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Bitter vetch seed oil (*Vicia ervilia* L.) – A new source of bioactive components

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Nowadays, the demand of underused plants which may be new sources of bioactive compounds is constantly growing. For that reason, the lipid composition of bitter vetch seed oil (*Vicia ervilia* L.) has been examined. The plant has Russian origin but has been introduced in southern Bulgaria. The oil content of the seeds is rather low (1.4%), but the lipids are rich in unsaponifiable matter (39.7%) – phytosterols (4.7%), tocopherols (4010 mg/kg) and carotenoids (684 mg/kg). Unsaturated fatty acids (73.4%) predominate in the glyceride oil and the main ones are oleic (52.0%) and linoleic (20.4%) acids. The major saturated fatty acid is palmitic with amount of 20.8%. The quantity of monounsaturated fatty acids (52.6%) is higher than that of polyunsaturated (20.8%) ones. β-Sitosterol (83.3%) is the main component in the sterol fraction, followed by campesterol (12.1%). Only two tocopherols (α- and γ-) are identified in the tocopherol fraction, in which γ-tocopherol predominates (87.6%). Total phospholipids in bitter vetch seeds are 0.7% and the major component is phosphatidylinositol (28.9%). Fatty acid composition of three classes of the phospholipids (phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine) in the seeds has also been examined. Oleic acid is the main fatty acid in the last two phospholipids (61.1 and 48.2%, respectively), while palmitic acid (46.8%) predominates in phosphatidylinositol.
These results depict that the oil from bitter vetch seeds is a promising source of bioactive components and can be successfully implemented in different functional food ingredients.

Keywords: Bitter vetch, Fatty acids, Sterols, Tocopherols, Phospholipids

Introduction

Currently the interest in finding new sources of fat-soluble biologically active components (such as fatty acids, mono-, di- and triacylglycerols, phospholipids, tocopherols, phytosterols, phytostanols, squalene, terpenoids, sphingolipids, carotenoids, chlorophylls, *etc.*) is a topic of many studies.[1,2] These compounds are important for the human consumption and are present mainly in the plants, specifically in the lipids of their seeds or grains. The searching of new alternative sources of these components among the flora is constantly growing. Nevertheless, myriad of plant species are not examined enough regarding the lipid composition of their seeds.

Bitter vetch (*Vicia ervilia* L.) is a legume crop from Fabaceae family, which has been grown since ancient times. This plant is widespread in the Mediterranean region in Europe, West and Central Asia, North Africa and the United States.[3,4] Bitter vetch is being described as a promising crop because of its easiness to be cultivated and harvested, and relatively good yield of the plant is obtained even in dry areas where the precipitation during the year is low.[3] Other than that, its seeds have high protein content (20-30%), carbohydrates (60.5 - 64.0%) also constitute a big part of the chemical composition of the seeds, and there is a small presence of glyceride oil (1.1-2.0%), fibers (4.0-6.0%) and ash (2.4-3.5%).[5,6,7,8,9,10] Bitter vetch seeds also contain some anti-nutritional factors (such as condensed tannins, canavanine and trypsin inhibitors) but their content is proved to decrease significantly after hydrothermal and extrusion processing of the seeds as well as soaking in diluted basic and acidic solutions.[11,12] The main fatty acid in the triacylglycerols of bitter vetch seed oil is linoleic acid (40.2-53.4%), followed by oleic (18.2-28.2%), palmitic (12.7-14.3%) and linolenic acid (9.3-10.1%).[13,14]

Globally the studies of bitter vetch seeds are mainly focused on their chemical composition (content of proteins, lipids, carbohydrates, fibers, ash and dry matter), presence of trace elements and fatty acid composition of their glyceride oil.

However, no in depth study was performed on the lipid composition of bitter vetch seed oil, including not only fatty acid composition, but also sterols, tocopherols, phospholipids, carotenoids and chlorophyll. For that reason, the aim of the present work was to characterize the lipid composition and to determine the fat-soluble biologically active components of bitter vetch (*Vicia ervilia* L.) seed oil.

Experimental

Plant material

Bitter vetch seeds were provided by Institute of Plant Genetic Resources "Konstantin Malkov" – Sadovo, Bulgaria. The plant identified as *Vicia ervilia* L. was with Russian origin but introduced in the southern part of Bulgaria (in the region of Plovdiv). The moisture of the seeds was 9.7% ± 0.2%. The glyceride oil of the seeds was obtained by extracting with *n*-hexane for 8h[15] and was found to be 1.4% ± 0.1%.

