CHARACTERIZATION OF HOMEMADE AJVAR - A PEPPER-BASED RELISH

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Ajvar is a pepper-based relish that represents a mixture of roasted sweet peppers. As a domestic agricultural food product known as vegetable caviar, ajvar is consumed in small portions, cold, in addition to the main meal, or as a spread. This paper investigated the composition of domestic ajvar made of *Capsicum annuum* L. sweet peppers. Bioactive compounds, mineral matters, moisture, ash, crude protein, and total fat content were analyzed. The identification of bioactive compounds is done using the UHPLC-DAD-ESI-MS/MS method. The content of mineral matters, in the ajvar sample, has been investigated by ICP-OES. Moisture and ash content are determined by using gravimetric methods, while the Kjeldahl method and Soxhlet extraction were used for crude proteins and total fats content determination, respectively. Out of the bioactive compounds several organic acids and flavonoid derivates were successfully identified in the ajvar sample. Investigation of mineral compositions showed high levels of sodium (3571.703 mg/kg) and potassium (2172.695 mg/kg), as well as the presence of some significant microelements such as zinc (10.740 mg/kg) and copper (4.480 mg/kg). The obtained results of moisture content (73.04%), ash (2.53%), crude proteins (1.78%), and total fat content (9.21%), have further confirmed that ajvar is quite nutritious and can fit into various diet plans.

Keywords: sweet ajvar, UHPLC-DAD-ESI-MS/MS, ICP-OES, bioactive compounds, minerals

Introduction

Ajvar is a Serbian brandy pepper-based relish that represents a mixture of roasted sweet peppers. The relish became a popular side dish throughout Yugoslavia, especially in the second half of the last century. It is considered vegetable caviar and usually is eaten cold, in small portions, in addition to the main meal, or as a spread. Ajvar is a Leskovac brand that is internationally protected [1] and the third product from Serbia that is registered with WIPO (World Intellectual Property Organization). The time for making ajvar across the Balkans is during the fall. The preparation method of traditional ajvar is part of the folklore of a region. Most manufacturers follow their recipe when preparing pasteurized baked vegetable caviar from different cultivars of peppers such as Amfora, Kurtovska kapija, Delfina, Ami, Palanačko Čudo, Elephant's ear, etc. Ajvar's popularity is also testified by the recent data about its increasing exports [2]. Ajvar requires good pepper quality which includes appropriate water content and enough dry matter of peppers. The morphological traits of pepper fruits, such as length, width, and pericarp thickness, are crucial factors that can impact the final quality of ajvar. High dry matter content and high pectin content are important for vegetable caviar preparation. Other ajvar components are salt, wine vinegar, sugar, oil, often garlic, etc. A sweet pepper usually used for ajvar's preparation is a Serbian pepper known as "Kurtovska kapija" a popular variety of sweet pepper, which belongs to the Capsicum genus (*Capsicum annuum var. annuum* (Grossum Group)). That is a flat large, bulky, thick, and extra sweet pepper. Pepper vegetable is highly nutritious due to its richness in carbohydrates, sugars, malic and citric acids, β-carotene, capsaicin, and vitamins, particularly vitamin C [3, 4]. The chemical composition of ajvar is variable depending on the ingredients as well as the heating rate. Nutritional, ajvar is rich in fatty acids content such as linoleic (49.10%), oleic (25.51%), and palmitic acid (15.68%) [5]. Because humans are unable to synthesize linoleic acid, it must be provided by some food intake. Oleic acid also has beneficial effects on cardiovascular health, skin repair, cancer, autoimmune diseases, and inflammation [5].

Domestic agricultural food products are often consumed and most of the population in Serbia has positive attitudes toward those products [6]. Food market globalization and internationalization, which is in progress, may influence the disappearance of traditional foods.

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Because of that the recipes of traditional foods are an important part of cultural heritage. In this study, we have investigated the presence of bioactive compounds, minerals, moisture, ash, protein, and total fat content to determine the nutritional properties of homemade sweet ajvar. Considering these facts, the results from this study could be useful for teachers, students, and scientists, as well as for the manufacturing, processing, and marketing of Ajvar´s relish.

