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Araştırma Makalesi ***Research Article***

Determination of Fatty Acid in Asparagus by Gas Chromatography

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Abstract

Asparagus contain a lot of macronutrients and micronutrients including folate, dietary fibre (soluble and insoluble) and phenolic compounds. Also asparagus is a good source of unsaturated linoleic and linolenic fatty acids which are precursors for Eicosapentanoic acid (EPA) and Docosahexanoic acid (DHA). Unsaturated fatty acids have important biological effects and they have important role in human health. The objective of this study was to analyze fatty acid composition of asparagus as a potential source of linoleic and linolenic acid - a precursor for EPA and DHA. For this reason we analyzed fifty seven samples of asparagus collected from the local market. We used AOAC 996.06 method and analyses were performed with gas chromatograph with flame-ionization detector (GC-FID). The highest concentration of fatty acid in the asparagus was linoleic acid (C18:2n6) which content in asparagus is 25.620±1.0%. Also, asparagus is good source of α-linolenic fatty acid (C18:3n3) and content of this fatty acid in asparagus is 8.840±0.3%. The omega-6 to omega-3 (n6/n3) ratio in asparagus was 3.19. Polyunsaturated fatty acids (PUFAs) were higher than monounsaturated fatty acids (MUFAs), and from saturated fatty acids, palmitic acid was most frequent with 24.324±1.0%. From our study we can conclude that asparagus is very good source of unsaturated fatty acids, especially linoleic and linolenic fatty acids.

Özet

Gaz Kromotografi Tekniği ile Kuşkonmazda Yağ Asidinin Tespiti

Kuşkonmaz folat, diyet lifi (çözünebilen ve çözünemeyen) ve fenolik komponentler dahil çok sayıda makro ve mikronutrientleri içermektedir. Ayrıca kuşkonmaz, doymamış linoleik ve linolenik yağ asitlerinin öncüleri olan Eikosapentanoik asit (EPA) ve Dokosahekzanoik asit (DHA) için iyi bir kaynaktır. Doymamış yağ asitlerinin önemli biyolojik etkileri vardır ve insan sağlığı için önemli role sahiptir. Bu çalışmanın amacı, kuşkonmazda yağ asit kompozisyonunu potansiyel kaynak olarak linoleik ve linoleik asiti -öncüleri EPA ve DHA- analiz etmektir. Bu nedenle, lokal marketlerden temin edilen yirmi beş adet kuşkonmaz örneği analiz edilmiştir. AOAC 996.06 metodu kullanılmış ve analizler gaz kromotografi alev iyonizasyon dedektörü ile (GC-FID) gerçekleştirilmiştir. Kuşkonmazdaki en yüksek yağ asit konsantrasyonu linoleik asit (c18:2n6) olup, kuşkonmazda %25,620±1,0 düzeyindedir. Ayrıca, kuşkonmaz α-linolenic fatty acid (C18:3n3) için iyi bir kaynaktır ve kuşkonmazda bu yağ asidi miktarı %8,840±0,3'dır. Kuşkonmazda omega-6 ve omega-3 (n6/n3) oranı 3,19'dur. Çoklu doymamış yağ asitler (PUFAs) tekli doymamış yağ asitlerinden (MUFAs) daha yüksek düzeydedir ve doymuş yağ asitlerinden palmitik asit %24,324±1,0 ile en sık tespit edilendir. Bizim çalışmamızdan, kuşkonmazın özellikle linoleik ve linolenik yağ asitleri olmak üzere doymamış yağ asitleri bakımından çok iyi bir kaynak olduğu sonucuna varılabilir.

Introduction

The natural fats and oils are mixtures of glycerides of fatty acids and they are naturally occurring organic compounds which are part of a large group of water insoluble substances called lipids. Fats and oils are insoluble in water and soluble in organic solvents (chloroform, ether, benzene, turpentine). Supplies of fats and oils in the world are reported to come from vegetable sources (68.1%), animal fat (28.2%) and marine fat (3.8%) (Akpan et al., 2006).

