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X International Scientific Agriculture Symposium "AGROSYM 2019"



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#### FOREWORD

#### A Word from the Editor-in-Chief

Dear colleagues,

In your hands is the Book of Proceedings of the X International Scientific Agricultural Symposium "AGROSYM 2019", which I hope you will find useful in your work. As many as 900 contributions, from 82 countries, have been accepted for oral or poster presentations. Symposium themes cover all branches of agriculture and are divided into 7 sessions: 1) Plant production, 2) Plant protection and food safety, 3) Organic agriculture, 4) Environmental protection and natural resources management, 5) Animal husbandry, 6) Rural development and agro-economy, 7) Forestry and agroforestry. Papers dealing with agricultural engineering and technology were included into one of the seven sessions depending on their focus.

In the plenary lectures were addressed interesting topics; one keynote was on biotechnology and two others dealt with organic farming in Australia and Europe. This confirms the role of AGROSYM as a forum for open discussions and exchanges on agriculture, food, the environment and rural development in the Balkans and beyond. Many of the papers identify a number of approaches and market-based incentives to encourage producers to achieve higher levels of performance (from both economic and environmental points of view) and as a result to meet the expectations of governments and consumers.

The successful management of agricultural resources to satisfy changing human needs, while maintaining or enhancing the quality of the environment and conserving natural resources, indicate a long-term agricultural development imperative. Advances in productivity, profitability and stability of modern cropping, animal and forestry systems will have to be achieved globally on an ecologically sustainable basis. Today, it is obvious that conventional methods of agricultural production, while providing sufficient food and various products to humanity, have led to a number of negative impacts, including the transgression of many planetary boundaries. These negative impacts raise serious questions about the long-term sustainability of high-input agriculture and call for a genuine transition towards sustainable agro-food systems, which achieve food and nutrition security for present and future generations within the safe operating space for humanity.

Full texts of the submitted communications will be available on the website of AGROSYM (<u>http://agrosym.ues.rs.ba</u>). Each paper included in the present Book of Proceedings was positively reviewed.

Much appreciation is due to the authors of all papers submitted and presented at the symposium as well as to all symposium participants whose ideas and contributions allowed rich and lively discussions during the various sessions. Many thanks to all reviewers, session moderators and colleagues for their help in editing the Book of Proceedings. Special thanks go to all co-organizers, partners and sponsors for their unselfish collaboration and comprehensive support.

Editor-in-Chief Dusan Kovačerić

Dusan Kovacevic, PhD East Sarajevo, 12 October 2019

## NUTRITIVE CHARACTERISTICS AND ANTIOXIDANT ACTIVITY OF PSEUDOGRAINS

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#### Abstract

Amaranth (Amaranthus sp., Amaranthaceae), quinoa (Chenopodium quinoa, Amaranthaceae) and canihua (Chenopodium pallidicaule, Amaranthaceae) are pseudograins originating from South America. They are widely used in the diet as well as chia seeds (Salvia hispanica, Lamiaceae) from South America and wheat (Triticum aestivum, Poaceae) and millet (Panicum miliaceum, Poaceae), characteristic for our climate. The aim of the study was to determine the nutritive characteristics, phenolic content and antioxidant activity of commercial samples of pseudograins (amaranth, quinoa and canihua) from our market, as well as a comparative analysis to chia seeds, common and khorasan wheat and millet. Basic nutritive value parameters and mineral contents were assessed for pseudograins and chia seeds. The content of total phenolic compounds (TPC) and total flavonoid compounds (TFC), antioxidant activity by DPPH and FRAP tests were determined for all samples. The results of the nutrient composition indicates that analyzed pseudograins are good sources of proteins (13.39%) and unlike chia seeds are characterized by relatively low fat content (5.97%). Analyzed pseudograins contain calcium, magnesium, zinc and iron in a significant amount. The highest TPC content was observed in chia seeds (395.7 mg GAE/100 g), following canihua (327.4 mg GAE/100 g), quinoa (161.9 mg GAE/100 g), common wheat (61.8 mg GAE/100 g), Khorasan wheat (51.1 mg GAE/100 g), while the lowest content was identified in millet (32.9 mg GAE/100 mg). TFC content was in a range from 0.001 % (common wheat, khorasan wheat) to 0.099 % (canihua). Antioxidant properties of pseudograins were lower compared to chia seeds, but similar to common wheat and khorasan wheat while even higher comparing to millet.

