

Main biologically active substances of amaranth grain

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Abstract. The purpose of the study was to determine the nutritional value and main biologically active substances in amaranth grain from different geographical regions of growth and its processed products. Nine commercially available amaranth samples were selected for the study: 1-3. amaranth grain originated from Russia, Peru and India, respectively, 4. extruded flour, 5. high-protein flour, 30% protein 6. flour with a protein content of 20%, 7. flour, enriched with fiber, 8. cake flour, 9. amaranth grain that has not undergone technological purification (samples 4-9 - country of origin - Russia). The protein content in amaranth samples varied from 12.6 to 33.8%, the fat content was in the range of 5.6-8.1%, and the carbohydrate content was 50.4-72.2% , the ash content was 2.06-6.12% and moisture 2.1-7.1%. The main hydrocinnamic acids in amaranth samples were identified: caffeic, ferulic and p-coumaric. The main flavonoids in the studied samples were rutin pentoside, rutin and nicotiflorin (kaempferol-3-rutinoside). The main fatty acids identified: linoleic, oleic, palmitic, stearic, vaccenic, alpha-linolenic (ω -3).

1 Introduction

Amaranth grain contains a unique set of phytonutrients - compounds that protect plants from adverse environmental conditions, including fungal, bacterial and viral infections. Main phytonutrients or biologically active components of pseudocereal grains are saponins, phenolic compounds (including flavonoids), phytosterols and some other compounds.

Polyphenolic compounds are contained in amaranth grain in the form of free molecules, their glycosylated forms and polymer molecules (for example, oligomeric flavonoids - proanthocyanidins, condensed tannins) [1]. In the most cultivated amaranth species: *Amaranthus caudatus*, *A. cruentus* and *A. hypochondriacus*, the following main groups of phenolic compounds have been identified: phenolic acids (ferulic, p-coumaric, p-hydroxybenzoic), flavonoids (rutin, quercetin), as well as tannins [1-4], both individually and as part of extracts [5-6].

Antioxidant, as well as hypolipidemic and hypoglycemic properties of amaranth grain extracts are largely determined by the presence of polyphenolic compounds in them. Accordingly, there is an increased interest in obtaining amaranth grain extracts highly

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enriched with polyphenolic compounds through deep processing of raw materials with the further prospect of their use in specialized multi-purpose food products [7-8].

Most of the studies conducted indicate the potential positive effects of betacyanins: amaranthine and isoamaranthine from amaranth grain [9-10]. The advantage of using betalains as food colorings compared to anthocyanins is their stability over a wider range of pH and temperatures [11]. Replacing potentially less healthy synthetic dyes, betalains, like some other natural pigments of plant origin, are increasingly used in the food and pharmaceutical industries.

The lipid content in amaranth grain is higher than in common cereals by 6-9%. According to some authors, the lipid content in amaranth is 2–3 times higher than in buckwheat grain and common cereals. Amaranth lipids consist of fatty acids, triglycerides, sterols, phospholipids, glycolipids, tocopherols and hydrocarbons. Amaranth lipids are characterized by a high content of unsaturated fatty acids, especially high content of linoleic acid. According to the literature, linoleic acid makes up more than 50% of the total fatty acids and is therefore the most abundant fatty acid, followed by oleic (more than 25%), palmitic (about 20%) and linoleic (about 1%) [12]. The total unsaturation of amaranth lipids exceeds 75%. The most common phytosterols are chondritic sterol and clerosterol (42%). Despite its high degree of unsaturation, amaranth oil is generally resistant to oxidation due to the protective effect of the tocopherols present. Phospholipids make up about 5% of the lipid fraction, the most common being lecithin, cephalin and phosphoinositol.

The purpose of our study was to determine the nutritional value and main biologically active substances in amaranth grain from different geographical regions of growth and its processed products.

2 Materials and methods

Protein content was determined by Kjeldahl method using a conversion coefficient from total nitrogen to protein equal to 6.25 (with pre-mineralization) according to national standard GOST 26889-86 using an automatic analyzer Kjeltac 8100 (FOSS Analytical AB, Sweden). The moisture content of the samples was determined using Mettler Toledo MJ33 moisture analyzer (Mettler Toledo, USA). Ash content was determined according to national standard GOST 15113.8-77. The mass fraction of ash was determined as the ratio of the mass of ash to the mass of the original sample, expressed as a percentage.