Asparagus (*Asparagus officinalis* L.) is a vegetable that has recently attracted attention for its potential health benefits. It is a member of the Asparagales family and is related to onions, leeks and garlic. Asparagus is rich in numerous macro- and micronutrients including being a good source of folate, dietary fibre (soluble and insoluble) and phenolic compounds (Lu, 2013). Also Asparagus oil is a good source of unsaturated linoleic and linolenic acids which are a precursor for EPA and DHA (Soyland and Drevon, 1993; Vidrih et al., 2009). Moreover, unsaturated fatty acids have important biological effects and are essential fatty acids; therefore nutritionists are interested in knowing the content of these fatty acids in different foods (Beare-rogers and Dieffenbacher, 1990).

Essential fatty acids are necessary for the formation of healthy cell membranes, the proper development and functioning of the brain and nervous system, and for the production of hormone-like substances called eicosanoids (thromboxanes, leukotrienes, prosta-glandins). Marine foods such as fish and shellfish are the main dietary sources of long chain n-3 PUFA, such as EPA and DHA. The precursor of these long chain n-3 PUFA is a-linoleic acid (18:3n-3), which is mainly found in flaxseed oil, perulla oil, walnut oil, soy oil, baked beans and most green leafy vegetables. Most animals can convert 18:3n-3 to 20:5n-3 and 22:6n-3, however this conversion does not occur in plants, therefore there are no C20 and C22n-3 LC PUFAs in vegetable-based diets. Vegetarians must obtain EPA and DHA by endogenous synthesis from 18:3n-3, by desaturation and elongation. Omega-6 and omega-3 fatty acids are not interchangeable; we must consume both. These two families of essential fatty acids compete for enzymes involved in their desaturation, thus the excessive consumption of foods rich in omega-6 fatty acids may compromise the conversion of alpha-linolenic acid to EPA, with adverse affects for health and disease. Current research suggests that the levels of essential fatty acids and the balance between them may play a critical role not only in growth and development, but also in the prevention and treatment of chronic diseases including coronary artery disease, hypertension, type II diabetes, arthritis and other immune/inflammatory disorders, and cancer (Pereira et al., 2001).

The objective of this study was to analyze fatty acid composition of Asparagus as a potential source of linoleic and linolenic acid - a precursor for EPA and DHA.

Materials and Methods

Fifty seven samples from *Asparagus officinalis* stem used as experimental material were collected from fifty seven local market; 7 samples from local market in Gevgelija, 13 samples from Skopje, 8 samples from Strumica, 2 samples from Bogdanci, 4 samples from Sveti Nikole, 9 samples from Veles, 9 samples from Tetovo and 5 samples from local market in Negotino. The collected plant material was placed in a polyethylene bag to prevent loss of moisture during transportation to the laboratory.

Extraction of the lipids

The samples were homogenized on ultraturax (T25 Basic, Ika Laboretechnik, Germany) and lipid extraction from the samples was carried out under the operating conditions specified in International Standard (ISO) 6492 (ISO 6492, 1999).

Methylation (preparation of methyl esters of fatty acids)

100 mg of extracted lipids from samples were transferred into dark glass vial from 22 ml (Supelco, USA, 27004) and than fatty acids methyl esters (FAMEs) were prepared according to AOAC Official Method 996.06 (William and Latimer, 2005).

Standards

The individual fatty acid methyl ester standards (FAMEs): capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), myristoleic acid (C14:1), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1n9c), linoleic acid (C18:2n6c), arachidic acid (C20:0), γ-linolenic acid (C18:3n6) and α-linolenic acid (C18:3n3) were purchased from Sigma (Sigma-Aldrich, Germany). The individual FAMEs were used to prepare the stock solution (50 mg/ml). Calibration curves were produced from six working standards (0.5 – 30 mg/ml) which were prepared from stock solution by diluting with n-hexane. All six working standards were analyzed in five replicate for

construction of calibration curve. Identification and contents of the fatty acid were carried out by comparing sample FAME peak retention times and peak area with those obtained for FAME mix standard. The calculation of results was made with Chemstation software.

Gas chromatograph (GC) condition

Analyses of the FAMEs were carried out on a GC-FID, (GC Agilent Technologies 7890 GC System, CN 11251075, USA). Column HP88 (J&W 112 -8867; 250°C; 60m x 250mm x 0,2 mm, Agilent, USA) was used for FAMEs analysis. The operation parameters of column are given in Table 1.

