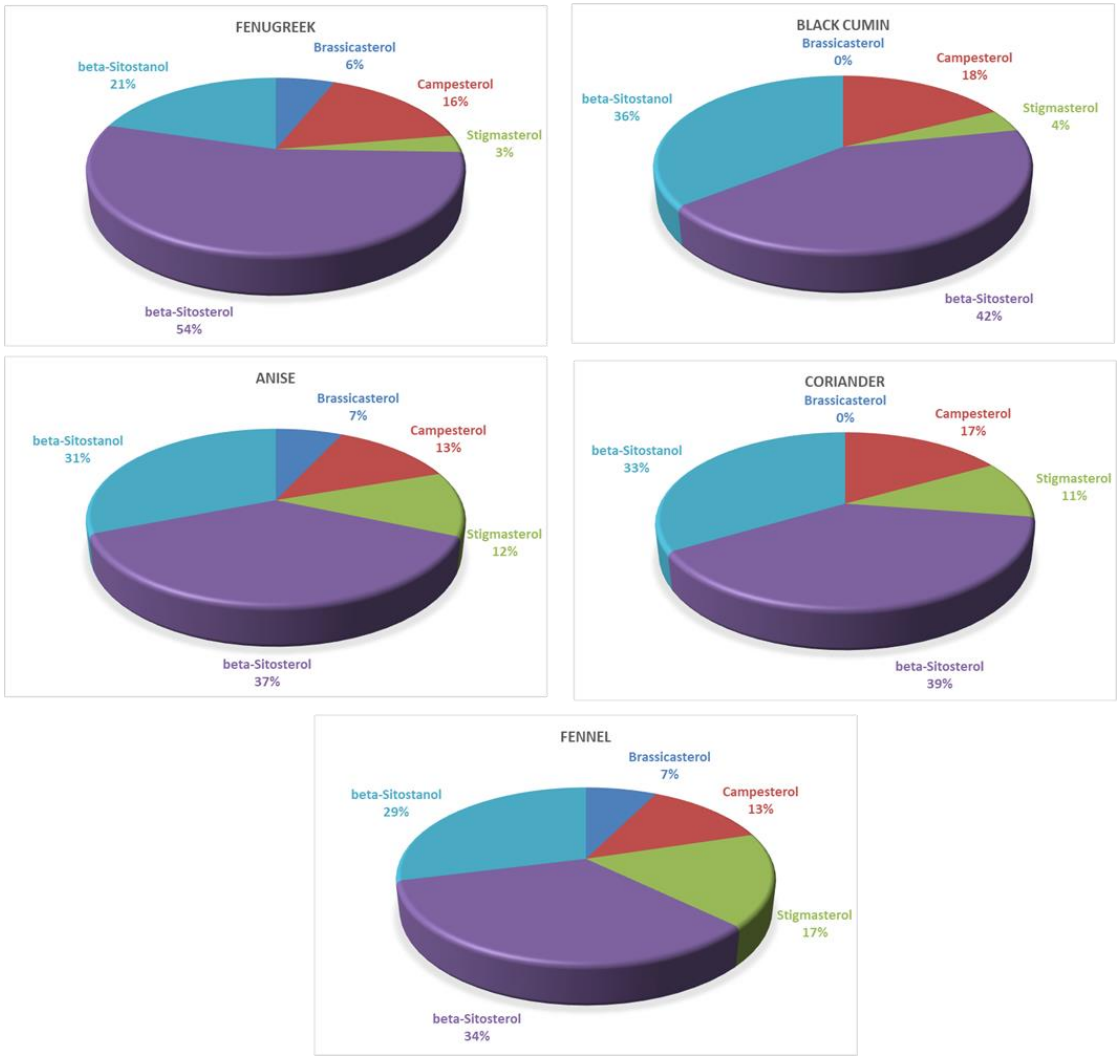

Figure 3. Profile (%) of the sterols determined by the method having silylation agents

4. Conclusions and Recommendations

In our study, especially fenugreek and coriander oils had the superior phytosterol content compared the mentioned results. In addition to that, the high crude oil content of black cumin can provide more benefits in terms of phytosterol content. Plant sterols are minor lipid components of plants, which may have potential health benefits, mainly based in their cholesterol-lowering effect. Plant sterols enriched foods are popular especially LDL-cholesterol-lowering properties nowadays. This study approved that medicinal plants can be used as an enrichment agent not only their medicinal properties but also with their nutritional facts.

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