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Bitter vetch seeds (*Vicia ervilia* L.) – a valuable source of nutrients

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The main nutrients of seeds from bitter vetch (*Vicia ervilia* L.) were determined. The seeds were with Russian origin, introduced in Bulgaria. They possessed high carbohydrate (66.2%) and protein (20.1%) content, but their oil content was extremely low (1.4%). The starch and dietary fibers were found to be 20.4 and 3.1%, respectively. The individual composition of water-soluble carbohydrates was determined by high performance liquid chromatography on an Agilent® LC 1220 instrument equipped with a refractive index detector. Total content of water-soluble carbohydrates was found to be 4279.0 mg/100 g. It was established that the only disaccharides in the seeds were sucrose (2649.8 mg/100 g) and cellobiose (149.4 mg/100 g), while the main monosaccharides were glucose (600.9 mg/100 g) and fructose (540.7 mg/100 g). The content of rhamnose and xylose was 198.3 and 140.1 mg/100 g, respectively. Amino acid composition of the seeds from bitter vetch was also determined and 17 amino acids were identified. The major amino acid was phenylalanine (46.2 mg/g), followed by lysine (34.8 mg/g) and histidine (30.0 mg/g). The moisture of the seeds was found to be 9.7% and the ash content was 2.6%. The content of the main nutrients in bitter vetch seeds is relatively high which determines their satisfying energy value – 357 kcal/100 g (1520 kJ/100 g) that corresponds to the energy value of the most commonly used legume seeds.

Keywords: bitter vetch, chemical composition, carbohydrates, amino acids

INTRODUCTION

Legume crops are considered as a very important food group because they are a major source of protein. They are mostly spread in Asia and America, being used as food and for industrial processing. Recently, protein deficiency has been observed in Europe, encouraging the cultivation of legumes that possess high protein content (25-40%) [1]. In Bulgaria, legumes occupy less area than cereals, and the main crops are chickpeas, peas, lentils, beans and soybeans, which are the richest in protein of all legumes. Proteins from leguminous plants are the cheapest and can be used as an additive to increase the protein content of foods for human consumption and feed for animals [2, 3]. Legumes are unique foods because of their rich content of other nutrients such as starch, fiber, oligosaccharides and minerals [1, 4].

Nowadays, there is an increasing interest in new alternative crops that could be a source of valuable nutrients for human consumption. As an alternative to the major legume species bitter vetch (*Vicia ervilia* L.) can be offered [5]. Bitter vetch is an annual crop from the family Fabaceae grown in the Mediterranean region, western and central Asia, North Africa and America [6-9]. The plant is mainly cultivated for forage and yield of seeds. The total protein content of bitter vetch seeds ranges between 20 and 30% [2, 5, 7, 8, 10-16] and their oil content is extremely low (1-2%) [1, 12]. According to Fernández-Fígares *et al.* (1995) [10] the main amino acids in bitter vetch seeds are glutamic acid (18.13 g/kg) and aspartic acid (9.47 g/kg). Sadeghi *et al.*, 2009 [15] also reported that the same amino acids prevailed in the seeds (19.63 and 13.83 g/100 g crude protein, respectively).

The information about the chemical composition of bitter vetch seeds is rather scarce. The previous examinations were mainly focused on their total protein and lipid content, crude fibers, dry matter and ash content, but there was lacking data about total carbohydrates, starch content, energy value of the seeds, as well as the content of water-soluble sugars and amino acid composition. For that reason, the aim of the present study is to perform a thorough determination of the chemical composition of bitter vetch (*Vicia ervilia* L.) seeds with Russian origin introduced in Bulgaria, in order to prove their nutritional value.

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MATERIALS AND METHODS

Samples

Bitter vetch seeds, identified as *Vicia ervilia* L., were obtained by the Institute of Plant Genetic Resources "Konstantin Malkov", Sadovo, Bulgaria (crop 2018). The seeds were with Russian origin (var. Krasnografskaya) introduced in the southern part of Bulgaria.

Chemical composition

The oil was extracted from ground seeds using hexane in a Soxhlet apparatus for 8 h [17]. Protein was calculated from the nitrogen content by Kjeldahl method using a factor of 6.25 [18]. The carbohydrate content was calculated by the following formula:

100 – (weight in grams [protein + lipids + water + ash] in 100 g of dry seeds) [19].

