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Research Article

Nutritional and phytochemical comparative analysis of conventional/organic maize grain before and after subjection to accelerated aging test - a preliminary study

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Abstract

This study aimed to ascertain the differences in the nutritional composition of maize grains by analysing their lipid, protein, fatty acid, triacylglycerol, and sugar content before and after undergoing an accelerated aging (AA) test. Additionally, phytochemicals (total pigments, phenolics and flavonoids), as well as antioxidant potential of maize grains were evaluated through ABTS$^{\bullet+}$ scavenging capacity and ferric reducing power (FRP) assays. Maize was cultivated in Serbia during the 2017 season, employing two growing systems (conventional and organic). Although minimal differences were observed in protein and lipid content, the grains proved to be an excellent source of polyunsaturated fatty acids and triacylglycerols with ECN44 fraction being predominant. The total soluble sugar content was low, while starch content was significantly higher. HPLC analysis confirmed a considerably higher prevalence of non-reducing disaccharides compared to reducing disaccharides. Maize grains emerged as a substantial source of chlorophyll *a* (0.53-4.38 µg/g DW), phenolics (1498.9-1931.3 mg FAE/kg DW), and flavonoids (85.7-381.9 mg QE/kg DW). All tested extracts exhibited satisfactory abilities to neutralize free ABTS$^{\bullet+}$. The FRP assay revealed a significantly higher ability to reduce Fe^{3+}-ions in conventionally produced grains. According to the Blunt-Altman test, all analysed parameters showed considerable similarity, except for chlorophylls *a* and *b*. Principal component analysis (PCA) revealed that grains exposed to the AA test were mainly separated based on chlorophyll *a*, C20:0, C18:1n-9, MUFA, carotenoids, lipids content. Conversely, conventional untreated grains were primarily distinguished by their pentose, hexose and chlorophyll *b* content, while in the case of organic samples, disaccharides (both reducing and non-reducing) and soluble sugars were the main differentiating factors. The present preliminary study can serve as foundational research for future investigations aimed at comprehending alterations in nutrients and

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phytochemicals induced by accelerated aging tests. However, it should be supplemented and expanded upon by subsequent studies in the future.

Keywords: accelerated aging; grain; nutrients; organic agriculture; phytochemicals; *Zea mays* L.

Introduction

Maize (*Zea mays* L., family Poaceae, order Maydeae) is the most evolutionarily developed and domesticated plant species in the world. Its primary economic importance arises from its diverse applications in human and animal nutrition, as well as industrial processing, yielding over 1,500 industrial and food products, including starch, sweeteners, oils, beverages, glue, industrial alcohol, and ethanol (Glamočlija, 2012). This extensive range of maize products is attributed to its favourable chemical composition, comprising approximately 71% starch, 10% protein, 4.7% oil and 2.5% cellulose, along with B-group vitamins, essential minerals, and fibres. However, maize is deficient in sources of Ca, Fe, folate, and lacks vitamins B12 and C (Ranum *et al*., 2014). Grain maturation (aging) leads to changes in its main nutritional and phytochemical constituents. Numerous enzymatic processes are triggered causing alterations in protein, sugar, and phytochemical content. However, the most significant changes are related to lipid content and profile (Önder *et al*., 2020; Al-Taher and Nemzer, 2023), as well as antioxidants (Kravić *et al*., 2021). The main reason for the alteration in grain lipids is the possible peroxidation process (Önder *et al*., 2020) while antioxidants are prepared to combat the increased production of reactive oxygen species (ROS) (Kravić *et al*., 2021).

The organic production system prohibits the use of genetically modified varieties. A key requirement for successful organic maize production is the utilization of hybrids well-suited to local conditions, specifically those tolerant to various abiotic and biotic stressors (Bekavac, 2012). Although there is a growing global demand for organic maize, the acreage dedicated to its production remains relatively small compared to conventional methods (Golijan and Marković, 2018). According to a report by Boiko *et al*. (2023), global maize grain production has increased by 33% over the past decade. Currently, maize is cultivated on 197,204,250 hectares worldwide, with an annual production totalling 1,148,487,291 tons and an average grain yield of 5.82 tons per hectare (FAOStat, 2023). Maize ranks second globally in terms of cultivated area and first in total production and grain yield per unit area. In Serbia, maize is the predominant crop, occupying 996,527 hectares and yielding 7.87 million tons annually, with an average grain yield of 7.90 tons per hectare (Živanović *et al*., 2021). Organic maize production in Serbia has only recently commenced and currently covers a smaller area (Golijan and Marković, 2018). In 2022, organic maize was cultivated on 591.18 hectares, ranking third after wheat and oats, with an average grain yield ranging from 5000 to 6000 kg per hectare, significantly lower than conventional production (Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, 2024). Although organic production entails 15-25% higher labour costs, a significant advantage is the premium price, which in many countries is 20-50% higher compared to conventional maize (Bekavac, 2012). While the market price of organic maize generally follows the same upward trend as conventional maize, this is not always consistent. However, in the long run, organic production can be up to 25% more profitable compared to conventional methods (Bekavac, 2012).

