Research Article

Fatty acid composition of vegetable marrows and zucchini leaves

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Abstract

The qualitative composition and quantitative content of fatty acids in leaves of vegetable marrows (*Cucurbita pepo* L. var. *giromontina* Alef.), zucchini (*Cucurbita pepo* L. var. *cylindrica* Paris) yellow- and green-fruits varieties were determined by using GC/MS. 14 fatty acids were identified as the result of the experiment. Unsaturated fatty acids were found to be dominated in the raw plant material. The total content of unsaturated fatty acids amounted to 60.47% in vegetable marrows leaves, 64.35% yellow zucchini leaves and 68.85% green zucchini leaves, among which linoleic and linolenic acid dominated. The related health lipid indices (IA, atherogenicity, IT, thrombogenicity and IH, health) were determined. It is shown that the use of such biological resources is actual and expedient for the purpose of alimentary correction of the physiological condition of a person.

Keywords

Introduction

Cardiovascular diseases are one of the most topical problems of scientific medicine at the beginning of the XXI century. They occupy a leading position among the causes of mortality in most economically developed countries (Arnoldi 2004). WHO experts estimate that one third decrease in mortality from cardiovascular diseases can be caused by the development of drug therapy and clinical interventions. At the same time, a two-thirds reduction of mortality provides a change in lifestyle habits, among which one can be called proper nutrition (FAO 2010; Ulbricht and Southgate 1991). An important factor in dietary adjustments is the special products based on raw materials of plant origin that are tailored to the physiological needs of the body. One of the key directions of the solution of the problem is the development and introduction of

foods with a balanced fatty acid composition into the diet (Levitsky 2002). The combination of saturated fatty acids (SFA) with polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) significantly reduces the atherogenic effect of SFA. It is generally recognized that the fatty component of the daily diet should provide no more than 30% of the energy requirement, including in equal quantities of individual fractions of fatty acids, that is, SFA:PUFA:MUFA = 1:1:1.

This composition of "ideal" fat is the basis for development of norms of physiological needs of the population in the basic nutrients and energy. It has been scientifically proven that the ratio of ω-6 to ω-3 PUFA should be 10:1, and in cases of lipid metabolism infringement 5:1 and even 3:1 (FAO 2010). For the elderly, the ratio of PUFA to

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SFA is 2:1 (Gladyshev 2012). Analysis of the results of the actual diet of the population of Ukraine shows that these acids actually come into the human body in relations to 10:1 to 30:1 (Smolyar 2006). Part of the ω-3 fatty acids that are useful for preventing age-related illnesses against other unsaturated fatty acids should be as much as possible. Residents of Japan, who consume a lot of seafood with high content of ω-3 fatty acids, differ not only in longevity, but also in lower incidence in older age. At the same time, the actual consumption of fats with the dominance of MUFA is redundant and the use of PUFA is insufficient in the countries of Europe and America (FAO 2010; Gladyshev 2012).

Aliphatic fatty acids play an important role in the human body: normalize the metabolism of lipids and proteins, increase the detoxification function of the liver, restore and maintain its cellular structure, improve blood rheology and microcirculation (FAO 2010).

In order to predict and further adjust the influence of food ration on human health, it is advisable to assess the atherogenicity of the food product. Indicators of atherogenicity, thrombogenicity and health that characterize the fatty acid composition of food intake and medicinal plant materials have been identified with the purpose of argumentation of expediency of appointment for long-term application (Sinyavskiy et al. 2016; Tait et al. 1991; Ulbricht and Southgate 1991). These indicators allow to estimate the risk level of atherosclerosis and thrombosis depending on the fatty acid composition of the product.

The atherogenicity index (IA) indicates the relationship between the sum of the main saturated fatty acids and that of the main classes of unsaturated (Sinyavskiy et al. 2016; Ulbricht and Southgate 1991). This index is calculated by the ratio of saturated and unsaturated fatty acids. For example, for olive oil, this figure is 0.14, for sunflower oil is 0.07.