The starch content was determined by using BS 13488:76 [20]. Crude fiber, ash content and moisture were determined according to AOAC (2016) [21].

Soluble carbohydrates

The soluble carbohydrates were identified by their extraction with water and were determined by high performance liquid chromatography (HPLC) on an Agilent® LC 1220 (USA) instrument equipped with Zorbax carbohydrate column (150 mm × 4.6 mm, 5μm, Agilent) and Zorbax Reliance Cartridge guard-column (Agilent) and refractive index detector (RID 1260) [22]. The mobile phase was acetonitrile/water (AcN/H_2O) (80/20) at 1.0 mL/min. All standards (individual pure monosaccharides with purity 98%) were purchased from Sigma Chemical Company (USA).

Amino acids

For the hydrolysis of the protein to free amino acids 300 mg of dried seeds were put in a glass ampule with 5 mL of 6N HCl solution. The ampule was thoroughly sealed and left in a drying chamber at 105°C for 24 h. The content of the ampule was transferred to a crystallizer and dried in a vacuum chamber at 40-50°C. After evaporation of the water, the residue was fully dissolved in 10 mL of 20 mM HCl. The solution was filtered through a paper filter and 20 μL of the collected filtrate was derivatized with AccQ-Fluor kit (WATO52880, Waters Corporation, USA). Initially, 60 μL of AccQ-Fluor borate buffer was added to the filtrate and homogenized. Then, 20 μL of AccQ-Fluor reagent was added, and the sample was homogenized again for 30 s. Before injection, the solution was heated in a water bath at 55°C. The resulting AccQ-Fluor derivatives of amino acids were separated by an ELITE LaChrome high performance liquid HPLC chromatograph (HPLC) (Hitachi) equipped with a DAD and a reverse phase C 18 AccQ-Tag (3.9 mm × 150 mm). The volume of injected sample was 20 μL, and the elution was done with a gradient system of two mobile phases: A – buffer (WATO52890, Waters) and B – 60% acetonitrile. Amino acids were detected at 254 nm and column temperature 37°C.

Energy value

The energy value of the seeds was determined following the FAO [19] procedure using the formula:

$$EV = C \times 4 + L \times 9 + P \times 4 \quad \text{(kcal/100 g)},$$

where EV – energy value; C – total carbohydrates, %; L – total lipids, %; P – total proteins, %.

RESULTS AND DISCUSSION

Chemical composition of the examined bitter vetch seeds (*Vicia ervilia* L.) is shown in Table 1.

Table 1. Chemical composition of bitter vetch seeds (*Vicia ervilia* L.)

Composition	Content
Oil content, %	1.4 ± 0.1
Proteins, %	20.1 ± 0.2
Carbohydrates, %	66.2 ± 0.4
Starch, %	20.4 ± 0.2
Fibres, %	3.1 ± 0.2
Ash, %	2.6 ± 0.1
Moisture, %	9.7 ± 0.2
Energy value, kJ/100 g (kcal/100 g)	1520 (357)

Bitter vetch seeds were characterized as a good source of proteins (20.1%) and carbohydrates (66.2%), but possessed low oil content (1.4%). Starch represented about 31% of total carbohydrates. Dietary fibers and ash content were 3.1 and 2.6%, respectively. The moisture of the seeds was found to be 9.7% and corresponded to the results about water content of different seeds [23]. The energy value of bitter vetch seeds was also evaluated – 1520 kJ/100 g (357 kcal/100 g). These results are in agreement with the energy value of different seeds from family Fabaceae such as bean, soybean, peas and chickpeas (337, 446, 326 and 325 kcal/100 g, respectively) [24].

Total protein and lipid content of the examined bitter vetch seeds were in agreement with the

results obtained by previous authors who reported that the oil content of the seeds was between 1.0 and 2.0% [1, 12] and the proteins ranged from 20.0 to 30.0% [2, 5, 7, 8, 10-16]. On the other hand, higher content of crude fat in the seeds was observed by Tabatabaei *et al*. (2000) – 2.43% [16]. The content of the dietary fibers was similar to that reported by Hadjipanayiotou *et al*. (1985) (3.5%) [12], but about twice lower than the results by Tabatabaei *et al.* (2000) (7.75%) [16]. Ash content of the seeds was lower than the results from previous studies where the ash content ranged between 3.20 and 3.97% [12, 15, 16]. Moisture content of the examined seeds was in agreement with previous studies where the dry matter was 92.00 – 94.52 g/100 g [12, 15, 16].