The overuse of fertilizers and pesticides has led to detrimental effects on soil quality, impacting the grain production of various crops in the food chain. Consequently, organic production methods have garnered increased attention, aiming to preserve and enhance soil fertility through the utilization of local renewable resources (Gomiero, 2018). For the past thirty years, researchers around the world have conducted numerous comparative scientific studies on the chemical composition and nutritional characteristics of organic and conventionally produced crops (Golijan and Sečanski, 2021). According to reports by various authors, organic foods contain higher levels of dry matter, total sugars, essential amino acids, vitamin C, macro- and microelements (especially Ca, Mg, Fe, P, Zn), organic acids, phenolic compounds, and a higher total antioxidant capacity. They also have fewer proteins and a higher proportion of amino acids, as well as significantly lower levels of nitrates (Worthington, 1998; Worthington, 2001; Bourn and Prescott, 2002; Heaton, 2001; Rembiałkowska, 2007; Baranski *et al.,* 2014; Golijan and Sečanski, 2021). However, the most recent data suggest that it is not possible to determine significant and consistent improvements in the nutritional properties of organic food compared to conventional food, although consumers hold a more positive attitude toward organically produced foodstuffs (Suciu *et al*., 2019).

Furthermore, results from numerous research studies indicate that the differences in nutritional composition between organic and conventional food vary significantly depending on the examined nutrient, the production season, the plant species, and the analysed plant parts. Consequently, drawing reliable conclusions about the influence of the production method on the nutritional composition of plants proves challenging (Magkos *et al*., 2003). Despite claims suggesting that the nutritional value of organic food is higher than that of conventional food, there is still no clear consensus.

Given all that has been mentioned, the aim of this study was to determine the content of several important nutrients (lipids, proteins, fatty acids, triacylglycerols, and sugars) and phytochemicals (phenolics and flavonoids) in the grains of the maize cultivar 'Rumenka', developed and grown in Serbia. The study considered two different cultivation systems – conventional and organic – both before and after exposing the grains to an accelerated aging test. Thus, the study also aims to provide possible insights into the influence of growing conditions on the chemical composition of (non)germinated grains.

Materials and Methods

Grain material

ZP 'Rumenka' maize was cultivated at the experimental field of the Maize Research Institute in Zemun Polje, Serbia. Winter wheat served as the pre-crop to maize. Following the harvest of wheat in 2016, the stubble was ploughed to a depth of 10 - 15 cm. Before the basic soil cultivation (carbonate chernozem type), NPK fertilization was applied at a rate of 120:80:60 kg/ha of active matter for conventional production, or 500 kg/ha of organic fertilizer DIX 10N for organic production. Basic soil cultivation occurred during autumn, in the middle of November, to a depth of approximately 25 cm. Supplementary soil cultivation was carried out during the spring of 2017, and just before maize sowing, pre-sowing preparation took place. The area of the calculation plot in organic maize was 3000 m^2, while in conventional maize was 300 m^2. Machine sowing of ZP Rumenka maize occurred in April 2017, adhering to the recommended density (0.7 m × 0.2 m). Care measures included the use of appropriate herbicides based on nicosulfuron and mesotrione for conventional production, and mechanical weed control, manual hoeing, and inter-row cultivation for organic production. Maize harvest was done manually in October, during the stage of full maize maturity, with a moisture content of 18-14%. The average air temperature during the maize growing season was 19.4 °C. The precipitation for the growing seasons was 279.8 mm.

The yield of organic maize was 140 kg of grain, while the yield in conventional maize was 106 kg. The weight of the seed sample saved from the rows was 2.5 kg for conventional maize and 1.7 kg for organic maize. After harvesting the maize, the cobs were dried to 13% moisture content. The dried maize cobs were shelled and the collected grains were stored in the storage facility of the Maize Research Institute in Zemun Polje (Serbia), without exposure to any pesticides.

Analytical methods

The collected grain samples were cleaned to remove the damaged ones and weed. Whole grains were ground, placed in vacuum bags, and stored in a dark and cold place (at 4 °C temperature) until further analysis. Before analysis, the grain samples were ground into a fine powder. All extractions and analyses were performed successively in 2017. In total, four samples were prepared: organic and conventional maize grains, as well as organic and conventional maize grains exposed to the accelerated aging test.

Accelerated aging test

The viability of organic and conventional maize grains was tested using an accelerated aging (AA) test. The grains were exposed to double stress conditions of high temperature (45 °C) and high relative humidity (100%) for 72 hours. Chemical parameters were measured both before and after the test.

Extraction procedures and determination techniques

Lipid extraction and determination

The extraction of total lipids from the whole milled grains of maize was performed for 10 minutes using a methodology previously described (Golijan *et al*., 2019), with an n-hexane/isopropanol mixture (3:2, v/v). The extraction process was repeated twice, and the resulting extracts were combined. The solvent was evaporated in an inert atmosphere at a temperature of 50 °C using a stream of nitrogen (N_2). The dry residue was measured, and the total lipid content was determined and expressed as a percentage (%), i.e., g/100 g dry weight (DW) of the sample. The fatty acid and triacylglycerol (TAG) content were determined using the previously described method (Golijan *et al*., 2019). The obtained results were expressed as the mass percentage of total fatty acids and TAGs per 100 g DW of the sample. Additionally, the ratio of nutritional, parameters, UFA and SFA, was calculated.

