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RESEARCH ARTICLE

Fatty Acids Composition and Bioactive Substances of Cold Pressed Oils from Strawberry Seed

Çilek Tohumundan Soğuk Sıkımla Elde Edilen Yağların Biyoaktif Bileşenleri ve Yağ Asidi Kompozisyonu

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ABSTRACT: Fatty acid and sterol compositions of strawberry seed oil (SSO) were determined. The fatty acid and sterol compositions were analyzed by GC. Tocols, tocotrienols and glycerides composition were designated on a high performance HPLC equipped with a reversed-phase HPLC columns. The physicochemical characteristics of strawberry seed oil were also studied. Our results showed that the strawberry seed oil was composed mainly of unsaturated fatty acids (92.36%), such as oleic acid (15.58%), linoleic acid (42.54%) and linolenic acid (33.48%). The total sterol constituents in strawberry seed oil were determined as 932.00 mg/kg and the major sterol was β-sitosterol (81.04%). The total β-sterol composition was 83.84%. Dominant triacylglycerol (TAG) molecules in SSO were determined to be OLnL (13.99%), LLL (8.05%) and PLnL (4.41%), respectively. Furthermore, the strawberry seed oil was rich in other bioactive compounds, such as phenolic compounds, tocopherols and sterols.

Keywords: Strawberry, seed oil, fatty acid, sterol, tocopherol

ÖZ: Çilek çekirdeği yağının (SSO) yağ asidi kompozisyonunu ve sterol içeriği belirlenmiştir. Yağ asidi kompozisyonu ve sterol içeriği, gaz kromatografisi (GC) ile analiz edilmiş, tokoferol ve gliserid içeriği, bir ters faz kolonu ile donatılmış yüksek performanslı sıvı kromatografisi (HPLC) cihazı ile belirlenmiştir. Çilek çekirdeği yağının fizikokimyasal özellikleri de incelenmiştir. Sonuçlar, çilek çekirdeği yağının esas olarak oleik asit (%15.58), linoleik asit (%42.54) ve linolenik asit (%33.48) gibi doymamış yağ asitlerinden (%92.36) oluştuğunu göstermiştir. Çilek çekirdeği yağındaki toplam sterol bileşenlerinin 932.00 mg/kg olduğu ve baskın sterolün β-sitosterol (%81.04) olduğu belirlenmiştir. Toplam β-sterol içeriği %83.84 olarak tespit edilmiştir. SSO'da baskın triaçilgliserol (TAG) moleküllerinin sırasıyla OLnL (%13.99), LLL (%8.05) ve PLnL (%4.41) olduğu belirlenmiştir. Ayrıca, çilek çekirdeği yağının fenolik bileşikler, tokoferoller ve steroller gibi diğer biyoaktif bileşikler açısından da zengin olduğu tespit edilmiştir.

Anahtar Kelimeler: Çilek, tohum yağı, yağ asidi, sterol, tokoferol

1. INTRODUCTION

Strawberries are popular products worldwide[1]. They are widely adopted to variable agro-climates across the temperate to sub-tropical regions of the world [2]. Strawberries (*Fragaria* spp.) are members of the Roseceae family and are generally grown and consumed fruits. They are classified in the genus *Fragaria* and strawberries have a very different taste and flavor. Fruits have an attractive aroma and appearance and are rich sources of bioactive compounds. While producing juices and seedless fruits, seeds and peels are removed to form a waste stream [1].

Strawberry fruits are exceptionally appreciated for their taste, health benefits and cancer prevention agent mixes, predominantly phenolics. Fruit antioxidants are derived from achaenium and tissue, yet achaenium involvement with the total antioxidant potential of fruit and bioaccessibility

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after admission is as yet obscure [3]. What is called the "seed" in many species is an achaenium, a fruit that contains the seed. The seed-like configuration is due to the hardening of the fruit wall (pericarp), which encloses the single seed so closely that it appears as a seed coat.