Fatty acid composition

Fatty acid composition of the oil was determined by gas chromatography (GC)[16] on HP 5890 gas chromatograph. Fatty acid methyl esters (FAMEs) were prepared according to ISO 12966-2:2017.[17] Identification was performed by comparison of the retention times with those of a standard mixture of FAME.

Sterol composition

Unsaponifiables were determined according to ISO 18609:2000.[18] Total sterols were determined spectrophotometrically at 597 nm.[19]

Individual sterols were determined on HP 5890 gas chromatograph with 25 m x 0.25 mm DB – 5 capillary column and FID. Identification was confirmed by comparison of retention times with those of a standard mixture of sterols.[20]

Tocopherol composition

Tocopherols were determined in the oil by high performance liquid chromatography (HPLC) on a Merck-Hitachi (Merck, Darmstadt, Germany) with fluorescent detector. Tocopherols were identified by comparing the retention times with those of a standard mixture.[21]

Phospholipid composition

Phospholipids were extracted with a mixture of chloroform : methanol (2 : 1, v/v).[22] Individual phospholipid classes were isolated by two-dimensional thin-layer chromatography (TLC).[23] Identification was performed by comparing the respective Rf values with those of standards. Phospholipids were scrapped and mineralized with perchloric and sulphuric acid, 1 : 1 (v/v). After that, they were measured spectrophotometrically at 700 nm.[24]

The fatty acid composition of the phospholipids was determined by GC.

Determination of carotenoids and chlorophyll

The total carotenoid and chlorophyll content were determined spectrophotometrically at 470 and 670 nm, respectively, on a Boeco S-26 spectrophotometer (Germany) according to the method described by Borello and Domenici (2019).[25]

Statistical analysis

All measurements were performed in triplicate (n = 3) and the results were given as mean value ± SD.

Results and discussion

In the present study the lipid composition (fatty acids, sterols, tocopherols, phospholipids, carotenoids and chlorophyll) of bitter vetch (*Vicia ervilia* L.) seed oil was determined.

The quality of the glyceride oils is a very important factor for assessing their nutritional value. The fatty acid composition of the bitter vetch seed oil was examined (Table 1).

Thirteen fatty acids were identified in the bitter vetch seed oil. Oleic acid (52.0%) was the main fatty acid in the triacylglycerols, followed by palmitic (20.8%) and linoleic (20.4%) acids. The content of the saturated stearic acid was found to be relatively low – 3.1%. The other fatty acids varied between 0.1 and 1.0%.

The results about the fatty acid composition of the examined bitter vetch seed oil differed from these obtained by Bakoglu et al. (2009)[14] and Kökten et al. (2010)[26] who reported that the main fatty acid was linoleic (40.16-53.77%). The content of oleic and palmitic acid in the same studies was also different (9.20-28.20% and 10.33-12.16%, respectively). A significant amount of linolenic acid was observed, too – from 9.26 to 19.69% compared to the results from the present study – 0.4%. On the other hand, Kaplan et al. (2014)[27] examined the fatty acid composition of ten varieties of *Vicia ervilia* from Turkey and observed that the amount of both oleic and linoleic acids were in a very close range (26.69-31.62% and 27.93-39.39%, respectively).

The quantities of saturated (SFA), unsaturated (UFA), mono- (MUFA) and polyunsaturated (PUFA) fatty acids are given in Fig. 1.

UFA predominated in the triacylglycerol fraction (73.4%), while the content of SFA was 26.6%. Among the UFA the amount of MUFA (52.6%) was as twice as higher than those of PUFA (20.8%). These results also differed from the previous studies where the quantity of PUFA varied from 62.75 to 83.1%.[14,26,27]

Regarding the fatty acid composition of the bitter vetch seeds oil can be said that it is close to those of olive oil in which according to International Olive Council (IOC) (2015) the allowable range for oleic acid is 55.00-83.00%, for linoleic is 2.50–21.00%, for palmitic is 7.50–20.00% and the content of linolenic acid have to be below 1.00%.[28]

Other very important fat-soluble components in the seed oils are sterols, tocopherols, phospholipids, carotenoids and chlorophyll. The content of the biologically active components in bitter vetch seed oil is shown in Table 2.

The amount of unsaponifiable matter in the oil was rather high (39.7%) and one of the main components in this fraction were sterols. Their content in the oil was 4.7% and they constituted 12.0% of the unsaponifiables. Bitter vetch seed oil contained mostly phytosterols and negligible amount of cholesterol. Phytosterols are important natural compounds which derived from glyceride oils of different plants, cereals, fruits and vegetables.[29] Tocopherols are potent antioxidants which break the chain reactions during the peroxidation of the UFA.[1] Total tocopherol content in the oil was extremely high – 4010 mg/kg in the oil (56 mg/kg in the seeds).

