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The Content of Antioxidant Compounds and VOCs in Sorghum Grain Grown in Central and Eastern Europe

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Abstract: Sorghum is a plant belonging to the *Poaceae* family. It is drought-resistant and has low soil requirements. In the face of climate change, it is increasingly cultivated in Europe. Poland is a country with great agricultural potential; it is thus important to develop effective and economic methods of agricultural production, which is confirmed by the introduction of sorghum into cultivation. The aim of this study was to characterize the composition of bioactive compounds (i.e., phenolic acids, flavonoids, carotenoids, and phytosterols) and VOCs in sorghum grain of two varieties, i.e., white 'Sweet Caroline' and red 'Sweet Susana' grown in the temperate climate (Pętkowo, Poland (52°12′40″ N 17°15′31″ E)). The following tests were carried out: analysis of phenolic acids, flavonoids, carotenoids, phytosterols, antioxidant activity (ABTS), free phenolic acids (FPAs); elemental analysis; and water, fat and starch content analysis. Based on the conducted research, it was concluded that Poland has appropriate conditions for growing sorghum, as the content of bioactive (antioxidant) compounds was at a similar level to those grown in tropical and subtropical climates. Of the nine phenolic acids and seven flavonoids determined, the highest concentrations in both sorghum grain varieties were found for ferulic, p-coumaric and protocatechuic acids. The content of ferulic acid was three times higher in Sweet Caroline grains than in Sweet Susana grains. Differences in the content of these compounds may be the result of genetic differences between the Sweet Susana and Sweet Caroline varieties.

Keywords: sorghum; Sweet Susana; Sweet Caroline; bioactive compounds; ABTS; FPAs; phenolic compounds; phenolic acids; flavonoids; carotenoids; phytosterols; macroelements; microelements; VOC

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1. Introduction

Sorghum (*Sorghum Moench*) is one of the oldest plants grown in dry areas, unsuitable for growing other plants in tropical and subtropical climates. Sorghum is an annual plant belonging to the *Poaceae* family, like corn, millet and other grains [1,2]. Sorghum is a short-day spring crop. Sorghum develops best at temperatures above 21 °C; seeds should be sown when the ground temperature reaches 16 °C. Sorghum is therefore an extremely thermophilic plant. It does not tolerate standing water. It tolerates drought very well due to the C4 photosynthetic cycle. In the face of climate change, and above all its warming,

especially observed in Central Europe, we are looking for plants that will suit the climatic conditions of this part of Europe.

Poland is a country with great agricultural potential; therefore, it is important to develop effective and economic methods of agricultural production, which is confirmed by the introduction of sorghum into cultivation. Due to the above, intensive breeding work has been carried out in recent years [3–5], which allowed the creation and registration of new varieties of hybrids and forms of sorghum that also produce fully ripe seeds in temperate climate conditions [6–14]. Due to the low popularity of sorghum in Europe, only a few studies have been carried out on plants grown in conditions similar to those on other continents [15,16]. Previous research has shown that the chemical composition of sorghum grain is similar to popular cereals from the same *Poaceae* family. Its main ingredients are starch, proteins, fats and carbohydrates [17–26]. An important aspect is that sorghum grain is gluten free [27–31]. Moreover, it is characterized by the content of many bioactive nutrients such as micro- and macroelements (K, P, Mg, Ca and small amounts of Fe) and vitamins (B, E, D, K) [32,33] and is also a source of non-nutritive compounds such as flavonoids, phenolic acids, carotenoids and phytosterols [29,34–54].

Taking into account that sorghum grain contains many desirable nutrients and bioactive compounds, and given that their content may vary depending on the variety and growing conditions, this article describes both the growing conditions of sorghum and the results of chemical analyzes of grain for two varieties of sorghum, i.e., red and white [15,36,37]. Additionally, the content of volatile organic compounds (VOCs) was also tested. VOCs are compounds originating from anthropogenic and biogenic sources [55,56]. In plants, these are the most structurally diverse and numerous secondary metabolites. They are used by plants for protection—repelling pests and attracting pollinating insects—and mediate other interactions between plants and the environment. VOCs are also mediators of plant interactions at both the individual and community levels [57–59]. The results of field and laboratory experiments indicate an increase in VOC emissions from crop species when the plants are physically damaged or exposed to other stressors [60–63]. VOC emissions are also related to plant species [64,65] and environmental factors [63,66–72]. Scientific research confirms the wide possibilities of using sorghum in various industrial fields [43–48,70–72].