The thrombogenicity index (IT) is an indicator that characterizes the tendency to form clots in the blood vessels. It is defined by the ratio of prothrombogenic (saturated fatty acids) and antithrombogenic (mono- and polyunsaturated fatty acids) factors (Sinyavskiy et al. 2016; Ulbricht and Southgate 1991). For example, for olive oil it is 0.32, for sunflower oil it is 0.28 (MUFA, PUFA – n6 and PUFA – n3).

Thus, excessive consumption of saturated fats and products having high atherogenic and thrombogenic indices significantly increases the risk of atherosclerosis and as a consequence of coronary heart disease, heart attack and stroke (Sinyavskiy et al. 2016; Ulbricht and Southgate 1991).

Health index (IH) indicates the ratio of the sum of polyunsaturated and monounsaturated fatty acids to saturated fatty acids (Tait et al. 1991). The greatest health index has vegetable oils such as soybean oil and olive oil. It is more than 7. Animal fats, which are characterized by low content of PUFA and MUFA, have a health index of less than 2.

The consumption of foods with high levels of thrombogenicity and atherogenicity increases the risk of cardiovascular diseases.

In this regard, the search for raw materials that have a positive impact on the quality of life and reduce the development of metabolic-alimentary and cardiovascular diseases is of some interest (Gunstone 2011).

The *Cucurbitaceae* family includes several important crops, such as melon (*Cucumis melo*), watermelon (*Citrullus lanatus*), cucumber (*Cucumis sativus*), pumpkin (*Cucurbita pepo)* and many *Cucurbita* species with edible fruits (Lim 2012). Analysis of the literature showed that fruits of the plants of the *Cucurbita* genus contained various biologically active compounds: carbohydrates, vitamins, minerals, amino acids, organic acids, phenolic compounds, terpenoids etc. (Lim 2012; Van Wyk 2005).

Vegetable marrow (*Cucurbita pepo* L. var. *giromontina* Alef.) and zucchini (*Cucurbita pepo* L. var. *cylindrica* Paris) are a monoecious species grown as a vegetable. The both species are annual herbaceous plants with sturdy running stalks; the leaves, large hispid and lobed, are supported by long, thick, completely hollow petioles. The fruits of the both cultivars are for the most part smooth and cylindrical; they are in general uniformly light green (vegetable marrow), yellow or dark green (zucchini), but some cultivars are variegated. They have soft and edible skin (Lim 2012).

The edible fruit, seeds and flowers of the both cultivars contain phytochemicals that impart many pharmacological properties (Lim 2012; Ivanova et al. 2012). Epicarp of fruits showed a higher carotenoid content and that the darker green colored fruit are indicative of higher carotenoid levels (Ivanova et al. 2012). The carotenoids were identified as β-carotene, lutein, neoxanthin, violaxanthin, lactucaxanthin, α-cryptoxanthin, β-cryptoxanthin, β-carotene 5,6-epoxide, α-carotene, 9Z-β-carotene and 15Z-β-carotene (Lim 2012). Fruits also contain chlorophylls a, a', b, b', pheophytins a, a', b and pyropheophytins a and b. The raw zucchini, vegetable marrow fruit with skin a rich source of water 95%, protein 1%, fats 0.1%, minerals (Ca, Fe, Mg, P, K, Na, Zn, Cu, Mn), vitamins (C, B_1, B_2, PP, B_5, B_6, E, K), fiber 0.9%, carbohydrates 2-3%, amino acids, etc. (Lim 2012).

Fruits of the both species are recommended in dietary food for convalescents or people with strict diets and used in infant food. They also have antisclerotic and considerable diuretic actions. Zucchini have tonic properties due to high content of carotene and vitamin C. The seeds of vegetable marrow have an anthelminthic activity. Fruits of the both species are eaten as a vegetable, steamed, boiled, grill, fried, baked, barbequed or hollowed and stuffed (Lim 2012; Van Wyk 2005).