Table 1. Fatty acid composition of bitter vetch seed oil.

Fatty acids		Content, %
$C_{12:0}$	Lauric	0.1±0.0
$C_{14:0}$	Myristic	1.0±0.1
$C_{14:1}$	Myristoleic	0.1±0.0
$C_{15:0}$	Pentadecanoic	0.4±0.1
$C_{16:0}$	Palmitic	20.8±0.5
$C_{16:1}$	Palmitoleic	0.4±0.1
$C_{17:0}$	Margaric	0.2±0.0
$C_{17:1}$	Heptadecenoic	0.1±0.0
$C_{18:0}$	Stearic	3.1±0.1
$C_{18:1}$	Oleic	52.0±0.6
$C_{18:2}$	Linoleic	20.4±0.4
$C_{18:3}$	Linolenic	0.4±0.1
$C_{20:0}$	Arachidic	1.0±0.1

Table 2. Content of biologically active components of bitter vetch seed oil.

Biologically active components	Content
Unsaponifiable matter, %	
- in the oil	39.7 ± 0.4
- in the seeds	0.6 ± 0.0
Sterols, %	
- in the oil	4.7 ± 0.2
- in the seeds	0.1 ± 0.0
Tocopherols, mg/kg	
- in the oil	4010 ± 262
- in the seeds	56 ± 4
Phospholipids, %	
- in the oil	45.8 ± 0.6
- in the seeds	0.7 ± 0.0
Carotenoids, mg/kg	
- in the oil	684 ± 16
- in the seeds	10.0 ± 0.2
Chlorophyll, mg/kg	
- in the oil	56 ± 5
- in the seeds	0.9 ± 0.1

Table 3. Sterol, tocopherol and phospholipid composition of bitter vetch seed oil.

Sterols	Content, %
Cholesterol	0.2 ± 0.0
Campesterol	12.1 ± 0.2
Stigmasterol	3.3 ± 0.1
β – Sitosterol	83.3 ± 0.4
Δ^7 – Stigmasterol	1.1 ± 0.1
Tocopherols	
α-Tocopherol	12.4 ± 0.3
γ-Tocopherol	87.6 ± 0.5
Phospholipids	
Lysophosphatidylcholine	22.6 ± 0.3
Lysophosphatidylethanolamine	8.3 ± 0.1
Phosphatidylinositol	28.9 ± 0.5
Phosphatidylcholine	14.2 ± 0.2
Phosphatidylethanolamine	7.0 ± 0.1
Diphosphatidylglycerol	7.0 ± 0.2
Phosphatidic acids	12.0 ± 0.3

This amount of tocopherols in bitter vetch seed oil was bigger than in one of the most common seed oils such as sunflower (737.0 mg/kg), soybean (1328.0 mg/kg), rapeseed (822.8 mg/kg) and corn oil (1006.3 mg/kg).[30] The quantity of the polar components – phospholipids was 0.7% in the seeds and 45.8% in the oil. Total carotenoids and chlorophyll were 684 and 56 mg/kg. They are natural-occurring colorants and what is more, the content of chlorophyll is a valuable indicator for the quality of the oil.[1]

Sterol, tocopherol and phospholipid composition of bitter vetch seed oil is given in Table 3.

Table 4. Fatty acid composition of phosphatidylcholine (PC), phosphatidylinositol (PI) and phosphatidylethanolamine (PE) in bitter vetch seed oil.