Keywords: pseudograins, chia, millet, wheat, phenolic compounds, antioxidant activity.

## Introduction

Amaranth (*Amaranthus sp.*), quinoa (*Chenopodium quinoa* Willd.) and canihua (*Chenopodium pallidicaule* Aellen) from Amaranthaceae are usually called pseudograins (pseudocereals) because they belong to the different family than the usual cereals like wheat, oat or rise (Poaceae). They are native to South America and have been used for thousands of years (Repo-Carasc-Valencia et al., 2010). Chia presents edible seeds of *Salvia hispanica* L., (Lamiaceae), annual plant from Central America (Da Silva et al., 2017). Pseudocereals as gluten-free grains, and chia seeds, contain various biologically active compounds like polyphenols of growing interest regarding their potential health benefits (Vollmannova et al., 2013; Valdivia-López and Tecante, 2015; Tang and Tsao, 2017).

Millet (*Panicum miliaceum* L., Poaceae) originates from India and represents one of the oldest cereals. It is traditionally used in European and Asian countries and just like pseudocereals,

millet is also gluten-free and is convenient for patients suffering from celiac disease (Alverez-Jubete et al., 2010). Moreover, the proteins in millet grains have higher biological values compared to wheat grain, due to the higher content of essential amino acids (leucine, isoleucine, methionine) (Kalinova and Moudry, 2006).

Another nutritionally valuable grain is an ancient khorasan wheat, kamut or oriental wheat (*Triticum turanicum* Jakubz.). Whole grain consumption can have the significant role in the prevention of cardiovascular disease, diabetes and other metabolic diseases. It has also been found that khorasan wheat products promotes the growth of probiotic strains in the gastrointestinal tract and improves metabolic, lipid and antioxidant status in healthy subjects and in patients with acute coronary syndrome (Sofi et al., 2013; Whittaker et al., 2015)

The aim of the study was to evaluate the nutritive characteristics, phenolic content and antioxidant activity of commercial samples of pseudograins (amaranth, quinoa and canihua) from our market, as well as a comparative analysis to chia seeds, common and Khorasan wheat and millet.

# Material and methods

Commercial samples of amaranth, quinoa, canihua, chia, millet, khorasan and common wheat were purchased from the market in Belgrade, Serbia. Three independent samples from different suppliers were used for the research. All samples were grounded, homogenized and stored at room temperature until the analyses.

# Nutritional analysis

Determination of moisture (water, loss on drying), ash and crude fiber in the samples was performed by the recommended methods by the Association of Official Analytical Chemists (AOAC 930.04; AOAC 930.05; AOAC 930.09; AOAC 977.02). Proteins were estimated from the nitrogen content by multiplying by factor 6.25. The analysis of fat content was done by Soxhlet extraction after acid hydrolysis and carbohydrates were calculated by subtracting the total sum of proteins, lipids, crude fiber and ash from 100% sample. The contents of nutrients was expressed in percentages. Total energy values were calculated in kcal multiplying the amount of fat by factor 9 and protein and carbohydrate by factor 4.

After dry ashing mineralization (AOAC method 999.11 B), the content of sodium, calcium, magnesium, phosphorus, iron, zinc and manganese were determined by the ICP-OES, Vista-PRO Simultaneous ICP-OES (Varian Inc.). The mineral content was expressed as miligrams per 100 g.

# Total phenolic content (TPC)

The total phenolics were determined in methanol (80% *V/V*) extracts of investigated samples spectrophotometrically by *Folin–Ciocalteu* method. Gallic acid was used for construction of calibration curve and the results were expressed as mg of galic acid equivalents/100 g of sample (Singleton et al., 1999).

# Total flavonoid content (TFC)

The content of the total flavonoids in methanol extracts was determined according to the procedure given in the monograph of Ph. Eur. 7.0. (Ph. Eur. 7,0)

# Antioxidant activity determination by DPPH scavenging activity

For the DPPH scavenging activity determination the solution of DPPH (0.0147 g DPPH in 20 ml of ethanol) was prepared *ex tempore*. The standard solution was Trolox in ethanol (2 mM/l) in the range 0.2-0.7 mM/l. The colour change was measured spectrophotometrically at 525 nm after 1 hour of incubation at the dark place. The results are presented as mM Trolox equivalents/100 g of sample (Brand-Williams et al., 1995).