At the same time, scientific data on chemical composition of vegetable marrows and zucchini leaves not available. Preformulation phytochemical studies have proven the presence of amino acids, polysaccharides, organic acids, vitamins, flavonoids, tannins in the leaves of vegetable marrow and zucchini. The mineral composition of vegetable marrows and zucchini leaves was previously studied by us (Iosypenko et al. 2019). Our study confirmed the literature data on the use of zucchini and vegetable marrow leaves. Infusions, decoctions and juice from zucchini and vegetable marrow leaves contribute to the normalization

of water-salt metabolism in the body and have a diuretic effect (Lim 2012).

Expanding the range of medicinal plant materials is an urgent task of pharmacy. The selected object is widely cultivated and has an adequate, affordable and cheap raw material base.

Thus, the study and use of zucchini and vegetable marrow leaves open up prospects for replenishing the registry of medicinal plant raw materials and substances based on them.

Aim of the research

The aim of this work was to identify and determine the quantitative content of fatty acids in vegetable marrows leaves, yellow zucchini leaves and green zucchini leaves by gas chromatography / mass spectrometry method (GC/MS) (Omelchenko and Kyslychenko 2005).

Materials and methods (experimental part)

The research used raw materials harvested in August 2018 in Kharkiv region (Ukraine). After harvesting the raw materials were dried, brought to a standard state in accordance with the general GACP requirements (WHO 2003).

The plants were identified by Department of Chemistry of natural compounds, National University of Pharmacy, Kharkiv, Ukraine. Voucher specimens of the plants have been deposited in Departmental Herbarium for future record.

All applied reagents were of the highest purity available and purchased from the Sigma–Aldrich Chemical Company.

The sample of plant raw material was grinded into a powder by laboratory mill, then about 0.5 g (accurately mass) was selected and placed into the glass vial and 3.3 ml of reacting mixture (methanol: toluene: sulfuric acid (44:20:2 v/v)) and 1.7 ml of internal standard solution (undecanoic acid in heptane solution) were added. The sample was maintained at 80 °C for 2 hours, cooled and centrifuged for 10 minutes at 5000 rpm. 0.5 ml of the upper heptane phase was taken, then the heptane phase containing methyl esters of fatty acids (Omelchenko and Kyslychenko 2005).

The chemical composition of the samples was analyzed on a Hewlett Packard HP-6890 chromatographic/mass spectrometer with a mass-selective detector HP-5972. The separation of the components of the mixture was carried out using a capillary column HP-5MS (apolar) of 30 m in length and an internal diameter of 0.25 mm, a thickness of the stationary phase is 0.25 µm (5% Diphenyl), a carrier gas flow rate of 1 cm^3/min, and polar column HP-INNOWAX (Polyethyleneglycol) 30m × 0.25mmø 0.25µm. The carrier gas is helium in the temperature programming mode. The column was maintained at 60 °C for 5 minutes, then heated at a temperature of 5 °C/min to 280 °C and maintained at a final temperature for 10 min. The volume of the sample that was injected is 1 µl. The temperature of the evaporator was 250 ºC, the temperature of the detector was 280 ºC, the electron impact beam ionization mode with electron energy was 70 eV, the scan was in the range of m/z from 40 to 450.

The quality composition of fatty acids was established by directly comparing them by the time of holding with similar indicators of authentic samples and full mass-spectrums with matching standard spectrums of clean substances from the NIST02 and Willey 138k electronic libraries.