Materials and methods

Ajvar preparation procedure

Rinsed, dried, and roasted peppers (100 pieces) are grill-baked at 230–260 °C. After cooling peppers are cleansed from skins, seeds, and excess overage pepper juice. Peppers are left in a sack or bag to drip overnight to shorten the cooking process. By manual meat grinder, all peppers are ground. The 400 ml of oil is heated in the pot to boiling and poured over the peppers, while cooking spices such as salt are added by taste. The ajvar is traditionally cooked at a high temperature while stirring continuously, making sure to scrape the bottom of the pot. When the ajvar is done, 2-3 tablespoons of vinegar are added, then stirred well, and put into hot, sterilized jars.

Identification of bioactive compounds

For the identification of bioactive compounds, the method of high-performance liquid chromatography in combination with mass spectrometry was used (UHPLC-DAD-ESI-MS/MS method). Before the UHPLC-DAD-ESI-MS/MS analysis, bioactive compounds from the Ajvar sample were extracted. It is measured at 0.5 g of Ajvar sample and dissolved in 10 ml of methanol/water mixture (80:20 ratio) and sonicated at room temperature (25 °C) for 30 minutes. After that, the obtained mixture was centrifugated at 3500 rpm for 15 minutes, the supernatant was transferred to a round-bottom flask, and the solution was evaporated using a rotary evaporator. The residue was then re-dissolved in 0.5 ml of the same methanol/water mixture and before analysis extract solution was filtered through a 0.45 µm syringe filter.

UHPLC-DAD-ESI-MS/MS analysis was done by using a liquid chromatography system (Thermo Scientific) composed of a quaternary pump with a degasser, a thermostated column compartment, an autosampler, and a diode array detector connected to Ion Trap Mass Spectrometer (Thermo Scientific) equipped with electrospray ionization. Xcalibur (version 2.2 SP1.48) and LCQ Fleet (version 2.7.0.1103 SP1) software were used for instrument control and data analysis. Separation was performed on Hypersil gold C18 column (Thermo Scientific, 50×2.1 mm, 1.9 µm).

The mobile phase consisted of (A) water (Fisher Chemical, LC-MS grade) + 0.1% formic acid (Carlo Erba) and (B) acetonitrile (Fisher Chemical, LC-MS grade). A linear gradient program at a flow rate of 0.25 mL/min was used for 1-5 min from 5 to 10% (B), 5-8 min from 10 to 20% (B), 8-8.5 min from 20 to 14% (B), 8.5-10.5 min from 14 to 20% (B), 10.5-16 min from 20 to 30% (B), 16-17.6 min from 30 to 100% (B), 17.6-25.6 min 100% (B), 25.6-30.2 min from 100 to 5% (B), and 30.2-35 min 5% (B).

The temperature of the column and injection volume were 25°C and 5µL, respectively. The analysis was followed at four wavelengths: 280, 360, 422, and 598 nm. The mass spectrometer was operated in a negative ion mode. The parameters of ESI-source were: capillary temperature 275 °C, tube lens voltage 115 V, capillary voltage 36 V, source voltage 4.5 kV, sheat and auxiliary gas flow (N_2) 50 and 8 (arbitrary units), respectively. The normalized collision energy of the collision-induced dissociation (CID) cell was set at 30 eV.

Determination of mineral content

The quantitative and qualitative analysis of the ajvar sample was done by using ICP-OES method (Inductively Coupled Plasma Optical Emission Spectrometry, ARCOS FHE12, SPECTRO). The operative instrument conditions are presented in Table 1.

Table 1. Operating conditions for ICP-OES

Plasma Power (W)	1400
Gas flow (L/min)	
-Coolant	13
-Auxiliary	0.80
Nebulizer type	Crossflow
Nebulizer flow rate (L/min)	0.95
Pump speed	30
Stabilization time (s)	0
Number of probes for each measuring	3
Plasma observation	axial

Calibration standard solutions for investigated elements were prepared by diluting of multielement standard IV solution (Merck) that contained Ag, B, Ba, Bi, Cd, Co, Cr, Cu, Fe, Li, In, Mn, Na, Ni, Pb, Tl, and Zn at a concentration of 1000 ppm, as well as and using of additional specific calibration standards for elements: As, Ca, Hg, K, Mg, P, Si, and Sr that were purified by Reagecon. For dilution of standard solutions distilled water (Fisher Chemical) was used. The carrier gas was Argon 5.0 (99.999% purity). Table 2 presents the wavelength detection of each element, the correlation coefficient (R), the limit of detection (LOD), and the range of linearity.