Fatty acids, %		PC	PI	PE
$C_{12:0}$	Lauric	0.1±0.0	0.1±0.0	0.1±0.0
$C_{14:0}$	Myristic	0.5±0.1	0.6±0.1	0.6±0.1
$C_{14:1}$	Myristoleic	-[a]	0.1±0.0	0.1±0.0
$C_{15:0}$	Pentadecanoic	0.3±0.1	0.6±0.1	0.3±0.1
$C_{15:1}$	Pentadecenoic	0.1±0.0	0.1±0.0	0.3±0.1
$C_{16:0}$	Palmitic	19.8±0.3	46.8±0.6	27.0±0.3
$C_{16:1}$	Palmitoleic	0.3±0.0	0.1±0.0	0.4±0.1
$C_{17:0}$	Heptadecanoic	0.2±0.0	0.5±0.1	0.2±0.0
$C_{17:1}$	Heptadecenoic	0.2±0.0	0.1±0.0	0.1±0.0
$C_{18:0}$	Stearic	3.1±0.1	4.8±0.2	4.3±0.2
$C_{18:1}$	Oleic	61.1±0.6	34.1±0.5	48.2±0.5
$C_{18:2}$	Linoleic	10.5±0.3	8.2±0.2	12.3±0.3
$C_{18:3}$	Linolenic	0.5±0.1	0.4±0.1	2.2±0.1
$C_{20:0}$	Arachidic	0.3±0.0	0.3±0.0	0.4±0.1
$C_{20:1}$	Gadoleic	0.8±0.1	0.6±0.1	0.6±0.1
$C_{21:0}$	Heneicosanoic	0.4±0.1	0.3±0.1	0.4±0.1
$C_{20:2}$	Eicosadienoic	0.1±0.0	-	0.1±0.0
$C_{20:3}$	Eicosatrienoic	0.8±0.1	0.9±0.2	0.6±0.1
$C_{20:4}$	Arachidonic	0.2±0.0	0.4±0.1	0.4±0.0
$C_{22:0}$	Behenic	0.2±0.0	0.2±0.0	0.3±0.0
$C_{22:1}$	Erucic	0.1±0.0	0.2±0.0	0.2±0.0
$C_{22:2}$	Docosadienoic	0.1±0.0	0.3±0.0	0.2±0.0
$C_{20:5}$	Eicosapentaenoic	0.3±0.1	0.3±0.0	0.6±0.1
$C_{24:1}$	Nervonic	-	-	0.1±0.0

[a] - Not identified

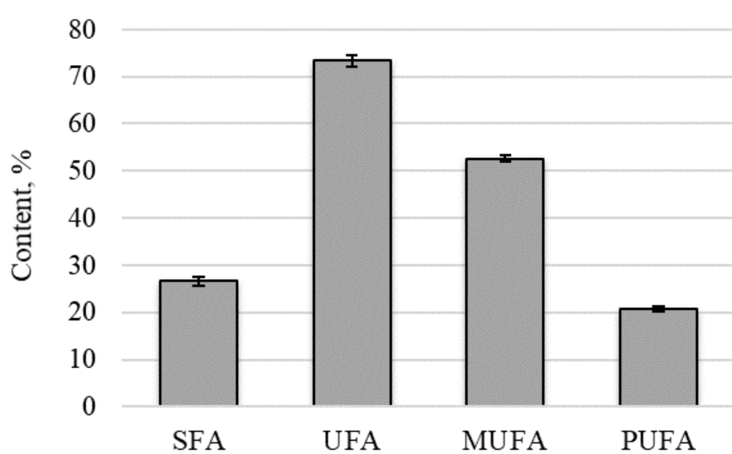

Fig. 1. Content of saturated (SFA), unsaturated (UFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids of bitter vetch seed oil.

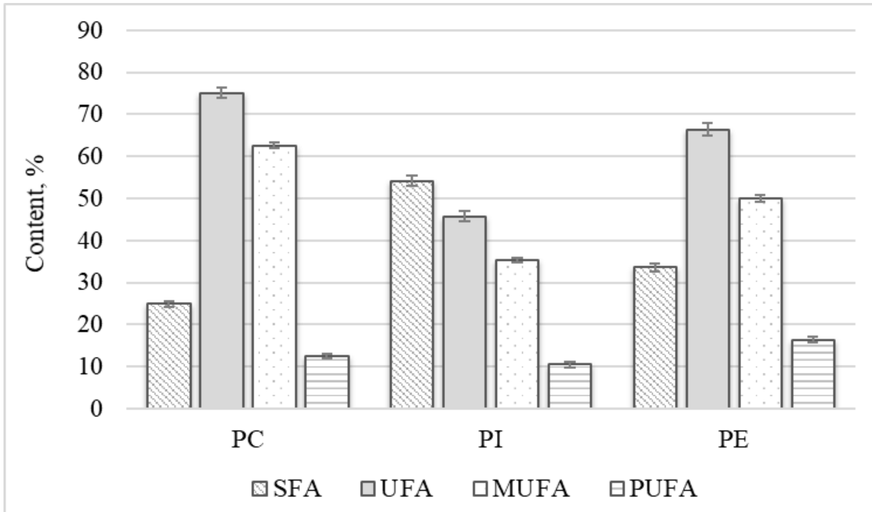

Fig. 2. Content of saturated (SFA), unsaturated (UFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids of three phospholipids from bitter vetch seed oil

[*] PC - phosphatidylcholine, PI – phosphatidylinositol, PE – phosphatidylethanolamine