Protein extraction and determination

Protein extraction and content determination were conducted following AOAC - standard method no. 960.52 (AOAC, 1997). The obtained results were quantified and expressed as a percentage (% g/100 g) on a dry weight (DW) basis of the sample.

Extraction and determination of total soluble sugars and starch

0.5 g of grain flour was extracted with 10 mL of 80% methanol (CH_3OH) for 45 minutes at room temperature. The extraction procedure was repeated three times. The total soluble sugars and starch content were determined using the previously described method (Golijan *et al*., 2021a). The obtained results are expressed as a percentage of glucose (g/100 g) on a DW basis of the sample.

Extraction and determination of individual soluble sugars

5 g of grain flour was weighed and mixed with approximately 40 mL of deionized water. Individual soluble sugars were determined according to the method described in Golijan *et al.* (2021a). The obtained results are expressed as percentage (g/100 g) of total soluble sugars.

Extraction and determination of free and bound phenolic (TPC) and flavonoid (TFC) fractions

For further analysis, 1 g of grain samples was mixed and extracted with 10 mL of 80% CH_3OH for 1 hour at room temperature in a dark place. The extraction procedure was repeated three times to obtain the free fraction, while the bound fraction was obtained after alkaline digestion of the samples (Golijan *et al*., 2021a). The resulting extracts were used to determine the content of free and bound phenolics and flavonoids, as well as the antioxidant activity of the maize grain samples. The standard Folin-Ciocalteu method was used and is described in Golijan *et al.* (2021a). The results for the TPC fractions (free and bound) are expressed as mg of ferulic acid equivalent (FAE) per kg DW of the sample. In a previous study on similar samples, ferulic acid was found to be the most abundant (Kravić *et al*., 2021) justifying the choice of this compound as the standard for result quantification. For total flavonoid content determination, aluminium chloride method was applied as previously described (Golijan *et al.*, 2021a). The obtained values for free and bound TFC fractions are expressed as mg of quercetin equivalent (QE) per kg DW of the sample.

Extraction and determination of pigments

The content of pigments (chlorophyll *a*, chlorophyll *b* and total carotenoids) was assessed following the method outlined by Laware (2015). Approximately 0.1 g of finely ground grain samples was extracted with 2 mL of 80% acetone, for 5 minutes, using a thermoshaker (600 rpm) (Thermomixer comfort, Hamburg) at room temperature. Subsequently, centrifugation was performed at 17,000 *g* for 15 minutes. The resulting supernatant 1 was transferred to a 10 mL centrifugal cuvette. This extraction process was repeated twice more, and the supernatants were pooled. The total volume was then adjusted to 10 mL with 80% acetone. To determine the pigments in the grain samples, the absorbance of the obtained extracts was measured at 663 nm, 646 nm, and 470 nm. The content of chlorophyll *a*, *b* and total carotenoids was calculated using the following formulas:

$$\text{Chlorophyll } a\ (\mu g/ml) = 12.21 - A_{663} - 2.81 \cdot A_{646}$$

$$\text{Chlorophyll } b\ (\mu g/ml) = 20.13 - A_{646} - 5.03 \cdot A_{663}$$

$$\text{Carotenoids } (\mu g/ml) = (1000 - A_{470} - 3.27 \cdot \text{chlorophyll } a - 104 \cdot \text{chlorophyll } b)/227$$

The Determination of ABTS$^{\bullet+}$ (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) scavenging capacity

The ability of methanolic extracts of maize grains to quench this radical cation was determined using the method described in Golijan *et al.* (2021a) and expressed as the percentage of ABTS$^{\bullet+}$ inhibition.

The Determination of ferric reducing power (FRP)

The ability of grains to reduce ferric ions was assessed at an absorbance of 700 nm following the methodology described in Golijan *et al.* (2021a). The resulting capacity for Fe^{3+} reduction was expressed as the absorbance value obtained at 700 nm- higher absorbance where a higher absorbance indicates a greater reduction ability of the sample.

Statistical analysis

One-factor analysis of variance with a balanced design was used to examine the significance of differences in the mean values of the observed parameters between different methods of maize cultivation. Tukey's test was used for post hoc comparisons. A significance level of 5% was applied in all tests. One-way ANOVA was conducted in IBM SPSS version 25, Armonk, NY, while Principal Component Analysis was performed using XLSTAT (2007) software.

Results

The nutrient content

When comparing organically and conventionally produced grains through the AA test, organic maize grains exhibited higher values for germination first count (78.50%), overall germination (84.25%), seedling above-ground part length (117.13 mm), and above-ground part dry weight (0.36 g). Conversely, conventionally produced grains showed a higher percentage of off-type seedlings (15.00%) and non-germinated grains (38.75%) as reported previously (Golijan *et al.*, 2022).

Furthermore, the results obtained from the current study (Table 1) reveal that the method of production exerts a distinct influence on nutrient composition, specifically proteins, lipids, fatty acids and triacylglycerols. The highest protein content was detected in the conventional grain (CM) and in the same sample after the accelerated aging test (CMaat).

In contrast to protein content, the production type did not affect the lipid content while the highest lipid content (4.04%) was detected in the OMaat grain sample exposed to the AA test.