Table 1. Operation parameters of column.
Tablo 1. Kolonun çalışma parametreleri.

	Rate °C/min.	Value °C	Hold Time min.	Run Time min.
Initial	/	70	1	1
Ramp 1	5	100	2	9
Ramp 2	10	175	2	18.5
Ramp 3	3	220	5	38.5

The injector and detector were kept at 250 °C and 300 °C, respectively. The gas flows which we used were: 1.4 mL/min carrier gas (He), 23 mL/min make up gas (N_2), 30 mL/min H_2 and 400 mL/min flame synthetic air. The split used was 200:1. Injections of 1 µL sample were carried out in duplicate.

Validation of the method

The method was validated by guidelines for validation of chromatographic methods (Taverniers et al., 2004). During the validation procedure were investigated linearity, precision and accuracy of the method, limit of detection (LOD) and limit of quantification (LOQ).

Results

Linearity of the method

The linearity of the method was estimated by performance of 5 replicates of FAME mix standard solution in a range between 0.5 and 30.0 mg/ml at six concentration levels. Table 2 indicates the retention time, equation and coefficient of correlation (r^2) of the calibration curve for the selected fatty acid.

Limit of detection and limit of quantification

From the mean noise value, by analysing six blanks, were calculated the results for LOD and LOQ and established by multiplying the mean noise value by 3 and 10, respectively. Table 3 are showing the value for LOD and LOQ.

Precision and accuracy of the method

The precision of the method was calculated through repeatability and reproducibility and the results expressed as the relative standard deviation (RSD, %) (Table 4). With six analysis of three samples in a day the repeatability of the method was establish, whereas the reproducibility was establish with three analysis of three samples in three consecutive days. The asparagus sample was fortified with FAME mix standard (10 mg/ml) for measure of recovery (%) Table 4. Through the recovery, accuracy of the method was verified.

Analysis of sample

Fatty acids detected in the asparagus were are follows; capric acid, lauric acid, myristic acid, myristoleic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, arachidic acid, γ-linolenic acid and α-linolenic acid (Table 4). In the present study saturated fatty acids (SFAs) in lower content than unsaturated fatty acids (UFAs) (Table 3). In the chromatogram 1 showed the retention time and peaks on fatty acid in asparagus.

Discussion

Linearity of the method

The obtained values of coefficient of correlation (r^2) were higher than 0.99 for all FAMEs, instead linoleic acid where r^2 was 0.9891. As observed of the data obtained from calibration curve the results were found to be linear over the concentration range studied.

Limit of detection and limit of quantification

The range of LOD values for target fatty acids obtained was from 0.02 to 0.09 µg/ml, while the range of LOQ was from 0.05 to 0.31 µg/ml. The lowest LOD was about 0.02 µg/ml for palmitic and α-linoleic acid and also LOQ 0.05 µg/ml and 0.07 µg/ml, respectively. These LOD and LOQ were low enough for determination of fatty acids in asparagus.

Precision and accuracy of the method

As can be seen from Table 4, RSD for repeatability ranged from 0.78% to 3.04%, and for reproducibility RSD values ranged between 1.43% and 5.14%. The method recovery ranged from 94.08% to 105.74%. From these results we can conclude that extraction, derivatization and analysis methods used are precise, reproducible and accurate.

Table 2. Linearity of the method.
Tablo 2. Metodun doğrusallığı.

Fatty Acids	Retention Time (min)	Calibration Curve Equation	Coefficient of Correlation (r^2)
C10:0	12.716	y = 1053979x - 95276	0.9995
C12:0	15.331	y = 1809994x - 538411	0.9999
C14:0	17.385	y = 1013164x - 180692	0.9974
C14:1	18.060	y = 484931x + 110777	0.9969
C16:0	19.561	y = 1656466x + 518106	0.9947
C16:1	20.208	y = 551094x + 177839	0.9946
C18:0	22.084	y = 1225351x + 285167	0.9967
C18:1n9c	22.779	y = 1206717x + 497589	0.9929
C18:2n6c	23.861	y = 565472x + 274563	0.9891
C20:0	24.655	y = 524268x + 219085	0.9998
C18:3n6	25.009	y = 1337303x + 277975	0.9983
C18:3n3	26.698	y = 665030x + 149937	0.9985

Table 3. Limit of detection and limit of quantification.
Tablo 3. Tespit ve ölçüm limitleri.