The preparation of the ajvar sample for the ICP-OES analysis was carried out by wet digestion. About 0.5 g of the previously homogenized samples were treated with 10 mL of nitric acid (65% w/w, p.a, Merck) and heated to complete mineralization. After mineralization the sample was cooled, and diluted with distilled water to 100 ml. Before ICP-OES analysis, the sample was filtrated through a 0.45 µm syringe filter.

Table 2. Calibration parameters: λ, nm; R; LD (μg/L) and the range of linearity (μg/L)

Element	Detection wavelength (nm)	Correlation coefficient (*R*)	Limit of detection (μg/L)	Linearity range (mg/L)
As	197.262	0.99982	2.55	2.55×10^{-3}–3.60
Ag	224.641 328.068	0.99993 0.99995	0.39	1.45×10^{-2}–12.00 3.99×10^{-4}–12.00
B	182.641 249.773	0.99999 0.99999	6.43	7.37×10^{-3}–12.00 6.43×10^{-3}–12.00
Ba	233.527	0.99995	0.183	1.83×10^{-4}–12.00
Bi	190.241 223.061	0.99991 0.99996	3.53	3.53×10^{-3}–12.00 3.68×10^{-3}–12.00
Ca	183.801 396.847	0.99995 0.99946	2.14	1.6–480.00 2.14×10^{-3}–2.41
Cd	214.438	0.99994	0.127	1.27×10^{-4}–12.00
Co	228.616	0.99980	0.327	3.27×10^{-4}–12.00
Cr	283.563	0.99998	0.435	4.35×10^{-4}–12.00
Cu	224.700 324.754	0.99999 0.99999	0.259	9.07×10^{-4}–12.00 2.59×10^{-4}–12.00
Fe	259.941	0.99997	0.118	1.18×10^{-4}–12.00
Hg	184.95	0.99992	0. 192	1.92×10^{-4}–12.00
In	325.609	1.00000	4	4×10^{-3}–12.00
K	404.721 766.491	0.99998 0.99999	0.378	0.798–300.00 3.78×10^{-4}–1.20
Li	323.261 670.780	1.00000 0.99997	5.75×10^{-2}	0.0798–12.00 5.75×10^{-5}–1.20
Mg	279.553 285.213	0.99997 0.99994	0.115	7.98×10^{-2}–12.00 5.75×10^{-5}–1.20
Mn	257.611	0.99992	3.57×10^{-2}	3.57×10^{-5}–12.00
Na	330.237 598.592	0.99994 0.99989	4.75	7.98–480.00 4.75×10^{-3}–12.00
Ni	231.604	0.99994	0.474	4.74×10^{-4}–12.00
P	214.914	1	1.59	1.59×10^{-3}–120.00
Pb	220.353	0.99998	1.78	1.78×10^{-3}–12.00
Si	251.612	0.9998	1.6	1.6×10^{-3}–60.00
Sr	407.771	0.99998	6.23×10^{-3}	6.23×10^{-6}–2.41
Tl	190.864	0.99998	1.72	1.72×10^{-3}–12.00
Zn	213.856	0.99997	8.2×10^{-2}	8.2×10^{-5}–12.00

Determination of moisture content

The analysis of moisture content was done by the gravimetric method (AOAC 925.40). The investigated sample was dried to the constant in the oven at a temperature of 105 °C.

Determination of ash content

This test was determined according to the direct gravimetric method (AOAC 923.03). The sample was annealed in the oven at 550 °C to the constant weight.

Determination of crude protein content

The investigation of protein content was done by the Kjeldahl method (AOAC 955.04D).

Determination of total fat content

The analysis of total fat content was done by traditional acid hydrolysis followed by Soxhlet extraction (AOAC 963.15) in a petroleum ether [7-8].

Results and discussion

UHPLC-DAD-ESI-MS/MS analysis

Extraction of the bioactive compounds was done using a solvent made of a mixture of methanol/water because it can successfully extract polar and semi-polar compounds that are of importance. The ultra-high-performance liquid chromatography (UHPLC) was combined with ion trap mass spectroscopy for the separation and identification of extracted bioactive compounds. Retention time, peak mass, ion fragments, and literature data were used to identify compounds. The obtained UV-Vis results from the diode array detector (DAD) were used to distinguish families of compounds, as each family has characteristic absorption bands.