The antioxidant properties of strawberries are related to either flesh or achenes. Given achenes that constitute a scant fraction of the total weight of the fruit, their contribution to the total antioxidant substance and capacity of the fruit is notable. While flavonoids, phenolic substances and anthocyanins are mostly in the flesh in the whole fruit, it is noticed that the antioxidant ability of strawberry is primarily attributable to achenes, indicating that the detoxification ability of strawberry reactive oxygen species accounts not only for the amount of antioxidants, but mostly for the type [3].

Strawberry (*Fragaria ananassa*) seeds are usually eaten all over the world with its fruit pulp. Despite the many studies on strawberry fruits, however, published papers on the bioactive compounds and their effects of strawberry seeds are minimal. Strawberry seed extract (SSE) has been documented in some cases to suppress oxidative stress, to remove fat accumulation, to exhibit antii-nflammatory stress [4], [5], anti hyperglycemic, hepatoprotective [6], and antidiabetic activities [7]. Such effects on health were linked to the antioxidant activity of phenolic substances, especially ellagitannins and anthocyanins [3].

Strawberry seed oil can be a good resource for essential fatty acids in nutrition and can theoretically be used in the treatment of certain obesity and cardiovascular disease related disorders [8]. A strong triglyceride-lowering activity of strawberry seed oil is particularly noticeable, and this activity is as effective as that of other drugs that reduce lipid level in the current market [8]. Strawberry seed oil is composed mainly of unsaturated fatty acids, such as oleic, linoleic and linolenic acid [9], which are classified as omega fatty acids, and also for its high content of phenolic compounds [10]. On the other hand, strawberry seeds display lower lipid concentrations [10]. Strawberry seed oil is a natural active ingredient, which is characterized by good oxidative stability and high biological activity.

The purpose of this study is to determine the fatty acid and sterol compositions of strawberry seed oil. The sterol and fatty acid compositions were analyzed by GC, tocols-tocotrienols and glyceride content were analyzed by HPLC on a reversed-phase HPLC columns. Also, some physical and chemical properties (yield, refractive index, antioxidant capacity, total phenolic, colour) were determined.

2. MATERIALS AND METHODOLOGY

2.1 Materials

Strawberry seeds were kindly provided by Göknur Juice Plant (Kozan-Adana, Türkiye). Strawberry seeds were removed from the settlings that is separated in the production of strawberry juice. The surfaces of strawberry seeds were cleaned by the brushing machine and the seeds were dried at about 60 °C.

2.2 Chemicals and reagents

A 37 component FAME mix (fatty acid methyl esters) standard mixture and Betulin (purity ≥98%) used for an internal standard were supplied by Sigma-Aldrich (St. Louis, Missouri, USA).

All of chemicals and other solvents were supplied by Delta Chemicals (Adana, Turkey).

2.3 Physicochemical characteristics of oils

2.3.1 Oils extraction

The seed oils were extracted using a cold press oil machine (Karaerler-NF500, Türkiye). The strawberry seed was pressed at 45°C with 15 Hz frequency. Oil press machine used in the study was preheated to set the temperature of 100 °C.

2.3.2 Refractive Index

Refractive index of SSO was measured by refractive index on a Krüss AR2008 refractometer (Germany), and given as nD 20°C.

2.3.3 Antioxidant capacity

Two different methods were used to evaluate the antioxidant potential of free (extractable) and bound (hydrolyzable) phenols using DPPH and ABTS radicals.

DPPH radical-scavenging test: The antioxidant activity was assessed as described according to a method described by Farhoosh et al. [11]. Specific toluene sample solution concentrations of 1 mL each were combined with 1 mL toluenic mixture comprising DPPH radicals (0.006 per cent by weight). Solution was vigorously swung, allowed to incubate in the dark for 60 min (until consistent absorption values were achieved). The DPPH radical reductions were calculated by measurement the absorption at 517 nm [12]. The capability of DPPH radical scavenging activity calculation was done using the following equation:

DPPH scavenging activity % = ((AB-AS)/AB) x 100

where AS is absorbance of the solution and AB is the absorbance of blank. A calibration curve was plotted with DPPH scavenging activity against Trolox concentration

ABTS test: The free radical scavenging activity was assessed as described with respect to the method specified by Re et al. [13]. Experiments were performed on the Cary60 UV-VIS Spectrophotometer (Santa Clara, CA 95051, USA). ABTS·cation radical was produced by the reacting with 7 mM/L ABTS in water and 2.45 mM/L potassium persulfate (1:1), and incubating in the dark at room temperature for 12-16 h. ABTS·+ solution was diluted with methanol to obtain an absorbance of 0.700 at 734 nm for SSO analysis. The absorbance was read at 30°C exactly 1 min after initial mixing and up to 6 min. Trolox was used as standard matter, and the results were expressed as mmol Trolox/L.