Fatty Acids	LOD (µg/ml)	LOQ (µg/ml)
C10:0	0.04	0.14
C12:0	0.05	0.16
C14:0	0.04	0.13
C14:1	0.05	0.17
C16:0	0.02	0.07
C16:1	0.06	0.21
C18:0	0.06	0.19
C18:1n9c	0.04	0.12
C18:2n6c	0.07	0.22
C20:0	0.09	0.31
C18:3n6	0.04	0.14
C18:3n3	0.02	0.05

Analysis of sample

The major SFA was palmitic acid 24.324±1.0%. From unsaturated fatty acids, essential PUFAs were higher than MUFAs (Table 5). The highest fatty acid in the asparagus was linoleic acid (C18:2n6) which content in asparagus was 25.620±1.0%. Also, asparagus is good source of α-linolenic fatty acid (C18:3n3) that belong in essential omega 3 fatty acid and content of this fatty acid in asparagus was 8.840±0.3%. The beneficial role of omega-3 PUFA related to inflammatory diseases and health has been known for many years. Both ALA and long-chain omega-3 PUFA help combat serious heart problems and immune disorders in humans. Thus, feeding omega-3 PUFA supplements to dairy cows could strengthen the immune systems of cows and improve the well-being of the people who consume milk from such cows. Supplementing rations of dairy cows with omega-3 PUFA increases the concentration of these fatty acids in their tissues and alters the concentrations of inflammation indicators in the blood, such as prostaglandin $F_{2\alpha}$ and tumor necrosis factor-α (TNF-α). It is not fully known if these effects of omega-3 PUFA can improve the health and breeding efficiency of high-producing dairy cows (Badinga and Torres, 2011). From Table 2 it can be seen that omega 3 fatty acids were lower than omega 6 fatty acids, 8.840±0.3 and 28.190±1.3% fatty acids respectively. The omega-6 to omega-3 (n6/n3) ratio in asparagus was 3.19. Optimal dietary intakes of the n6/n3 ratio should be around 1–4/1 (Callaway, 2004; Patterson et al., 2012; Simopoulos, 2003).

The ratio of omega–6/omega–3 of 4/1 appears to be the optimal ratio for brain-mediated functions and the same ratio, in the secondary prevention of cardiovascular disease was associated with a 70% decrease in total mortality (Simopoulos, 2003). In patients with colorectal cancer rectal cell proliferation were reduced with ratio of omega–6/omega–3 of 2.5/1, whereas the same amount of omega–3 PUFA with ratio 4/1 had no effect (Simopoulos, 2003). On the other hand, low intakes of omega 3 fatty acids along with high intake of omega 6 fatty acids could potentiate inflammatory processes and consequently predispose to or exacerbate many inflammatory diseases (Patterson et al., 2012).

Table 4. Repeatability, reproducibility and accuracy of the method.
Tablo 4. Metodun tekrarlanabilirliği, ölçülebilirliği ve doğruluğu.

Fatty Acids	Repeatability (n=6), RSD, % Sample			Reproducibility (n=6), RSD, % Sample			Recovery %
	1	2	3	1	2	3	
C10:0	1.39	1.48	1.26	2.47	2.49	3.06	95.14
C12:0	1.04	0.95	0.97	2.64	2.38	2.41	97.01
C14:0	2.04	1.93	1.87	4.04	3.12	3.56	103.42
C14:1	2.36	2.35	2.01	3.71	3.71	3.29	101.15
C16:0	1.15	1.37	1.43	2.78	2.61	2.95	94.72
C16:1	0.78	0.92	0.87	1.99	1.74	1.62	96.13
C18:0	0.92	0.93	1.16	2.05	1.49	1.56	96.44
C18:1n9c	2.34	2.33	2.57	4.38	4.52	4.80	98.23
C18:2n6c	2.56	2.08	2.44	3.06	3.27	2.91	105.74
C20:0	1.48	1.32	1.36	1.54	1.54	1.71	94.08
C18:3n6	1.32	1.01	1.17	1.48	1.43	1.81	99.04
C18:3n3	3.04	2.47	2.53	4.76	5.14	4.72	102.51

Table 5. Fatty acid composition in Asparagus.
Tablo 5. Kuşkonmazda yağ asit kompozisyonu.