The content of detected fatty acids (FAs) in all treatments during the examination of grain viability partially varied (Table 1). No difference was observed for myristic acid content except in the case of organic grains before the AA test. A higher content of palmitic and palmitoleic acids was achieved in CM, while the content of stearic, oleic, linoleic and arachidic remained the same in all treatments. The content of linolenic and docosanoic (behenic) acids increased after the AA test in both samples, respectively. On the other hand, the AA test provoked a small but significant decrease in the content of eicosenoic acid. Regarding the production type, there was partial agreement between the fatty acid content, which is similar to the research by Golijan *et al*., (2019), except for linoleic acid, whose content was more promising in OM.

Table 1. Content (%) of total lipids, total proteins, fatty acids and triacylglycerols in maize grains before and after AA test

Parameter %	OM	CM	OMaat	CMaat
Total proteins	10.06±0.05a	10.90±0.46b	10.07±0.28a	10.81±0.15b
Total lipids	3.11±0.15a	3.28±0.25a	4.04±0.12b	3.45±0.15a
		Fatty acids		
C14:0	0.03±0.002a	0.04±0.004b	0.04±0.002b	0.04±0.004b
C16:0	9.53±0.12a	10.12±0.24ab	9.71±0.19a	10.39±0.36b
C16:1	0.08±0.007a	0.1±0.004b	0.09±0.003ab	0.12±0.007c
C18:0	1.8±0.1a	1.85±0.09a	1.87±0.08a	1.88±0.1a
C18:1n-9	30.98±0.99a	30.79±0.83a	31.31±0.54a	31.51±0.97a
C18:2n-6	55.96±1.65a	55.31±0.9a	55.07±0.63a	54.12±0.67a
C20:0	0.47±0.03a	0.52±0.03ab	0.64±0.03c	0.59±0.03bc
C18:3n-3	0.69±0.04a	0.7±0.02a	0.62±0.04a	0.67±0.04a
C20:1	0.19±0.012b	0.17±0.01ab	0.15±0.01a	0.15±0.01a
C22:0	0.11±0.01a	0.12±0.01ab	0.13±0.003bc	0.14±0.006c
SFA	11.96±0.26a	12.67±0.37ab	12.41±0.31ab	13.05±0.5b
MUFA	31.25±1.01a	31.06±0.84a	31.55±0.55a	31.78±0.99a
PUFA	56.68±1.69a	56.09±0.93a	55.8±0.7a	54.9±0.74a
UFA/SFA ratio	7.35	6.88	7.04	6.64
		Triacylglycerols		
ECN42	29.83±0.43a	29.54±2.09a	29.36±1.05a	30.25±1.97a
ECN44	37.92±2.39a	37.79±0.68a	37.83±1.94a	37.45±1.55a
ECN46	22.6±2.1a	22.72±2.4a	22.87±0.97a	22.49±0.52a
ECN48	8.44±0.55a	8.64±0.76a	8.67±0.53a	8.57±0.15a
ECN50	1.21±0.07a	1.31±0.19a	1.27±0.05a	1.24±0.06a

*Notes (legend): C14:0 – myristic acid, C16:0 – palmitic acid, C16:1 – palmitoleic acid, C18:0 – stearic acid, C18:1n-9 – oleic acid, C18:2n-6 – linoleic acid, C18:3n-3 – linolenic acid, C20:0 – arachidic acid,C20:1 – eicosenoic acid, C22:0 – behenic acid, SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids; ECN – equivalent carbon number; OM – organic maize, CM – conventional maize, OMaat – organic maize grain after accelerated aging test, CMaat – conventional maize grain after accelerated aging test; values with the same letter in the same row do not significantly differ (p < 0.05, Tukey's test).

The content of triacylglycerols (TAGs), based on the equivalent number of carbon atoms (ECN42; ECN44; ECN46; ECN48; ECN50), as the main components of lipids, did not differ significantly in the examined samples (Table 1), regardless of the type of production. A similar outcome for ECN50 was achieved in the research by Golijan *et al.* (2019). However, the content of ECN42, ECN44, ECN46, and ECN48, obtained in the research, differed depending on the production systems, indicating a distinct influence of agroecological factors (caused by different production years). Interestingly, the AA test did not alter the TAG content either.

Based on the obtained results (Table 2), the content of free sugars, starch, monosaccharides (pentoses and hexoses), and disaccharides was partially affected by the type of production and seed treatments. A higher content of soluble sugars was achieved in organic production (OM 2.34 %), while the rise in starch content in organically produced grains was insignificant. Additionally, the content of reducing (0.48%) and non-reducing (1.51%) disaccharides was higher in organic grains. In contrast, the content of monosaccharides (both pentoses and hexoses) was higher in conventional grains (1.48% for pentoses, 1.07% for hexoses).