The obtained chromatogram of UHPLC-DAD-ESI-MS/MS analysis with separated and labeled compounds is shown in Figure 1. In Table 3 the identified compounds are numbered from 1 to 14, and as it can be seen they belong to different types of compounds.

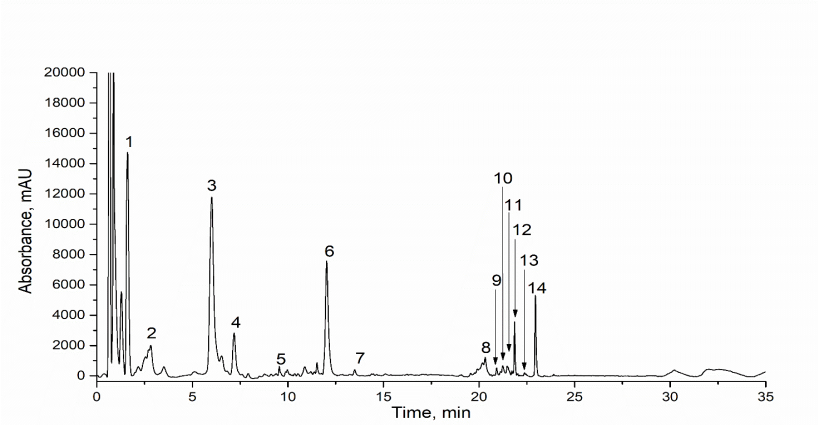

Figure 1. UHPLC chromatogram of Ajvar extract recorded at 280 nm in a methanol/water solution.

Table 3. Identified bioactive compounds in Ajvar extract

Peak	R_t, min	UV bands, nm	$[M-H]^-$	MS/MS	Identified compound
1	1.57	222	191.05	173.04; 171.03;127.04; 93.03; 85.02	Quinic acid
2	2.79	243	191.02	173.00; 129.01; 111.00	Citric acid
3	6.04	220; 302	175.02	115; 87	Ascorbic acid
4	7.21	221; 295	175.02	115; 87	Ascorbic acid
5	9.57	220; 258	282.09	150.04	Guanosine
6	12.06	219	164.06		D, L-phenylalanine
7	13.52	220; 292	326.13	164	Fructosyl phenylalanine
8	20.34	257; 352	609.14	445	Quercetin dihexoside (Isomer I)
9	20.90	255; 361	609.15	445	Quercetin dihexoside (Isomer II)
10	21.28	241	741.20	609	Rutin pentoside
11	21.47	236; 270	593.16	430.11; 283.03; 285.05;	Quercetin 3,7-di-O-α-L-rhamnopyranoside
12	21.84	265; 332	563.13		Apigenin 7-O-β-D-apiofuranosyl (1→2)-β-D-glucopyranoside
13	22.36	263; 347	447.06	327; 298	Luteolin-8-glucoside
14	22.97	250; 351	579.13		Narigenin-7-O-β-D-(3″-p-coumaroyl)-glucopyranoside

The first peak in the chromatogram at a retention time of 1.57 min and *m/z*191.05 was identified as quinic acid. The presence of a UV absorption band at a wavelength of 222 nm is typical for organic acid found in the literature [9, 10]. Peak 2 at a retention time of 2.79 min and *m/z*191.02 was identified as citric acid. This organic acid, behind pepper, has been found in many vegetables from the Solanaceae family [11]. In addition to the long-term thermal processing of ajvar, vitamin C was identified, for which there is data that up to 70% is lost during the thermal processing of food. Two isomeric forms of ascorbic acid were recorded at retention times 6.04 and 7.21 min with an almost identical molecular ion *m/z* 175.02. The following compound identified as quanosine was recorded at 9.57 min and molecular ion *m/z* 282.09. The ion fragment at *m/z*150 is the result of the loss of pentose moiety. This is not the first appearance of guanosine in red pepper, because it was previously detected in the study reported by Morales-Soto *et al.* [12]. Peak 6 at retention time 12.06 with *m/z* 164.06 was identified as DL-phenylalanine. The presence of this compound in *C. annuum* L. had been reported by Ritota *et al.* [13]. Peak 7 with *m/z* 326.16 was detected at a retention time of 13.52 min. The presence of ion fragment with *m/z* 164, indicates the loss of the hexose moiety which confirms that peak 7 corresponds to fructosyl phenylalanine. Peaks 8 and 9 with *m/z* 609.14 *i.e*. 609.15 were identified as quercetin dihexoside isomer I and II, respectively. The presence of ion fragments at *m/z* 445 represents a loss of sugar moiety. Based on the mass spectrometry data, peak 10 at retention time 21.28 min with *m/z* 741.20 and the presence of ion fragment at m/z 609 was identified as rutin pentoside. Peak 11 was identified as quercetin 3,7-di-O-α-L-rhamnopyranoside. At retention time 21.84 min with *m/z* 593.13 peak 12 was identified as apigenin 7-O-β-D-apiofuranosyl(1→2)-β-D-glucopyranoside. The