2.3.4 Total Phenol Content

Total phenolic content was defined by following a procedure explained by Singleton and Rossi [14] with the Folin-Ciocalteu reagent. The phenolic compounds from the strawberry seed oil (0.5 g) were extracted in methanol (5 mL) by inversion for 10 minutes. In short, 0.50 mL of the diluted sample was reacted for 2 min with a 2.5 mL of 0.2 mol/L Folin-Ciocalteu reagent. After that, 2 mL saturated sodium carbonate solution (about 7.5 g/100 mL) was added into the reaction mixture. After incubation at room temperature for 60 minutes the absorbance was read at 765 nm. Gallic acid was used as a reference standard, and the findings were expressed as milligram gallic acid equivalent (mg GAE)/L dry weight of strawberry seed oil.

2.3.5 Tocopherols

All conditions were set according to the method developed by Surai et al. [15]. Using this method, alpha, beta+delta and gamma tocopherols were determined [15], [16].

2.3.6 Fatty acid composition

TSE EN ISO 12966-2 and Method 4 were used with a modification for determining fatty acid composition [17], [18].

2.3.7 Glyceridic composition

HPLC analysis of triacylglycerols: The analysis of triacylglycerols was performed by Agilent Infinity II 1260 HPLC on a reversed phase column equipped with a refractive index detectors (RID) by modifying the method defined by COI methods [19] and Essid et al. [20]. A comparison with a reference chromatogram was performed to classify the triacylglycerols [20], [21].

Theoretical Triacylglycerols Composition: The triacylglycerol (TAG) profile identification using the fatty acid composition is important to determine the position and distribution of fatty acids bound to the glycerol molecule. The theoretical number of molecules in the internal position of a fatty acid compared with 100 molecules of that acid (relative proportion) was calculated in this analysis [20], [21].

2.3.8 Sterol analysis

Determination of individual and total sterol compositions - Gas chromatographic method - Part 1: Animal and vegetable fats and oils (TS EN ISO 12228-1) was used for sterol analysis [22].

2.3.9 Colour

The color of the strawberry seed oil was measured with the help of a clour spectrophotometer (Konica Minolta-CM-5 Spectrophotometer, Tokyo, Japan). Colour values were given in the CIELAB colour space for illuminant D65 and a 10° angle of vision. lightness (L*), red-greenness (a*) and yellow-blueness (b*), chroma (C*) and hue (*h*), were registered.

3. RESULTS

3.1 Physicochemical properties

The physicochemical properties of the cold pressed strawberry seed oil were presented in Table 1. It was determined that the yield of strawberry seed oil obtained by cold press was 12.65% on average. In previous studies, it was determined that the amount of strawberry seed oil obtained by cold press machine was 7.6% [10], 14.61% [23] and 17.75% [9]. It was thought that these differences on the quality and yield of the seed oil obtained by cold press may vary depending on the seed variety, seed maturity, pre-treatments applied to the seed (fresh, dried, roasted, etc.) and the characteristics of the cold press.

Table 1: Some characteristics of cold pressed SSOs.