The aim of the study was to characterize the basic composition of bioactive compounds and VOCs in the grain of two sorghum varieties grown in a temperate climate (Central and Eastern Europe). An additional goal was to demonstrate differences in the chemical composition of the analyzed varieties in order to indicate the target direction of cultivation.

2. Materials and Methods

2.1. Conducting the Field Experiment

The tested material was sorghum grain harvested from experimental plots runed by the Institute of Natural Fibers and Medicinal Plants—National Research Institute at the Experimental Station in Pętkowo, Poland (52°12′40″ N 17°15′31″ E). The grains of two varieties, white 'Sweet Caroline' and red 'Sweet Susana', were analyzed. These varieties were selected due to the variety of seed colors (red/white), cold tolerance and short growing season.

The plots were established on soil of average agricultural use; therefore, it was enriched with nutrients.

The experiments were conducted in 3 repetitions for each variety. In the autumn of the year preceding cultivation, plowing was carried out to an average depth, and in spring, the plot surface was leveled using a cultivator and a harrow. Just before sowing, mineral fertilization was applied at the following doses: 100 kg · ha^{-1} N, 60 kg · ha^{-1} P_2O_5, 120 kg · ha^{-1} K_2O and 30 kg · ha^{-1} MgO, and harrowed. The seeds were sown in mid-May, in rows every 0.5 m and 0.12 m in the row. A five-row sowing of green sorghum was used around the experimental plots in order to obtain an experiment in the form of a compact field, which allowed the reproduction of large-area cultivation and elimination of differences in the results obtained inside and on the edges of the field. No chemical plant

protection products were used in the experiments. Weeds were removed mechanically only at the initial stage of plant growth; later, it was no longer necessary. The grain was harvested when the seeds were fully ripe in October. The collected panicles were dried and threshed. The obtained seed material was then used to conduct laboratory analyses.

2.2. Testing Methods

2.2.1. Analysis of VOCs

VOCs were extracted from the headspace of 20 mL vials containing 4000 g of sorghum grain by means of the solid-phase microextraction (SPME) method using 200 mm of 53/30 mm DVB/Car-boxen/PDMS fiber. Extraction was carried out at 50 °C for 25 min. Afterwards, the SPME fiber was inserted into the injection GC port for thermal desorption at 260 °C for 2 min. For HS-SPME analysis, we used a Thermo Scientific Trace 1310 gas chromatograph and TSQ 8000 Evo (Schaumburg, IL, USA) mass spectrophotometer. The column used for analyses was an Rxi-5MS (30 m × 0.25 mm × 0.25 µm) from Restek. The method employed in this study adhered to rigorous parameters. The injector port temperature was maintained at 260 °C, ensuring efficient vaporization and injection of the sample. Helium 5.0, a commonly used carrier gas, was delivered at a constant flow of 1.0 mL/min, guaranteeing consistent sample movement through the column. The oven temperature, a crucial factor influencing separation, was meticulously controlled. Starting at 40 °C, the temperature was ramped up at a rate of 10 °C/min to 180 °C and further increased to 260 °C at 40 °C/min, held steady for 5 min. These parameters, as reported by formed the foundation of the analysis [56].

Accurate quantification of VOCs was achieved by comparing the area under their total ion current peaks with a carefully calibrated internal standard, tridecane, injected into the samples. This method allowed for precise measurement, expressed as the ratio of areas, termed relative units (RU). To ensure identification fidelity, mass spectra of compounds were meticulously compared with those in the NBS 75K and NIST 98 libraries. Retention indices, calculated based on the chromatographic analysis of chain alkanes (C8–C20), served as critical data points for compound confirmation, enhancing the reliability of the findings.