The composition of the soluble carbohydrates of bitter vetch seeds was examined for the first time and determined by high performance liquid chromatography (HPLC) on an Agilent® LC 1220 instrument equipped with a refractive index detector. Their individual composition is presented in Table 2.

Table 2. Composition of soluble sugars in bitter vetch seeds (*Vicia ervilia* L.).

Sugars, mg/100 g	Content
Fructose	540.7 ± 4.9
Glucose	600.9 ± 7.4
Xylose	140.1 ± 15.5
Rhamnose	198.3 ± 7.1
Sucrose	2649.8 ± 82.7
Cellobiose	149.4 ± 22.5
Total	4279.0 ± 111.0

Total content of water-soluble carbohydrates was found to be 4279.0 mg/100 g. It was observed that the main monosaccharides were glucose (600.9 mg/100 g) and fructose (540.7 mg/100 g) while the content of rhamnose and xylose was 198.3 and 140.1 mg/100 g, respectively. Carbohydrates are a major source of energy for the human body and the main representatives of these compounds present in the bitter vetch seeds are oligosaccharides such as sucrose (2649.8 mg/100 g). Other disaccharide found in the seeds was cellobiose (149.4 mg/100 g). High content of sucrose was also observed in soybean (64.2 mg/g), garden pea (62.3 mg/g) and peanuts (81 mg/g) by Kuo *et al.* (1988) [25].

Amino acid composition of bitter vetch seeds (*Vicia ervilia* L.) was also determined by HPLC chromatograph (Hitachi) equipped with a diode array detector (DAD) and a reverse phase C 18 AccQ-Tag (3.9 mm × 150 mm). The results are shown in Table 3.

Table 3. Amino acid composition of bitter vetch seeds (*Vicia ervilia* L.)

Amino acid, mg/g	Content
Aspartic acid	15.7 ± 0.1
Serine	28.5 ± 0.2
Glutamic acid	15.4 ± 0.1
Glycine	15.1 ± 0.1
Histidine	30.0 ± 0.2
Arginine	26.0 ± 0.3
Threonine	19.7 ± 0.1
Alanine	15.5 ± 0.1
Proline	10.0 ± 0.1
Cysteine	10.0 ± 0.2
Tyrosine	16.2 ± 0.1
Valine	23.6 ± 0.1
Methionine	5.6 ± 0.05
Lysine	34.8 ± 0.2
Isoleucine	29.6 ± 0.2
Leucine	4.8 ± 0.1
Phenylalanine	46.2 ± 0.3

Seventeen amino acids were identified in the seeds. The major amino acid was phenylalanine (46.2 mg/g), followed by lysine (34.8 mg/g) and histidine (30.0 mg/g). On the other hand, leucine and methionine were identified with the lowest content – 4.8 and 5.6 mg/g. The contents of the other amino acids were observed to range between 10.0 and 29.6 mg/g. The present results differed from those in previous studies where the authors reported that the main amino acids in bitter vetch seeds were glutamic and aspartic acids [10, 15]. This difference is probably due to the agro-meteorological conditions and the regions where the plants were grown. The relatively high content of lysine and cysteine (10.0 mg/g), which are essential amino acids in the nutrition, suggests that bitter vetch seeds can participate successfully in the human diet.

CONCLUSION

The main nutrients of bitter vetch (*Vicia ervilia* L.) seeds were determined. They possess high carbohydrate and protein content, but their oil content is extremely low. Total content of water-soluble carbohydrates was relatively low and the only disaccharides in the seeds were sucrose and cellobiose, while the main monosaccharides were glucose and fructose. Seventeen amino acids were identified in the seeds and the major ones were phenylalanine, lysine and histidine. The content of the main nutrients in bitter vetch seeds was observed to be relatively high which determined their satisfying energy value that corresponds to the

energy value of the most commonly used legume seeds.

Overall, bitter vetch seeds have a high nutritional value and could be successfully used as a valuable source of nutrients.

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