Index of atherogenicity (IA) was calculated according to the Ulbricht and Southgate formula (Sinyavskiy et al. 2016; Ulbricht and Southgate 1991).

$$IA = \frac{C_{12:0} + 4 \times C_{14:0} + C_{16:0}}{\sum MUFA + \sum PUFA}$$

The thrombogenicity index (IT) was calculated based on the Ulbricht and Southgate formula (Sinyavskiy et al. 2016; Ulbricht and Southgate 1991).

$$IT = \frac{C_{14:0} + C_{16:0} + C_{18:0}}{0.5 \times \sum MUFA + 0.5 \times \sum PUFA(\omega-6) + 0.5 \times \sum PUFA(\omega-3) + \frac{\sum PUFA(\omega-3)}{\sum PUFA(\omega-6)}}$$

Health index (IH) was calculated according to the formula (Tait et al. 1991).

$$IH = \frac{\sum MUFA + \sum PUFA}{C_{12:0} + 4 \times C_{14:0} + C_{16:0}}$$

It is known that unsaturated fatty acids give cell membranes the fluidity necessary to maintain their structural and functional state. Cell membrane fluidity depends on the ratio of saturated fatty acids to unsaturated acids and the degree of unsaturation of the latter (Sakhno 2010). Therefore, the next assessed parameter was the double bond index, which was calculated by the method proposed by Lyons with the co-authors (Sakhno 2010).

$$IDB = \sum P_j \times n_j / 100,$$

P_j – the content of fatty acids, %,
n_j – the number of double bonds in each acid.

Statistical processing and data analysis were performed using Statistic 7.0, Excel 7.0 statistical program package for Microsoft Office for Windows.

Results and discussion

Chromatograms of fatty acids, obtained during the analysis of the leaves of the studied species, are given on Fig. 1-3, and the summary results of the definition are presented in Table. 1.

As a result of the study, quantitative content of vegetable marrows leaves and yellow zucchini leaves for 14 fatty acids was determined, from which 7 unsaturated and 6 saturated fatty acids were identified, 12 fatty acids were found in green zucchini leaves of which 6 were unsaturated and 5 were saturated. The unsaturated coefficient, which was defined as the

Table 1. A comparative analysis of the fatty acid of the leaves of vegetable marrows, yellow zucchini, green zucchini and common pumpkin (Batyuchenko et al. 2013).

№	Common name of fatty acid	Chemical nomenclature	Quantitative content of methyl esters of fatty acids, % of the total			
			vegetable marrows	yellow zucchini	green zucchini	common pumpkin
Saturated acids						
1	Lauric (dodecanoic)	C 12:0	–	–	–	0.43
2	Myristic (tetradecanoic)	C 14:0	2.30	2.43	3.00	–
3	Pentadecanoic (pentadecyl)	C 15:0	–	–	–	0.37
4	2-Oxipalmitic (oxyhexadecanoic)	C 16:0	–	–	–	1.54
5	Palmitic (hexadecanoic)	C 16:0	17.98	19.18	16.60	30.14
6	Stearic (octadecanoic)	C 18:0	4.87	5.60	3.45	7.24
7	Arachidic (eicosanoic)	C 20:0	2.35	2.35	–	2.10
8	Behenic (docosanoic)	C 22:0	5.58	2.19	2.95	1.84
9	Lignoceric (tetracosanoic)	C 24:0	3.65	1.40	1.90	1.87
Monounsaturated acids (ω-9)						
10	Myristoleic	C 14:1	0.60	0.53	0.72	–
11	Palmitoleic (hexadecenoic)	C 16:1	0.68	1.15	1.12	3.96
12	Oleic (octadecenoic)	C 18:1	5.70	9.25	4.68	3.56
13	Gondoic (eicosenoic)	C 20:1	0.14	0.10	–	–
14	Erucic (docosenic)	C 22:1	3.90	5.00	2.30	–
Polyunsaturated acids (ω-3 and ω-6)						
15	Linoleic (octadecadienic, ω-6)	C 18:2	16.20	14.55	27.53	5.06
16	Linolenic (octadecatrienic, ω-3)	C 18:3	33.25	33.77	32.50	41.88
Unidentified components						
15	*--**	–	2.80	2.50	3.25	–
The amount of saturated fatty acids			36.73	33.15	27.9	45.53
The amount of unsaturated fatty acids			60.47	64.35	68.85	54.47
The amount of unidentified fatty acids			2.80	2.50	3.25	–
Total			100.0	100.0	100.0	100.0
Unsaturation ratio			1.65	1.94	2.47	1.20

ratio of the amount of unsaturated acids to the amount of saturated acids, is 1.65, 1.94 and 2.47, respectively. The length of carbon chains of fatty acids was 14 to 22 atoms. The acids C-16 and C-18 rows accounted for 78.68-85.88%, which is not unique and is typical of many systematic groups of plants, because these acids are mainly involved in the formation of cell membranes (Plemenkov 2001; Semenov 2000).