The deconvolution and quantification of chromatograms using sophisticated software like AMDIS 2.6 provided a detailed VOC profile of sorghum grain.

2.2.2. Analysis of Bound Phenolic Acids and Flavonoids

Sorghum grain samples, each weighing 0.20 g, were subjected to a two-step hydrolysis process—first alkaline and then acid hydrolysis. In the alkaline phase, samples were treated with 1 mL distilled water and 4 mL 2 M aqueous sodium hydroxide, heated at 95 °C for 30 min, neutralized with 2 mL 6 M aqueous hydrochloric acid, and then cooled. Flavonoids were extracted using diethyl ether, transferred to vials, and the solvent was evaporated. Acid hydrolysis followed, involving supplementation with 3 mL 6M aqueous hydrochloric acid, heating, extraction with diethyl ether, and solvent evaporation. Prior to analysis, samples were dissolved in 1 mL methanol.

Quantitative analysis was conducted using an Aquity H class UPLC system equipped with a Waters Acquity PDA detector (Waters, Milford, MA, USA). Chromatographic separation employed an Acquity UPLC® BEH C18 column, with elution carried out gradient-wise using acetonitrile with 0.1% formic acid and 1% aqueous formic acid mixture. Concentrations of flavonoids and phenolic acids were determined using internal standards at specific wavelengths (320 nm and 280 nm, respectively). Compound identification relied on comparing retention times with standards and repeating the analysis with standard-spiked samples (limit of qualifications: 0.01 mg/kg).

The study identified various phenolic compounds in sorghum grain, including kampferol, gallic acid, luteolin, quercetin, and others, each with distinct retention times. Compounds were identified based on a comparison of the retention time of the analyzed peak with the retention time of the standard and by adding a specific amount of the standard to the analyzed samples and a repeated analysis. The detection level is 1 µg/g. The retention

times of assayed acids are as follows: kampferol 6.11 min, gallic acid 8.85 min, vanillic 9.71 min, luteolin 11.89 min, protocatechuic acid 12.23 min, vanillin acid 14.19 min, apigenin 16.43 min, catechin 18.09 min, 4-hydroxybenzoic acid 19.46 min, chlorogenic acid 21.56 min, caffeic acid 26.19 min, syringic acid 28.05 min, naringenin 31.22 min, vitexin 35.41 min, rutin 38.11 min, quercetin 39.58 min, p-coumaric acid 40.20 min, ferulic acid 46.20 min, sinapic acid 48.00 min and t-cynnamic acid 52.40 min. The recovery rates for the analyzed phenolic compounds were as follows: kampferol 86 ± 5.3%, gallic acid 92 ± 4.4%, vanillic 79 ± 8.5%, luteolin 96 ± 2.7%, protocatechuic acid 90 ± 4.8%, vanillin acid 88 ± 5.1%, apigenin 93 ± 3.8%, catechin 89 ± 5.7%, 4-hydroxybenzoic acid 96 ± 3.78%, chlorogenic acid 92 ± 2.8%, caffeic acid 86 ± 6.7%, syringic acid 94 ± 3.9%, naringenin 88 ± 4.8%, vitexin 95 ± 3.8%, rutin 93 ± 4.9%, quercetin 97 ± 1.9%, p-coumaric acid 89 ± 3.6%, ferulic acid 91 ± 4.9%, sinapic acid 94 ± 5.1% and t-cynnamic acid 97 ± 2.9 [73,74].

2.2.3. Determination of the Free Phenolic Acids (FPAs) and Antioxidant Activity (ABTS Method)

The analysis of the free phenolic acids was determined as described by Przybylska-Balcerek et al. [72].