Extraction and determination of total fat. Fat extraction was carried out according to the method described in the work [13]. The fatty acid composition was determined by gas chromatography with a flame ionization detector. Data collection and processing were carried out using Agilent ChemStation Rev.B.04.03 and Microsoft Excel 2007 software, respectively. The ratio of fatty acids was determined by internal normalization method.

Flavonoid profiling was performed using an Ultimate 3000 liquid chromatography system with a diode array spectrophotometric detector (DAD) and a TSQ Endura triple quadrupole mass spectrometric detector (MSD). Hydroxycinnamic acids were determined by HPLC-DMD method: Phenomenex Luna C18 150*4.6 mm column. Data processing for both agalises was carried out using Thermo Xcalibur 4.2.47 software.

Statistical analysis

Statistical processing of the obtained results was carried out using the SPSS Statistics 20 software package, using the nonparametric Mann-Whitney rank test and Student's t test. The critical significance level of the null statistical hypothesis (p) was taken equal to 0.05.

3 Results

Nine commercially available amaranth samples were selected for the study: 1-3. amaranth grain originated from Russia, Peru and India, respectively, 4. extruded flour, 5. high-protein flour, 30% protein 6. flour with a protein content of 20%, 7. flour, enriched with fiber, 8. cake flour, 9. amaranth grain that has not undergone technological purification (samples 4-9 - country of origin - Russia).

Chemical composition

Table 1 presents the chemical composition of amaranth samples.

Table 1. Amaranth samples chemical composition.

Sample	Content, %				
	Fat	Protein	Ash	Moisture	Carbohydrates
1. Amaranth grain (Russia)	7.5±0.1	15.6±0.8	2.55±0.02	2.1±0.1	72.2±5.6
2. Amaranth grain (Peru)	6.8±0.1	15.0±0.7	2.06±0.02	5.9±1.0	70.2±4.0
3. Amaranth grain (India)	5.9±0.2	19.2±0.96	2.72±0.03	5.4±0.6	66.8±3.5
4. Extruded flour	6.0±0.1	12.6±0.6	2.00±0.02	2.9±0.2	76.5±5.0
5. High-protein flour (30% protein)	5.6±0.1	33.8±0.7	6.12±0.06	4.1±0.1	50.4±2.6
6. Flour (20% protein)	7.5±0.3	18.6±0.9	3.27±0.03	5.0±0.1	65.6±3.0
7. Flour enriched with fiber	6.7±0.1	24.5±1.2	4.22±0.04	6.0±0.1	58.5±5.3
8. Cake flour	8.1±0.1	15.7±0.8	2.93±0.03	7.1±0.1	66.2±5.6
9. Amaranth grain without technological purification	7.3±0.2	15.1±0.7	4.81±0.05	3.5±0.2	69.3±4.8

The protein content in amaranth samples varied from 12.6 to 33.8% and, in general, was slightly higher than that declared by the manufacturer, the fat content was in the range of 5.6-8.1%, and the carbohydrate content was 50.4-72.2%, the ash content was 2.06-6.12% and moisture 2.1-7.1%.

The content of hydroxycinnamic acids (HCAs) is presented in table 2.

Table 2. Content of hydroxycinnamic acids in amaranth samples, mg/kg.

Sample	Content, mg/kg				
	[M – H]- 517.15*	Caffeic acid	Ferulic acid	p-Coumaric acid	Total HCAs
1	123.8±8.9	n/d	4.3±0.3	n/d	128.1±9.4
2	n/d	traces	8.6±0.6	1.3±0.2	48.1±0.4
3	n/d	5.5±0.5	8.9±0.8	0.9±0.1	48.4±0.5
4	n/d	5.3±0.4	8.4±0.7	0.5±0.1	39.2±0.4
5	n/d	6.1±0.6	9.0±0.8	1.3±0.1	75.7±0.8
6	n/d	5.8±0.5	9.4±0.9	1.4±0.1	116.5±9.6
7	n/d	9.4±0.8	8.8±0.7	2.0±0.2	71.6±0.6
8	n/d	6.3±0.5	9.7±0.7	1.2±0.1	67.4±0.6
9	112.1±7.8	n/d	3.8±0.3	n/d	240.2±14.7

Note: *Unidentified HCA derivative, also found in scarlet amaranth [14].