Parameters		
Refractive Index	nD20	1.5362 ± 0.0004
Antioxidant Activity		
– DPPH	mg Trolox/L	11055.17 ± 120.45
– ABTS	mmol Trolox/L	2.25 ± 0.14
Total phenol content	mg GAE/L	1010.45 ± 67.73
Tocopherol		
– α-tocopherol	μg/g	23.96 ± 8.50
– γ-tocopherol	μg/g	242.21 ± 25.59
Total sterol composition	mg/kg	932.00 ± 3.62
Colour		
– L*	D65	19.44 ± 0.01
– a*	D65	17.14 ± 0.03
– b*	D65	33.07 ± 0.07
– C*		37.24 ± 0.07
– h		62.61 ± 0.02

It has been determined that the refractive index of strawberry (*Fragaria ananassa*) seed oil was an average of 1.5362 (Table 1). In a study by Burnett et al. [24], it was reported that the refractive index of oils obtained from strawberry (*Fragaria chiloensis*) seed varies between 1.465-1.485. The reason for this difference in refractive index was that the strawberry varieties from which the seed was obtained were different.

The total amount of phenol compound in the analyzed strawberry seed oil was 1010.45 mg GAE/L and was determined in gallic acid (Table 1). In previous studies, Pieszka et al. [25] found that the total phenolic content was (mg/kg) 18.2 caffeic acid equivalent [25], van Hoed et al. [1] were 15800 mg/kg in strawberry seed oils obtained by cold press. These changes in the total amount of phenol were caused by the different types of seeds, the calculations being made as different acids (gallic or caffeic acid, etc.) and the differences in the maturity levels of the seeds. Phenolic composition in achenes and flesh has been reported to differ, achenes having up to 10 times higher antioxidant activity and amount of phenolic compounds, particularly derivatives of ellagic acids [3], [26]–[28]. The concentration of polyphenols was found as 1429 mg/100 g in strawberry seeds, while strawberry pulp contains only 375-998 mg/kg of polyphenols [29], [30]. Among determined compounds, there are two main polyphenol groups: ellagitannins and flavanols, which mean content was 8.50 and 5.82 g/kg dry matter [29].

The α-tocopherol was determined to be 23.96 μg/g and the γ-tocopherol was determined to be 242.21 μg/g in strawberry seed oil (Table 1), but β-tocopherol and δ-tocopherol could not be detected. In the study conducted by da Silva & Jorge [10], they could not determine α-tocopherol and β-tocopherol, and it was determined that γ-tocopherol and δ-tocopherol were 71.77 mg/kg and 14.57 mg/kg, respectively [10]. Tocopherols, which are natural antioxidants in oils, are divided into two groups: methyl tocols and methyl tocotrienols. They inhibit oxidation reactions. Like other antioxidants, tocopherols prevent or delay oxidation of other substances by being oxidized themselves. α-tocopherol is the most effective tocopherol in humans and shows biological vitamin E activity [31].

The values listed in Table 1 for Hunter a* (redness), and b* (yellowness) and L*- (lightness) reflect the color spectrum of the oils. As seen in Table 1, cold pressed strawberry seed oil is light colored. The a*

values of strawberry seed oils obtained by cold press are slightly green. When the b* values are examined, it was seen that the strawberry seed oil has a yellowish colour.

3.2 Fatty acid content (FA)

The fatty acid content of strawberry seed oil is given in Table 2. As can be seen when the table is examined; The unsaturated fatty acid (UFA) content was determined to be 92.36%. When unsaturated fatty acids were evaluated, it was determined that the ratio of polyunsaturated fatty acids (PUFA) containing two or more double bonds was 76.30% and monounsaturated fatty acids (MUFA) was 16.06%. Saturated fatty acid (SFA) content was found to be 6.92%.

Table 2: FAs (%) of cold pressed SSOs.

Fatty Acids (FAs)	Fatty Acid abbreviation	FAs Concentration (%)
Lauric acid	C12:0	0.01 ± 0.00
Myristic acid	C14:0	0.05 ± 0.01
Palmitic acid	C16:0	4.32 ± 0.03
Palmitoleic acid	C16:1	0.20 ± 0.01
Heptadecanoic acid	C17:0	0.05 ± 0.01
Heptadecenoic acid	C17:1	0.07 ± 0.02
Stearic acid	C18:0	1.63 ± 0.04
Oleic acid	C18:1	15.58 ± 0.01
Linoleic acid	C18:2	42.54 ± 0.05
Linolenic acid	C18:3	33.48 ± 0.03
Arachidic acid	C20:0	0.77 ± 0.01
Eicosenoic acid	C20:1	0.22 ± 0.02
Eicosadienoic acid	C20:2	0.07 ± 0.01
Eicosatrienoic acid	C20:3	0.18 ± 0.01
Docosadienoic acid	C22:2	0.03 ± 0.01
Lignoceric acid	C24:0	0.09 ± 0.02
Σ SFA		6.92
Σ MUFA		16.06
Σ PUFA		76.30
Σ UFA		92.36

Data are expressed as means (n=3). SFA, MUFA and PUFA stand for saturated, monounsaturated and polyunsaturated fatty acids, respectively.