last two compounds in the chromatogram, peaks 13 and 14, with *m/z* 447.06 and 579.13 were identified as luteolin-8-glucoside and naringenin-7-O-β-D-(3"-p-coumaroyl)-glucopyranoside, respectively.

ICP-OES analysis

The results of investigations of the content of mineral matters in the ajvar sample are presented in Table 4. Based on the obtained results, it can be concluded that of all investigated macroelements the sodium content was the highest (3571.703 mg/kg). Sodium is a crucial component of the body's bicarbonate buffer systems and plays a significant role in water distribution through osmosis and maintaining acid-base balance in the blood. Furthermore, it is essential for neuromuscular function as it facilitates the transport of glucose and other nutrients [14]. After sodium, the highest content was recorded for potassium (2172.695 mg/kg). Potassium is essential for membrane transport, energy metabolism, and the normal functioning of cells, which is expected given that it is the main constituent mineral of fresh *Capsicum annuum* L. sweet peppers [9]. In addition to sodium and potassium, phosphorus has been present in a significant amount (852.360 mg/kg). The obtained results are in agreement with the previously published study [15, 16], wherein fresh *Capsicum annuum* L. sweet peppers the phosphorous, magnesium, and calcium were detected in significant amounts after sodium and potassium content.

Table 4. Content of minerals in Ajvar (mg/kg)

Element	Macroelements	Microelements	Heavy metals
Ca	0.000		
K	2172.695		
Mg	71.618		
Na	3571.703		
P	852.360		
Ag		0.000	
B		3.840	
Ba		9.190	
Bi		4.910	
Co		0.000	
Cr		0.000	
Cu		4.480	
Fe		0.000	
In		0.000	
Li		0.066	
Mn		0.000	
Ni		0.000	
Si		0.000	
Sr		0.000	
Tl		0.000	
Zn		10.740	
As			0.010
Cd			0.000
Hg			0.000
Pb			0.855

The calcium presence was not detected in the ajvar sample which can be related to thermal peppers treatment [17]. After sodium, potassium, and phosphorous, magnesium in the amount of 71.618 mg/kg contributes to the increased nutritional value of ajvar. Magnesium is a component of bones and influences the nervous system and muscle activity as well [18]. A magnesium deficiency can lead to nervous disorders and bone deterioration. The recommended daily dose of magnesium is 280-350 mg [19].

Based on the previously published study it is known that fresh *Capsicum annuum* L. sweet peppers are rich in microelements [15], and their presence was also detected in the ajvar sample. Compared to the results reported in the mentioned study, in this investigation, a higher yield of Zn, and Cu was detected, while the presence of Mn and Fe was not detected. Zinc is necessary for the function of a series of enzymes. Its presence in the body reduces the toxicity of cadmium and copper. Zinc absorption in the body varies widely (10-90%) but very high zinc concentrations can increase the sensitivity to carcinoma. Its recommended daily intake is 15 mg per day, which again varies with the age of the person [20, 21]. The value of 10.740 mg/kg implies that ajvar is very rich in this microelement content. Copper is naturally present in most food products in the form of copper ions or copper salts. In general, the concentration of copper in food is about 2 mg/kg or less [15-23], and the highest concentration is present in meat, fish, chocolate milk, and green vegetables. Significantly higher values were determined in ajvar (4.480 mg/kg), and if we consider that by JECFA daily copper requirements are 0.05 mg/kg bodyweight, the amount of the ajvar during a daily diet should be adapted to the needs of the body. Insufficient

ingestion of copper in the body can lead to greater consequences for human health as well as the case with an increased copper intake which can cause symptoms such as vomiting, drowsiness, acute hemolytic anemia, kidney and liver damage, neurotoxicity, and increased blood pressure.