As can be seen from Table 1, the total content of saturated fatty acids was 36.73% in the leaves of vegetable marrows, was 33.15% in yellow zucchini leaves and 27.9% was in green zucchini leaves. The fatty acids composition of many systematic groups of plants contains palmitic acid as the main saturated acid, its content in the investigated raw material was 17.98%, 19.18% and 16.60%, respectively. The myristic, arachidic, behenic and lignoceric acids were detected in small quantities. In the composition of fatty acids of vegetable marrows and yellow zucchini leaves arachidonic acid was found, which is absent in green zucchini leaves.

The acids with the largest number of carbon atoms were behenic (C22:0) and lignoceric (C24:0). Their content in vegetable marrows leaves is almost 2 times higher than in yellow zucchini leaves and green zucchini leaves.

Unsaturated fatty acids dominate the fatty acids of the studied raw materials the total content of which in the leaves of vegetable marrows was60.47%, in the leaves of yellow zucchini was 64.35%, in the leaves of green zucchini was 68.85%. Most of the total fatty acid content accounts for unsaturated acids of the C-18 series - 49.45%, 48.32% and 60.03% respectively. These acids dominate in the lipids of many higher plants (Plemenkov 2001; Semenov 2000).

Aliphatic monoenic, dienic and trienic fatty acids with cis-configuration of double bonds were identified in the studied raw material (Plemenkov 2001; Semenov 2000). Oleinic acid (C18:1ω-9) prevailed from ω-9 monoenic fatty acids, its content was 5.70% in the leaves of vegetable marrows, it was 9.25% in leaves of yellow zucchini and it was 4.68% in leaves of green zucchini. Gondoic acid (C20:1ω9) was present in vegetable marrows leaves and in yellow zucchini leaves in a small amount, its content was 0.14% and 0.10% respectively. It was not present in the leaves of green zucchini. A number of other monoic acids, which were contained in insignificant amount (<1%), were also found in the composition of fatty acids of the studied raw material. Linoleic acid (C18:2ω-6) was found from the family of ω-6 dienic fatty acids, its content was 16.20% in vegetable marrows leaves, it was 14.55% in yellow zucchini leaves, and it was 27.53% in green zucchini leaves. From the ω-3 trienic fatty acid family linolenic acid has the highest content. Its content in the leaves of vegetable marrows amounted to 33.25%, in yellow zucchini leaves it was 33.77% and in green zucchini leaves it was 32.50%. This acid is widespread in plants, a significant amount of α-linolenic acid is found in the lipids of chloroplast membranes (Plemenkov 2001). Controlling the content of essential fatty acids (18:2: 18:3) allows to design technologies for preventive treatment of various pathological processes, to design the formulations of biocorrectors. The content of erucic acid, which is regulated in many countries, did not exceed 2.30-5.0%, which is within acceptable limits (FAO 2010).
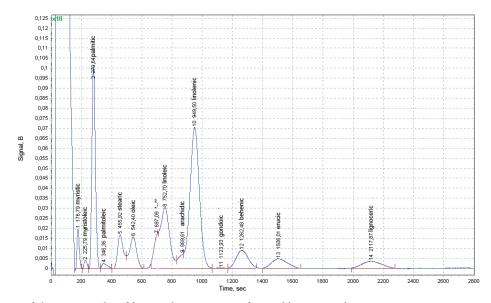
Figure 1. Scheme of chromatography of fatty acid composition of vegetable marrows leaves.
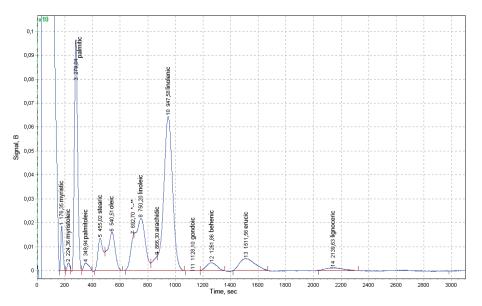