Table 2. Content of soluble sugars, starch, mono- (pentose and hexose) and disaccharides in maize grains before and after AA test

Parameter %	OM	CM	OMaat	CMaat
Soluble sugars	2.34±0.18d	1.53±0.07b	1.85±0.09c	1.15±0.06a
Starch	57.28±0.37a	58.32±1.36a	65.97±0.05b	59.17±1.47a
Pentoses	1.08±0.07a	1.48±0.13b	1.00±0.09a	1.13±0.12a
Hexoses	0.84±0.06a	1.07±0.05b	0.82±0.06a	0.87±0.04a
Non-reducing disaccharides	1.51±0.07c	1.17±0.08a	1.45±0.06bc	1.33±0.05ab
Reducing disaccharides	0.48±0.04b	0.38±0.03a	0.4±0.02a	0.39±0.03a

*Notes (legend): OM – organic maize, CM – conventional maize, OMaat – organic maize grain after accelerated aging test, CMaat – conventional maize grain after accelerated aging test; values with the same letter in the same row did not significantly differ (p < 0.05, Tukey's test).

The content of bioactive compounds

The content of examined pigments (chlorophyll *a* and *b*, carotenoids) varied among the samples grown under different conditions without any observed regularity. Chlorophyll *a* and carotenoids were more intensively biosynthesized in organic grains (Table 3) while chlorophyll *b* was not detected in this sample. After exposing the grains to the AA test, the grain sample grown under conventional production showed a more pronounced response, with a significantly higher content of chlorophyll *a* (5.89 μg/g) and carotenoids (1.03 μg/g), while chlorophyll *b* was detected only in the organic sample (2.04 μg/g) exposed to the AA test.

Table 3. Content of chlorophylls *a*, *b* and carotenoids in maize grains before and after AA test

Parameter (μg/g dry weight)	OM	CM	OMaat	CMaat
Chlorophyll *a*	3.53±0.14b	0.53±0.09a	4.38±0.49c	5.89±0.14d
Chlorophyll *b*	/	5.48±1.27a	2.04±0.23b	/
Carotenoids	0.23±0.02a	/	/	1.03±0.02b

*Notes (legend): OM – organic maize, CM – conventional maize, OMaat – organic maize grain after accelerated aging test, CMaat – conventional maize grain after accelerated aging test; values with the same letter in the same row do not significantly differ (p < 0.05, Tukey's test).

Pigments, as coloured compounds, play a major role in the manifestation of antioxidant potential. Alongside other compounds such as phenolics and polyamines, they contribute significantly to the plant's antioxidant potential. The results of the analysis revealed a wide distribution of phenolics and flavonoids in all treatments (Figure 1).

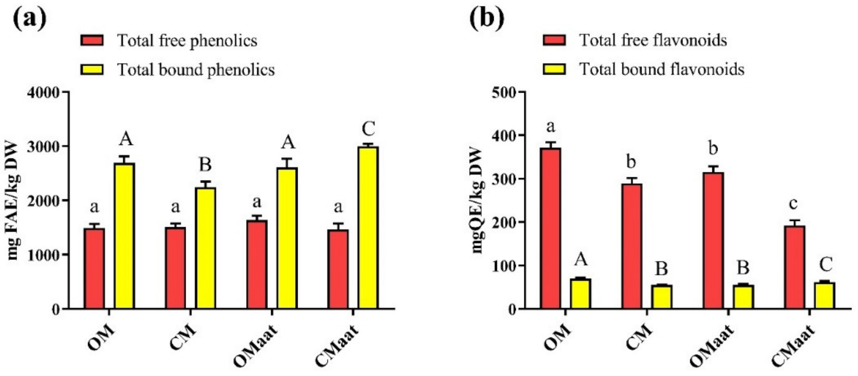


Figure 1. Total phenolic content (TPC) (a) and total flavonoid content (TFC) (b) of extract of organic / conventional maize grains and the same samples exposed to the accelerated aging test
The different case letters indicate the significant difference between samples (*Tukeys* test, $p<0.05$)

The concentration of free phenolics remained consistent regardless of the growing conditions, but organic production stimulated higher production of bound phenolics compared to conventional conditions (Figure 1 a). A similar trend was observed for TFC, with significantly higher levels recorded under organic system conditions for both free and bound fractions (Figure 1 b). Interestingly, the AA test induced the biosynthesis of phenolics, resulting in the highest observed TPC in conventional grain samples exposed to the AA test. However, in the case of total flavonoids, organic grains exposed to the AA test exhibited a higher TFC value compared to conventional grains. The observed increase in TPC in the samples exposed to the AA test is likely a result of stressful conditions to which the grains were subjected. However, there is no clear regularity, as not all results obtained under the conditions of the AA test were statistically significantly increased. Therefore, further, and more extensive research is warranted.

Given the variation in phytochemicals present in plants and different possible mechanisms of antioxidant actions it is strongly recommended to employ at least two different antioxidant assays as done in the current study. The results for the quenching ability (ABTS radical cation assay) of the samples and the reducing ability of the samples (ferric reducing power- FRP) are presented in Figure 2. Considering both the production systems and the AA test, grain samples obtained from conventional production exhibited higher antioxidant activity in both assays. Specifically, the conventional grain sample showed the highest FRP ability, while the same sample exposed to the AA test demonstrated the highest ability to quench ABTS radical cations.