Fatty Acids	Fatty acid composition in Asparagus, % (n=24)*	Standard deviation (%)
C10:0	1.777 ± 0.1	0.082
C12:0	1.250 ± 0.1	0.084
C14:0	3.575 ± 0.2	0.169
C14:1	0.887 ± 0.05	0.045
C16:0	24.324 ± 1.0	0.398
C16:1	8.511 ± 0.7	0.626
C18:0	8.924 ± 0.3	0.239
C18:1n9c	12.840 ± 0.6	0.451
C18:2n6c	25.620 ± 1.0	0.946
C20:0	0.882 ± 0.1	0.075
C18:3n6	2.570 ± 0.1	0.074
C18:3n3	8.840 ± 0.3	0.250
Omega 3[1]	8.840 ± 0.3	0.250
Omega 6[2]	28.190 ± 1.3	1.013
Saturated[3]	40.733 ± 1.3	0.865
Monounsaturated[4]	22.237 ± 1.2	1.037
Polyunsaturated[5]	37.030 ± 1.6	1.219
Unsaturated[6]	59.267 ± 2.8	2.236

*Each value is the mean ± minimum/maximum content of fatty acids in 24 Asparagus samples.
1: $C_{18:3n3}$
2: $C_{18:2n6c}$, $C_{18:3n6}$
3: $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{20:0.}$
4: $C_{14:1,}$ $C_{16:1,}$ $C_{18:1n9c,}$
5: $C_{18:2n6c,}$ $C_{18:3n6,}$ $C_{18:3n3}$
6: MUFA + PUFA

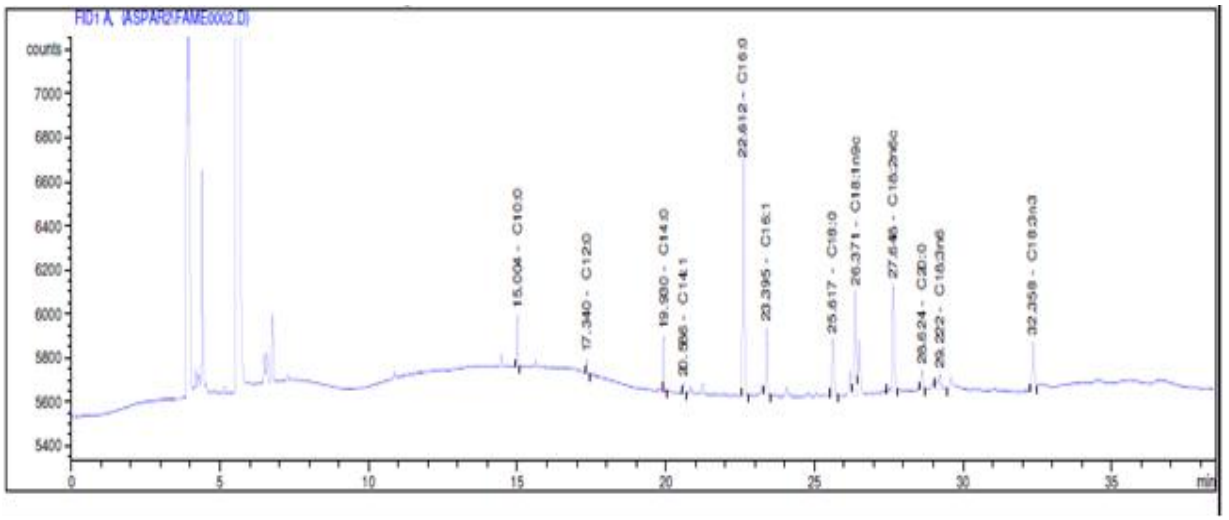

Figure 1. GC-FID chromatogram of fatty acid composition in asparagus.
Şekil 1. Kuşkonmazda yağ asit kompozisyonunun GC-FID kromotogramı.

In conclusion, asparagus is good source of polyunsaturated fatty acids, especially linoleic and linolenic fatty acids. Therefore, asparagus can be used as an important source of essential fatty acid in human nutrition.

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