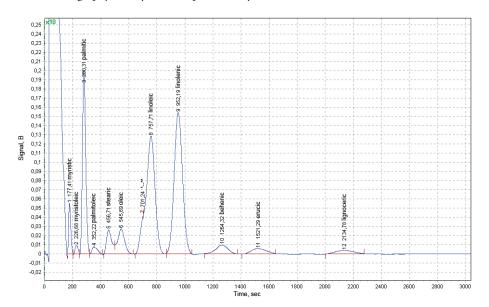**Figure 2.** Scheme of chromatography of fatty acid composition of yellow zucchini leaves.

Figure 3. Scheme of chromatography of fatty acid composition of green zucchini leaves.

Table 2. Indices of atherogenicity, thrombogenicity and health of the lipophilic fraction of the leaves of vegetable marrows, yellow zucchini and green zucchini.

Indexes	Raw plant material		
	vegetable marrows	yellow zucchini	green zucchini
Index of atherogenicity	0.45	0.45	0.42
Index of thrombogenicity	0.82	0.83	0.65
Index of health	2.22	2.23	2.41
Index of double bonds	1.43	1.46	1.61

Normally, the ratio of atherogenic and antiatherogenic lipids should not exceed 3.5. The analysis of the atherogenic and thrombogenic indices of the studied raw material showed their almost complete coincidence and significantly lower level than 3.5 (Table 2), which is an important argument for the feasibility for the long-term use of these plant objects as an active pharmaceutical ingredient or medicinal product.

By the magnitude of the double-bond index, the studied raw material was arranged in the following order: green zucchini leaves (1.61), yellow zucchini leaves (1.46) and vegetable marrows leaves (1.43). These data indicate the adaptability of plants to temperature differences during their cultivation.

Polyunsaturated fatty acids are not synthesized in the human body, but only come with food, therefore, the study of their presence and content in the researched objects is relevant. A wide range of saturated and unsaturated fatty acids, from which all the substances are predominantly unsaturated fatty acids, makes it possible to recommend the investigated raw materials in the complex therapy of metabolic disorders, for the prevention and treatment of cardiovascular diseases, etc.

A comparative analysis of the fatty acid composition of the *Cucurbita* plants leaves showed the prospects for their pharmacological study.

Conclusion

1. The fatty acid qualitative composition and quantitative content of the vegetable marrows leaves, yellow zucchini leaves and green zucchini leaves was determined by the GC/MS method.
2. The quantitative content of 14 fatty acids was determined, of which 13 were identified.
3. The palmitic acid prevails among saturated fatty acids, and linolenic acid dominates among unsaturated fatty acids in all investigated samples.
4. The greatest accumulation of linolenic acid is observed in the vegetable marrows leaves, yellow zucchini leaves and green zucchini leaves. It was 33.25%, 33.77 and 32.50%, respectively.
5. There are no arachidonic and gondoic acids in the green zucchini leaves.
6. The characteristic profile of fatty acid of the total fatty acid composition can probably be explained by the peculiarities of the metabolism of these acids in each of the studied types of raw materials.
7. Data for the atherogenic and thrombogenic indices, as well as the fatty acid composition of the investigated raw material indicate the prospect of its wide use for the design of functional products, including baby food products with directed anti-atherogenic and hypolipidemic properties.
8. The conducted researches allow to predict the use of the investigated raw materials for the prevention and treatment of cardiovascular diseases, metabolic diseases and inflammatory processes.

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