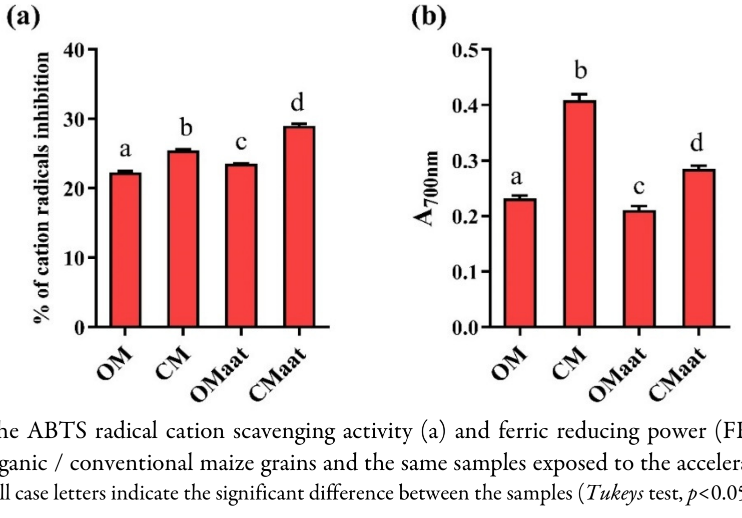
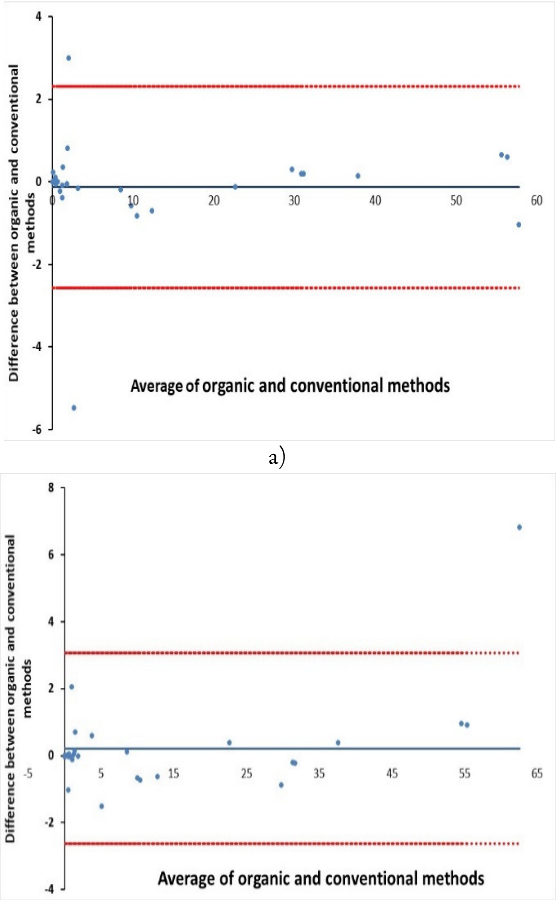


Figure 2. The ABTS radical cation scavenging activity (a) and ferric reducing power (FRP) (b) of the extract of organic / conventional maize grains and the same samples exposed to the accelerated aging test
Different small case letters indicate the significant difference between the samples (*Tukeys* test, $p<0.05$)

The statistical analysis data

The Bland-Altman diagram was used to show the similarities between the two growing methods. This diagram highlights the differences between the two applied systems in terms of the analysed parameters. Sublimating the results, all parameters, except for chlorophylls *a* and *b*, are located between the stacking lines, indicating that these two parameters are the only ones affected by the production method (Figure 3). In the AA test, starch content was the only parameter that showed significant differences in treatments.

A principal component analysis (PCA) was used to precisely delineate the similarities or differences among the treatments of organic and conventional maize production, as well as the effects of the AA test. This analysis also enabled the simultaneous determination of the correlation between the observed parameters (Figure 4). A biplot was constructed for this purpose, illustrating the loading of each parameter (arrows) and almost all four variants of the investigated treatments (OM, CM, OMaat, CMaat) (blue dot).



a)

b)

Figure 3. Bland-Altman plot analysis with limits of agreement (dashed line): two cultivation systems (organic and conventional) of maize production (A), and accelerated aging test (B) of maize grain Bland-Altman plot of agreement between organic and conventional methods