# Antioxidant activity determination by FRAP test

Ferric reducing antioxidant power (FRAP) assay is a method for determination of total reduction ability. During analysis the antioxidant donates electrone to the  $[Fe^{3+}-TPTZ]$  complex (Fe<sup>3+</sup>-2,4,6-tris(2-pyridyl)-*s*-triazine), that is subsequently reduced to blue  $[Fe^{2+}-TPTZ]$  at low pH values. After the incubation at 37°C for 40 minutes, the absorbance is measured at 593 nm. The Trolox was used for the construction of the calibration curve (0.1-0.8 mM/l). The results are presented as mM Trolox equivalents/100 g of sample (Benzie IFF, Strain, 1999).

# HPLC analysis

HPLC analysis of flavonoids was performed on the Agilent 1100, using the Zorbax Eclipse XDB-C18 analytical column ( $4.6 \times 250$  mm, 5 µm particle size), with the photodiode array detector set to 370 nm. Dried methanol extracts were dissolved in methanol (10 mg/ml). The samples were gradiently eluted with a two phase system: phase A = water/phosphoric acid (99.97:0.03), pH=2.75; and phase B = 10% A in acetonitrile, flow rate of 0.8 ml/min, at 25°C. The identification of components was performed by comparing the spectra with reference standards and quantification of rutin by constructing the calibration curve.

All analyses were done in triplicate and results were expressed as average mean.

# **Results and discussion**

The results of nutritional analysis of commercial samples of pseudograins, chia seeds, millet and khorasan wheat are given in Table 2. The amount of water was in the range 7.89-12.03% that was in accordance with the previous data (Repo-Carrasco-Valencia et al., 2010; Da Silva et al., 2017). The highest content of total ash was determined in the chia seeds (4.16%) and the lowest in the millet (0.7%). The results of total ash were also in agreement with former investigations (Repo-Carrasco-Valencia et al., 2010; Da Silva et al., 2017).

Pseudograins are important source of valuable proteins (Repo-Carrasco-Valencia et al., 2010; Da Silva et al., 2017; Kalinova and Moudry, 2006; Villa et al., 2014). Amaranth (19.91%) and chia (18.38%) contained the highest content of proteins, while lower contents were obtained in quinoa (8.31%) and millet (9.63%). However, obtained protein contents for quinoa (8.31%) and canihua (10.5%) were lower, while the amaranth (19.91%) protein content was higher than previously published data (Repo-Carrasco-Valencia et al., 2010).

Relatively low content of total lipids is one of the main nutritive characteristics of pseudograins. As expected, the obtained lipid content was 3.89-9.59%. On the contrary, chia seeds are characterised with high lipid content. So, obtained average content (30.09%) of total lipids in chia seeds is in accordance with literature data (Da Silva et al., 2017; Coates and Ayerza, 2009). Importantly, chia seed lipids contain very high amounts of polyunsaturated fatty acids, especially essential omega-3 alpha-linolenic acid (Coates and Ayerza, 2009). Observed higher total lipid content in chia seeds compared to other samples, corresponded the higher energy value than in other analysed samples.

Kilorasan wheat								
Content, %								
	Water <sup>a</sup>	Ash	Protein	Lipids	Available	Crude	Energy value	
Sample			3		s	noer	kcal/100 g	
Amaranth	10.06	1.94	19.91	3.89	63.63	0.57	369.17	
Quinoa	12.03	2.15	8.31	6.29	70.92	0.3	373.53	
Canihua	11.76	2.13	10.5	9.59	65.84	0.27	390.86	
Chia	7.89	4.16	18.38	30.09	38.53	0.95	498.45	
Millet	11.27	0.7	9.63	4.11	74.18	0.11	372.23	
Khorasan	10.94	2.12	14.4	2.21	67.13	3.2	346,01	

**Table 1.** Nutritional values of commercial samples of pseudograins, chia seeds, millet and khorasan wheat

<sup>a</sup> loss on drying;

The mineral content of analyzed samples is given in Table 2. The highest average content of calcium was observed for chia seeds (597.15 mg/100g). The obtained value was about three to four times higher than in amaranth (166.71 mg/100 g), about ten times higher than in quinoa (60.52 mg/100g), and even one hundred times more than in millet (5.25 mg/100g). Also, noticed variations in phosphorus contents were in the range 37.26 mg/100 g (millet) to 820.61 mg/100 g (chia seeds). Similarly, the lowest (51.73 mg/100 g) and the highest (337.06 mg/100 g) magnesium contents were recorded in the millet and chia seeds, respectively. Opposite to other samples, the quinoa had a relatively higher concentration of sodium (average 47.11 mg/100 g). Variations in iron content were in the range 0.93-4.04 mg/100g. The highest levels of iron were noticed for amaranth and chia, while the quinoa had the highest average content of manganese.