It was found that the main fatty acids were oleic acid (C18:1n-9) (15.58%), linoleic acid (C18:2n-6) (42.54%) and linolenic acid (C18:3n-3) (33.48%), respectively and the ratio of linoleic acid/α-linolenic was 1.27. The main saturated fatty acid was determined as palmitic acid (C16:0) (4.32%).

Usually, all fatty acids together accounted for 60–80 percent of the total fatty acids with a ratio of 1:1 to 2:1 between the two. Such composition is suitable for dietary ingredients or food supplements to improve n-3 fatty acid intakes [32].

In previous studies, it was observed that the palmitic acid changed between 3.2-6.99% [9], [24], [32–39], oleic acid 13.29-17.8% [32–36], [38], [39], linoleic acid 39.54-54.9% [25], [32–36], [38], [39] and linolenic acid 24.0-39.50% [10], [25], [32– 36], [38], [39]. When the findings obtained in our study were compared with these studies, it was determined that they were within the limits of previous studies. It was thought that the differences in the fatty acid composition were due to the origin and variety of the fruits. However, unsaturated fatty acid ratio was found to be very high in all studies.

3.3 Sterols

Sterols are important components in the unsaponifiable substances of seed oils. In our study, 17 different sterol components were defined (Cholesterol, Cholestanol, Brassicasterol, 24-methylene cholesterol, Campesterol, Campestanol, Stigmasterol, Δ7-campesterol, β-sterol, Δ5-23 stigmastadianol, Clerosterol, β-sitosterol, Sitostanol, Δ5-avenastenol, Δ5-D24 stigmastadionol, Δ7 stigmastenol, Δ7-avenasterol) and analyzed in strawberry seed oil. These components in strawberry seed oil were given in Table 3 and Figure 1.

As can be seen in Table 1 and 3, the total sterol composition in strawberry seed oil was determined as 932.00 mg/kg (Table 1) and the major sterol was β-sitosterol (81.04%) and total β-sterol composition was 83.84% (Table 3). Apart from β-sitosterol, the main sterols were determined to be Campesterol (6.68%), Δ7-stigmastenol (4.36%), Stigmasterol (2.75%), Δ7-avenasterol (2.16%) and Sitostanol (1.75), respectively (Table 3). In a study by Pieszka et al. were found the total sterol composition as

4643.1 µg/g and the β-sitosterol composition as 2656.6 mg/kg (57.22%) in strawberry seed oil [36].

Table 3: Sterols (%) of cold pressed SSOs.

Sterols	Sterols Concentration (%)
Cholesterol	0.19 ± 0.01
Cholestanol	0.00 ± 0.00
Brassicasterol	0.00 ± 0.00
24- methylene cholesterol	0.00 ± 0.00
Campesterol	6.68 ± 0.04
Campestanol	0.00 ± 0.00
Stigmasterol	2.75 ± 0.02
Δ7-campesterol	0.00 ± 0.00
β-sterol – Δ5-23 stigmastadianol	0.00 ± 0.00
– Clerosterol	0.00 ± 0.00
– β-sitosterol	81.04 ± 0.02
– Sitostanol	1.75 ± 0.03
– Δ5-avenastenol	0.00 ± 0.00
– Δ5-D24 stigmastadionol	1.05 ± 0.01
Δ7 stigmastenol	4.36 ± 0.02
Δ7-avenasterol	2.16 ± 0.01