β-Sitosterol (83.3%) was the major sterol in the fraction, followed by campesterol (12.1%). There was a small quantity of cholesterol (0.2%) in the oil and the amount of the other sterols ranged from 1.1 to 3.3%. Generally, phytosterols have the ability to reduce the cholesterol levels in the human body and to lessen the risk of coronary heart disease.[29] The sterol composition of bitter vetch seed oil is close to those of other seed oils such as sunflower, olive and corn oil, where β-Sitosterol was also the main component.[31]

Only two tocopherols were identified in the examined oil – α- and γ-tocopherol, where the latter predominated in the fraction (87.6%). However, α-tocopherol was considered to be the most important among the others tocopherol homologues because of its physiological activity.[2] Tocopherol composition of bitter vetch seed oil drastically differed from the composition of sunflower oil - 91% of α-tocopherol; 5.4% of β-tocopherol and only 3.6% of γ-tocopherol.[30]

Seven phospholipids were identified in the fraction and the main ones were phosphatidylinositol (28.9%) and lysophosphatidylcholine (22.6%), followed by phosphatidylcholine (14.2%) and phosphatidic acids (12.0%). The content of the other phospholipids was from 7.0 to 8.3%. The high content of lysophosphatidylcholine in the bitter vetch seeds can be explained by the hydrolysis of the phosphatidylcholine.[32]

The fatty acid composition of three phospholipid classes (phosphatidylcholine (PC), phosphatidylinositol (PI) and phosphatidylethanolamine (PE)) in bitter vetch seeds was also determined (Table 4).

The main fatty acid in PC and PE was again oleic acid (61.1 and 48.2%, respectively), followed by the saturated palmitic acid (19.8 and 27.0%, respectively). On the other hand, the major fatty acid in PI was found to be palmitic acid (46.8%), then followed by oleic (34.1%) one. Other fatty acids which were present in relatively big amount in the phospholipids were linoleic (from 8.2% in

PI to 12.3% in PE) and stearic acids (from 3.1% in PC to 4.8% in PI). The content of the rest of the fatty acids was in negligible amounts from 0.1 to 2.2%. It was observed an increase of the levels of oleic acid in the following direction: PI < PE < PC; for palmitic acid was the opposite: PC < PE < PI; for linoleic acid was: PI < PC < PE; and for stearic acid: PC < PE < PI.

Compared to the fatty acid composition of the triglycerides, the quantity of linoleic acid in the triacylglycerols was higher than the amount of the same acid in PI and PE, but was lower than those in PC. The content of palmitic acid was smaller in the triacylglycerol fraction than in PI and PE, but it was almost the same in PC. The quantity of linoleic acid in the triglycerides was as twice as higher than in the phospholipids and those of stearic acid remained almost in the same levels in both triacylglycerols and phospholipids.

The content of SFA, UFA, MUFA and PUFA of the three examined phospholipids from bitter vetch seed oil is given in Fig. 2.

UFA predominated in PC (75.1%) and PE (66.4%), while in PI SFA were in the highest amount (54.2%). Among the UFA, the content of MUFA (from 35.3% in PI to 62.6% in PC) was higher than those of PUFA (from 10.5 in PI to 16.4% in PE). It is noticeable that the fatty acid composition of PI is distinct from the other phospholipids. This was in agreement with the findings of Coulon et al. (2020) who reported that PI possessed a high content of saturated fatty acids and the most abundant one in this phospholipid was palmitic acid which represented up to 40% of the total fatty acids of the phospholipid.[33] The contents of SFA and UFA in PC and PE were similar to the SFA and UFA levels of the triacylglycerols. But the amounts of the same total fatty acids in PI were completely different from the triglycerides of the bitter vetch seed oil.

Conclusions

A thorough examination on lipid composition of bitter vetch seed oil was performed for the first time. The oil content of the seeds is low, but the lipids are rich in phytosterols, tocopherols and carotenoids. Unsaturated fatty acids are in bigger quantity in the glyceride oil and the main ones are oleic and linoleic acids. Palmitic acid is the main saturated fatty acid. The amount of monounsaturated fatty acids is higher than that of polyunsaturated ones. In the sterols β-sitosterol predominates; in the tocopherols – it is γ-tocopherol; and in the phospholipids – it is phosphatidylinositol. Oleic acid is the major fatty acid in the phosphatidylcholine and phosphatidylethanolamine, while palmitic acid predominates in phosphatidylinositol.

Based on the current results can be concluded that the oil from bitter vetch seeds is a new alternative source for obtaining of different fat-soluble bioactive components.

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