Sample	Calcium	Phosphorus	Magnesium	Sodium	Iron	Manganese	Zinc
-				mg/100g			
Amarant h	166.71	499.20	256.91	2.00	4.04	3.92	4.24
Quinoa	60.52	378.98	193.42	47.11	2.33	5.39	2.74
Canihua	41.23	184.37	117.59	8.53	1.47	3.67	2.29
Chia	597.15	820.61	337.06	5.70	3.85	3.61	3.89
Millet	5.25	37.26	51.73	2.38	0.93	0.21	2.79

**Table 2.** The content of minerals in commercial samples of pseudograins, chia seeds and millet

The total phenolic content (TPC) in investigated samples was in the range 32.90-395.75 mg GAE/100 g and total flavonoid content (TFC) 0.91-98.90 mg/100 g (Table 3). The highest content of phenolic compounds was in chia (395.75 mg GAE/100 g) and in canihua (327.36 mg GAE/100 g) samples, while the lowest in millet (32.90 mg GAE/100 g). Although the chia seed contained the highest value of phenolic compounds the obtained results were lower than previously described (757 mg GAE/100 g) (Hirose et al., 2010). The total phenolic content in amaranth (65.55 mg GAE/100 g) was similar to the Khorasan (51.05 mg GAE/100 g) and common wheat (61.82 mg GAE/100 g) samples. The highest amounts of total flavonoids were measured in canihua (98.90 mg/100 g) and quinoa (86.41 mg/100 g) samples.

Additionally HPLC method was used to identify the phenolic compounds in ethyl acetate extracts obtained after the hydrolysis of bounded phenolics and glycosides. Identified compound was present in very small amounts. In the ethyl acetate extracts of canihua and quinoa seeds quercetin aglycone was identified at 370 nm.

	r			
Sample	TPC <sup>a</sup>	TFC <sup>b</sup>	FRAP	DPPH
-	mg GAE/100g	mg/100 g	mM TE/100g	
Amaranth	65.55	2.06	5.8	2.55
Quinoa	161.92	86.41	3.0	0.85
Canihua	327.36	98.90	5.5	1.70
Chia	395.75	4.14	13.7	3.65
Millet	32.90	2.48	1.7	- <sup>c</sup>
Khorasan	51.05	0.91	6.4	9.06
Wheat	61.82	1.08	5.2	10.38

Table 3. Total phenolic and flavonoid content and antioxidant activity of commercial samples of pseudograins, chia seeds, millet, khorasan and common wheat

<sup>a</sup> total phenolic content; <sup>b</sup> total flavonoids content; c no antioxidant effect

The antioxidant activity of pseudograins, chia seeds, millet, khorasan and common wheat was not pronounced (Table 3). The total reduction ability measured by FRAP assay was in the range 1.7-13.7 mM TE/100 g and the DPPH radical scavenging activity 0-10.38 mM TE/100 g. The highest antioxidant activity in FRAP assay was obtained with chia seed (13.7 mM TE/100 g) and the lowest with millet sample. On the contrary khorasan and common wheat samples showed the highest scavenging activity in DPPH test (9.06 and 10.38 mM TE/100 g).

## Conclusion

Despite its growing popularity, this study is one of the scarce that offers insight in nutritive value of commercial samples of pseudograins on our market. Opposite to nutritional composition, there are observed differences in total polyphenol content and consequently in antioxidant activities among amaranth, quinoa and canihua samples. Also, there are prominent differences in all analyzed parameters among pseudograins and chia seeds, compared to millet. No differences were observed between khorasan and common wheat. In conclusion, obtained results of favorable nutritive value support the importance of pseudograins and chia seeds intake as part of our variety and balanced diet.

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