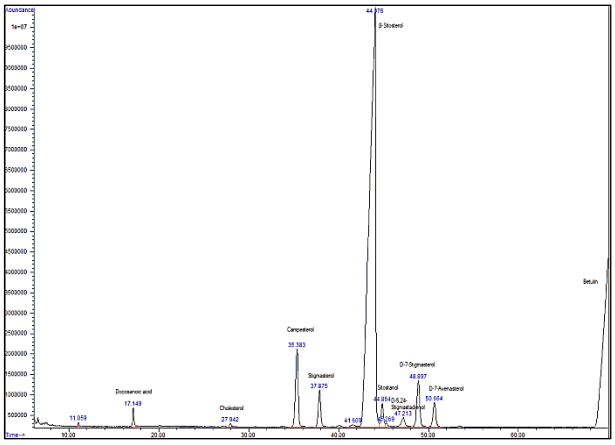

Figure 1: GC chromatogram of the sterols composition of cold pressed SSO.

In other previous studies, the total sterol composition was found to be 9 mg/kg [25], [34], [40] and β-sitosterol composition ranged between 711.0-3566.5 mg/kg [34], [40].

Both tocols and phenolics are antioxidants with strong protective effects on cardiovascular diseases, whereas phytosterols, such as β-sitosterol, suppress cholesterol absorption in the intestine, thus show the cholesterolemia-lowering properties [41].

3.4 Triglycerides

Triacylglycerols (TAG) composition analysis results of cold pressed SSO obtained in our study are given in Table 4 and compared with olive oil (Figure 2).

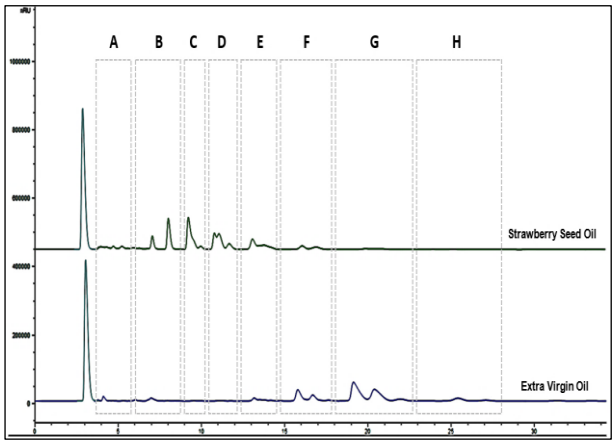

Figure 2: Triacylglycerols (TAG) chromatogram of cold pressed SSO and comparison with olive oil (A-Monoglycerides, B-Diglycerides, C-ECN40, D-ECN42, E-ECN44, F-ECN46, G-ECN48, H-ECN50).

The fatty acids are esterified in the native triacylglycerols (TAG) molecule to three stereospecific positions on the backbone of glycerol. The stereospecificity of fatty acids in TAGs are characteristic for native oils and fats [42]. Dominant TAG molecular species in olive oil were OOO (triolein), OOP (dioleopalmitin) OLO (oleolinleoolein) [42]. However, as can be seen from the findings obtained in our study (Table 4), the dominant TAG molecules in cold pressed SSO were determined to be OLnL (13.99%), LLL (8.05%) and PLnL (4.41%), respectively.

The ECN42 triglyceride content is applied to detect the presence of small amounts of seed oil (rich in linoleic acid) in olive oil and olive pomace oil. As seen in our study, the ECN42 value was determined as 27.27% and it is rich in linoleic and linolenic acids. Also, as seen in Figure 2; It was determined that strawberry seed oil contains monoglycerides, diglycerides and equivalent carbon number between 40-46 TAG types (ECN40-ECN46). However, olive oil contains mostly TAG types between ECN44-ECN50.

4. CONCLUSIONS

The strawberry oil used in this study was produced from seeds of *Fragaria ananassa* grown in Turkey. The high content of linolenic acid and linoleic acid in SSOs can be used to achieve an essential fatty acids balance in the food formulation, human diet and personal care products.

The strawberry seed oil is a wealthy source of mono- and poly- unsaturated fatty acids, tocopherols and phytosterols, which could find wide application in the food (functional food ingredients, food supplements and nutraceuticals), pharmaceutical and cosmetic industries.

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