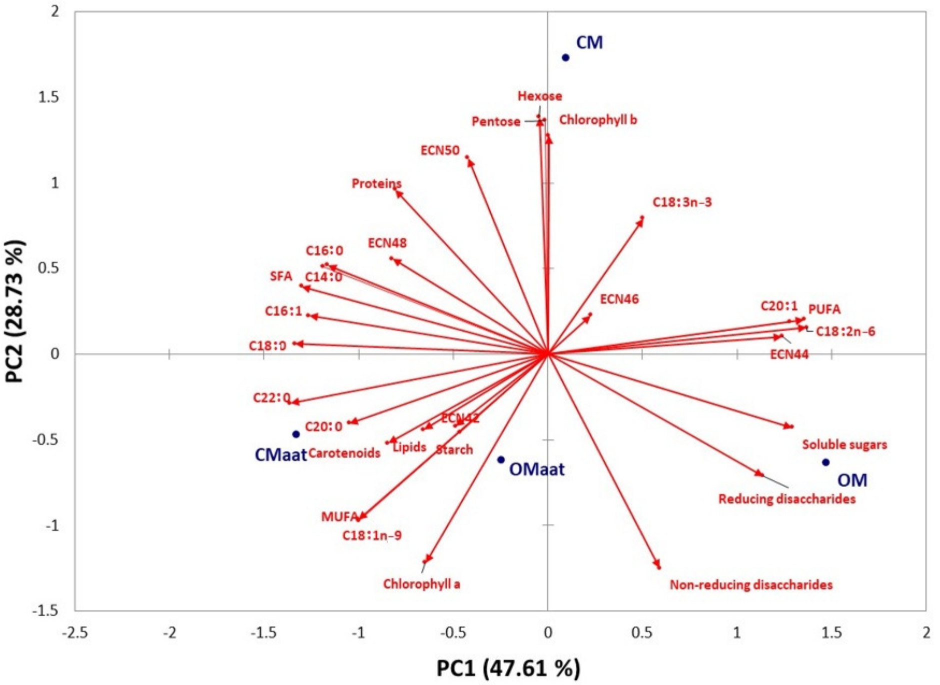


Figure 4. Principal component analysis scores of the observed parameters

The length of the vector approximates the variance of the features, while the angles between them (cosines) approximate their correlations. Points close to each other correspond to cultivation methods that have a similar influence on the PCA components. It can be observed that both samples under the AA test were separated from untreated grains, mostly influenced by the following parameters: carotenoids, lipids, starch, and chlorophyll *a* content, as well as the presence of several FAs (oleic, arachidic and bechenic) and ECN42 TAGs. On the other hand, untreated samples were separated from one another. Conventional grains were mostly influenced by the content of chlorophyll *b*, ECN44 and ECN 46 TAGs, as well as several UFAs (linolenic and eicosenoic). However, the organically produced grain sample was mostly influenced by the content of different sugars (soluble, reducing and non-reducing). The growing methods where the AA test was applied were more similar to each other than the remaining two methods where the test was not applied. In variants with accelerated aging, chlorophyll *a*, C18:1n-9, MUFA, carotenoids, lipids, C20:0 dominated. The OM system resulted in high values of disaccharides (both reducing and non-reducing) and soluble sugars. Conversely, the CM system was characterized by a higher content of pentoses, hexoses and chlorophyll *b* (Figure 4).

Discussion

The nutrient content

The accelerated aging test is one of the most relevant indicators of grain viability (Woltz *et al.,* 2001). Grain viability not only indicates the proportion of viable grains but also reflects the grain's capacity to develop healthy seedlings under unfavourable field conditions (ISTA, 2014). The grain vigour of maize (variety 'Rumenka') was previously assessed using the grain AA test. Research conducted by Golijan *et al.* (2021b, 2022) suggests that the method of production significantly impacts grain viability.

Protein content in the examined grains varied between different production systems but remained consistent after the AA test. The elevated protein content in the conventional sample can be attributed to the production method, specifically the positive influence of mineral fertilizer regulation on protein content (Al-

Budeiri *et al.*, 2021). However, no differences in this parameter were observed after the AA test. This observation aligns with the results of Wang *et al.* (2022) who also reported no alternation in protein content after sweet maize seeds were exposed to the AA test.

Overall, the majority of maize grains contain between 3.9 and 5.8% lipids (Aguirrezabal *et al.*, 2015), which is in partial agreement with the obtained results. Literature suggests that numerous agroecological factors can significantly influence lipid content (Aguirrezabal *et al.*, 2015; Majewska *et al.*; 2018, Golijan *et al.*, 2019). A similar lipid content (3.21-7.71%) in conventional grains was reported by Ullah *et al.* (2022). Mansilla *et al.* (2019) found that exposing grains to stressful conditions can influence lipid content, a phenomenon possibly observed in our organic grain samples.

Ashraf *et al.* (2016) pointed out that production methods do not affect fatty acid content, contrary to Kaplan *et al.* (2017), who argued the positive effect of nitrogen fertilizers. According to Goffman *et al.* (2001), fatty acid content and ratio are crucial for health safety- a higher content of unsaturated FAs compared to SFAs is preferable, consistent with this research. To determine the nutritional value of examined grains, the UFA/SFA ratio was calculated (Table 1). As literature suggests, this parameter should exceed 1.6 (Kostić *et al.*, 2017). It can be observed that all samples had values significantly higher than the suggested reference ratio. This generally makes maize grain a good source of preferable FAs. However, it should also be mentioned that, apart from PUFAs' predominance, it is important to maintain a balanced content of ω-3 and ω-6 UFAs (Kostić *et al.*, 2017) in some food stuffs for optimal nutritional balance related to lipids. The fact that the production system did not alter TAG content contradicts literature suggesting that the AA test significantly decreases TAG content due to lipid peroxidation (Önder *et al.*, 2020; Al-Taher and Nemzer, 2023). This opposing trend may be attributed to better storage conditions and prevention of possible lipid peroxidation and TAGs' deterioration.

The observed changes in sugar content may result from the hydrolysis of disaccharides caused by enzyme activity. Gao *et al.* (2020) suggested that the application of organic fertilizers increases the synthesis of hormones in grains, such as ABA (abscisic acid), GA (gibberellins) and the enzyme α-amylase, thereby improving grain quality by altering its chemical composition. Specifically, the improvement of grain quality occurs alongside the induction of amylase activity, whereby proteins, amino acids, and starch, potentially used for their mobilization, increase to provide the energy needed by the embryo for germination, as observed in OMaat treatments. Consequently, this could explain the highest starch content achieved in organic maize grain under the AA test (65.97%). However, other authors reported no significant changes in starch content for different maize hybrids exposed to artificial/natural germination process (Wang *et al.*, 2022). This can indicate that used variety is much more important compared to production system applied for expected starch content. Additionally, organic grains under the AA test exhibited higher soluble sugar content (1.85%).