The main hydroxycinnamic acids in amaranth samples were identified: caffeic, ferulic and p-coumaric. Total hydroxycinnamic acid derivatives content ranged from 39.2 to 240.2 mg/kg dry sample.

The flavonoid profiles are presented in Table 3.

Table 3. Content of flavonoids in amaranth samples, mg/kg.

Content, mg/kg	Sample								
	1	2	3	4	5	6	7	8	9
Rutin-rhamnoside [M+H] ⁺ 757	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	138.0± 9.7
Rutin-rhamnoside, isomer [M+H] ⁺ 757	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	31.0± 0.3
Rutin + pentose [M+H] ⁺ 743	n/d	n/d	n/d	n/d	n/d	17.9± 1.3	5.6± 0.4	n/d	299.0± 13.7
Quercetin 3-pentosyl-glucoside [M+ H] ⁺ 595	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	37.0± 0.3
Rutin	n/d	3.4± 0.2	5.4± 0.4	2.7± 0.2	13.7± 0.8	41.1± 2.1	20.7± 1.2	9.8±0.8	80.0± 6.6
Nicotiflorin (kaempferol-3-rutinoside)	n/d	n/d	3.8± 0.2	1.0± 0.1	8.0± 0.5	n/d	n/d	6.3±0.4	n/d
Isorhamnetin-3-rutinoside	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	29.0± 1.5
Total	n/d	3.4± 0.2	9.2± 0.5	3.7± 0.2	21.7± 1.3	59.0± 2.4	26.3± 1.6	16.2± 0.9	614.0± 24.2

4 Conclusion

The main flavonoids in the studied samples were rutin pentoside, rutin and nicotiflorin (kaempferol-3-rutinoside). Their total content varied in the range from 0 to 614 mg/kg of sample. The maximum content of flavonoids and hydrocinnamic acids was found in sample 9, which was amaranth grain that had not undergone technological purification (hydrocinnamic acid content - 240.2 mg/kg, flavonoid content - 614.0 mg/kg).

Table 4 shows the fatty acid (FA) composition of selected amaranth samples.

Table 4. Main fatty acids in amaranth samples.

Fatty acid	FA index	Samples								
		1	2	3	4	5	6	7	8	9
Palmitic	16:0	20.9	19.2	19.6	20.6	19.8	20.6	20.3	16.1	15.9
Stearic	18:0	3.29	4.20	3.33	3.74	3.50	3.57	3.74	3.03	2.66
Oleic	18:1 9-cis	33.1	27.8	34.0	23.4	23.4	23.7	23.2	36.2	27.5
Vaccenic	18:1 11-trans	1.30	1.00	1.39	1.15	1.12	1.14	1.15	1.49	1.40
Linoleic	18:2	38.0	44.2	37.8	47.0	48.4	47.1	47.5	39.7	44.6
α -Linolenic	18:3 ω -3	0.12	0.70	0.94	1.12	0.95	0.96	0.88	0.65	0.10

Linoleic acid accounted for 38-48% of the total amount of fatty acids, the second place took oleic acid – 23-36%, the content of palmitic acid varied from 16-21%, the content of stearic acid was in the range of 2-4%, vaccenic acid 1.0 -1.5%, alpha-linolenic (ω -3) 0.12-0.96%.

Antioxidant, as well as hypolipidemic and hypoglycemic properties of amaranth grain extracts are largely determined by the presence of polyphenolic compounds in them. Accordingly, there is an increased interest in obtaining amaranth grain extracts highly

enriched with polyphenolic compounds through deep processing of raw materials with the further prospect of their use in multi-purpose functional foods. Accordingly, the data obtained can be effectively used in selecting promising amaranth raw materials with the highest content of biologically active substances, including polyphenols, in order to further obtain concentrated fractions and determine their contribution to certain types of biological activity.

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