In addition to the necessary microelements, some are considered to be toxic microelements like Pb, Cd, As, and Hg. In the Ajvar sample presence of Pb and As was detected, while the concentration of Hg and Cd was below the limit of detection. Also, values of lead and arsenic were far below limited values by valid regulation [23]. By the valid regulative [23], the allowed lead values in vegetable products go up to 1 mg/kg, while certain products, such as spices or concentrated tomatoes limit lead concentration to 2 mg/kg.

Physical-chemical ajvar analysis

Moisture, ash, crude protein, and fat content were determined by appropriate methods to obtain more information regarding the composition of the ajvar sample. The results of physical-chemical characterization are presented in Table 5.

Table 5. Physical-chemical ajvar analysis

	Content in %	Determination method
Moisture	73.044	Gravimetric method, AOAC 925.40
Ash	2.526	Gravimetric method, AOAC 923.03
Crude protein	1.780	Kjeldahl method, AOAC 955.04D
Total fat	9.211	Soxhlet extraction, AOAC 963.15

The obtained results were similar to the previously published results regarding the moisture, ash, crude protein, and total fat content [25]. The slight differences may be the result of a different preparation recipe. The results were generally in compliance with the current Serbian and EU legislation. However, regular monitoring is crucial to prevent excessive accumulation in the food chain.

Conclusions

In summary, the obtained results suggest that ajvar is a valuable source of several bioactive compounds, as well as that it has a high level of some minerals that are of importance for human health. Potassium, which is detected in a high concentration is essential for the health of the heart and muscles, and together with zinc and copper that are also detected are significant for the normal function of the live cells. Finally, the analysis of moisture (73.04%), ash (2.53%), crude proteins (1.78%), and total fat content (9.21%) additionally confirmed that sweet ajvar is a nutritious addition to many dishes that provide health benefits.

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Izvod

KARAKTERIZACIJA DOMAĆEG AJVARA – NAMAZA OD PAPRIKA

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Ajvar je domaći poljoprivredni prehrambeni proizvod koji predstavlja smešu domaćih pečenih slatkih paprika. Obično se konzumira u malim porcijama, hladan, uz glavno jelo ili kao namaz. Cilj ovog rada bio je da istraži sastav domaćeg ajvara napravljenog od slatke paprike vrste *Capsicum annuum* L. U tu svrhu, analizirano je prisustvo bioaktivnih jedinjenja, mineralnih materija, određen je sadržaj vlage, pepela, sirovih proteina i ukupni sadržaj masti. Identifikacija bioaktivnih jedinjenja izvršena je korišćenjem tečne hromatografije visokih performansi u kombinaciji sa masenom spektrometrijom. Sadržaj mineralnih materija u uzorku ajvara ispitan je primenom optičke emisione spektrometrije sa indukovano spregnutom plazmom (ICP-OES). Sadržaj vlage i pepela određen je gravimetrijskim metodama, dok su za određivanje sadržaja proteina i ukupnih masti korišćene metoda po Kjeldahu i Soxhlet ekstrakcija, respektivno. U uzorku ajvara uspešno je identifikovano prisustvo nekoliko organskih kiselina i derivata flavonoida. Istraživanje mineralnog sastava pokazalo je visoke nivoe natrijuma (3571,703 mg/kg) i kalijuma (2172,695 mg/kg), kao i prisustvo nekih značajnih mikroelemenata kao što su cink (10,740 mg/kg) i bakar (4,480 mg/kg). Dobijeni rezultati sadržaja vlage (73,04%), pepela (2,53%), sirovih proteina (1,78%) i ukupnih masti (9,21%) dodatno su potvrdili da je ajvar prilično hranljiv i može se uklopiti u razne dijetetske programe.

Ključne reči: ajvar, UHPLC-DAD-ESI-MS/MS, ICP-OES, bioaktivna jedinjenja, minerali

Credit author statement

Sasa Savic: Conceptualization, Supervision, Writing – review & editing.
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