There was no significant difference between the content of monosaccharides and disaccharides in the OMaat and CMaat grains (Table 2). When comparing the treatments, a higher ratio of mono/disaccharides was observed in organic than in conventional grains, consistent with the findings of Golijan *et al.* (2021c). According to the literature, this is explained by a higher synthesis of starch, as the grains mature faster and more extensively, associated with increased hormone synthesis (Wang *et al.*, 2016).

The bioactive compound content

The observed alternations in pigment content (chlorophylls and carotenoids) could potentially be attributed to the increased degradation of chlorophylls, leading to a subsequent decrease in carotenoid concentration. According to Janečková (2019), the degradation of chlorophylls (*a* and *b*) takes place much faster than that of carotenoids. This is due to the significantly higher content of chlorophylls in immature grains compared to mature grains, with rapid reduction occurring during maturation. These factors can contribute to variations in pigment content, as observed in this research. The presence of pigments belonging to the carotenoid class is important, as they are part of the grain's defence system based on chemical rather than enzymatic mechanisms (Önder *et al*., 2020).

Phenolics are usually found in grains in two distinct forms- as extractable or bound fractions and can be located in different parts of the grain including the testa, endosperm, and embryo (Önder *et al*., 2020). Regarding the TPC content, there was no clear regularity related to TPC values, which aligns with previous literature findings (Önder *et al*., 2020). However, in the case of TFC, the AA test induced a decrease in flavonoid content compared to untreated samples, consistent with the observations of Önder *et al.* (2020). In fact, the authors noted that varieties had a higher influence on phytochemical content compared to the AA test (Önder *et al*., 2020). However, this cannot be directly applied to the current study, as only one cultivar was analysed.

Research by Tian *et al.* (2022) has indicated that certain agro-technical measures strongly influence the phenolic acid content and antioxidant activity of cereal grains, partially aligning with our findings. One reason is that in organic agriculture, where the use of industrial pesticides is restricted, plants may face a greater risk of pests and diseases. In response, a more intense synthesis of antioxidant compounds is possible due to the activation of the plant's defence system. Another reason is the limited availability of certain nutrients, especially nitrogen, since the use of synthetic fertilizers is not allowed. In such cases there may be a greater synthesis of some phytochemical compounds that are part of the plant's defence system (Zuchovski *et al*., 2011). The same authors stated that a higher concentration of phenolic compounds in organic grains could be associated with grain size. Namely, it is assumed that in small grains, there is a smaller proportion of endosperm and a larger proportion of outer layers rich in antioxidants, which is not the case in this research. According to Zuchovski *et al.* (2011), the organic production system can lead to an increase in antioxidant concentration due to nutrient deficiency stress.

By measuring the antioxidant activity of grain samples, it is possible to assess their ability to combat oxidative stress factors. Antioxidants present in grains can prevent lipid oxidation, genetic material deterioration, and the accelerated aging of grains themselves (Kravić *et al*., 2021). Comparing conventional and organic production system, a similar effect was confirmed by Capouchová *et al.* (2020), in the determination of the antioxidant potential of oat grains, and by Kwiatkowski *et al.* (2022) in their research on cereal grains (wheat, spring barley, and oats), where a significantly higher antioxidant potential was observed for conventionally produced grains. According to the literature, this difference may be related to plant species (Fardet *et al*., 2008). During the germination process, increased production of reactive oxygen species (ROS) has been observed (Kravić *et al*., 2021), which can be neutralized by phenolic compounds as natural antioxidants. The biosynthesis of phenolics is one of the seed responses to the intensive imbibition of seeds during the AA test performance (Kravić *et al*., 2021). This is the most probable reason for observed increased activity in the ABTS radical cation quenching assay for grains that underwent the AA test, consistent with the findings of Kravić *et al.* (2021).

Conclusions

The study results indicated no clear evidence of the influence of production type on nutrient and phytochemical content. Maize grains were identified as a good source for human diet in terms of certain parameters, particularly unsaturated fatty acids, and starch. The germination of seeds triggered the biosynthesis of several bioactive compounds to counteract damage caused by oxidative stress conditions. Statistical analysis confirmed the potential application of several parameters to distinguish between both production systems and the influence of the AA test on maize grain composition. These findings can serve as fundamental data for future research endeavours aiming to establish patterns related to the impact of germination tests on metabolite changes in the grains of selected maize cultivars.

Authors' Contributions

J.G.P.- Conceptualization, investigation, data curation, formal analysis, Writing- original draft; R.P.- GC and HPLC analyses, validation; S.G.- Writing- original draft, literature search; D.D.M.- Writing- original draft, formal analysis, software, statistical analysis, data curation; S.L.- Conceptualization, supervision, project administration; R.Đ.- Formal analysis, investigation; D.J.- Formal analysis, data curation; A.Ž.K.- Conceptualization, supervision, Writing- review and editing, funding acquisition, project administration.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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