For the ABTS generation from ABTS salt, 3 mM of $K_2S_2O_8$ (Sigma-Aldrich, Inc., St. Louis, MO, USA) was reacted with 8 mM ABTS salt in distilled, deionized water for 16 h at room temperature in the dark. The ABTS solution was then diluted with a pH 7.4 phosphate buffer solution containing 150 mM NaCl (PBS) (Sigma-Aldrich, Inc., St. Louis, MO, USA) to obtain an initial absorbance of 1.5 at 730 nm. The fresh ABTS solution was prepared for each analysis. The kinetic reaction was determined over a 2 h period with readings every 15 min; the reactions were complete in 30 min. The samples and standards (100 µL) were reacted with the ABTS solution (2900 µL) for 30 min.

Trolox was used as a standard. The results were expressed in the ABTS (µmolTROLOX/g DM) sample [71].

2.2.4. Analysis of Carotenoids

The process began by extracting carotenoids from sorghum grains (0.4 mg) through a saponification method. The extracted material was triturated with a mixture of acetone and petroleum ether (1:1). Plant tissue separation followed, and the remaining extract underwent washing with water, resulting in an ether extract containing a mixture of carotenoid pigments. This extract was then concentrated in a vacuum evaporator at 35 °C until an oily residue formed. Subsequently, the residue was digested in 2 mL of methanol (Merck, Darmstadt, Germany) for further analysis. The analysis of carotenoids was conducted using Acquity UPLC (Waters, Milford, MA, USA) equipped with a Waters Acquity PDA detector. Chromatographic separation was achieved on an Acquity UPLC® BEH C18 column (100 mm × 2.1 mm, particle size 1.7 µm) from Waters, Ireland. The elution process utilized a gradient of solvent A (methanol), solvent B (water), and tert-butyl methyl ether (TBME) at a flow rate of 0.4 mL/min. To maintain consistency, both the column and samples were thermostated, with the column set at 30 °C and the test temperature at 10 °C. Solutions were degassed using a Waters device during the analysis. The injection volume for samples was 10 µL, and detection was performed at a wavelength of λ = 445 nm [73,75].

2.2.5. Analysis of Phytosterols

Measures of 100 mg of ground grains were placed into 17 mL culture tubes. Measures of 2 mL of methanol were added to the tubes. Measures of 0.5 mL of 2 M aqueous sodium hydroxide were added to the tubes. The tubes were tightly sealed. The next step was microwave treatment. The culture tubes were placed inside 250 mL plastic bottles. The plastic bottles were tightly sealed. The bottles were placed inside a microwave oven (Model AVM 401/1WH, Whirlpool, Benton Harbor, MI, USA) operating at 2450 MHz and 900 W maximum output. Samples were irradiated at 370 W for 20 s, and after about 5 min, they were irradiated for an additional 20 s. After this process was neutralization. After 15 min,

the contents of the culture tubes were neutralized with 1M aqueous hydrochloric acid. On the end was extraction with pentane was carried out within the culture tubes, and this was performed three times with 4 mL of pentane each time. The combined pentane extracts were evaporated to dryness in a nitrogen stream. Before chromatographic analysis, the sample extract was dissolved in 1 mL of MeOH. A measure of 50 µL of the dissolved sample was analyzed using an Aquity H class UPLC system equipped with a Waters Acquity PDA detector (Waters, USA). Chromatographic separation was performed on an Acquity UPLC® BEH C18 column (100 mm × 2.1 mm, particle size 1.7 µm) (Waters, Dublin, Ireland). Elution was performed with a mixture of methanol/acetonitrile/water (85:10:5) at a flow rate of 0.4 mL/min. [73,75].

2.2.6. Elemental Analysis

In the study, the material was mineralized using a CEM Mars 5 Xpress microwave system, a sophisticated technology designed to break down complex samples. The material, placed in 55 mL vessels, underwent a meticulous process involving nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). The mineralization occurred in three stages, each precisely controlled for power, time, and temperature. This method not only ensures thorough digestion but also optimizes the process for efficiency and accuracy. Following mineralization, the materials were meticulously filtered through 45 mm filters to remove impurities. The filtrate was then adjusted to a final volume of 50 mL using deionized water. The concentrations of specific trace elements (Cu, Fe, Mn, Zn, and Se) were analyzed using various advanced spectrometric methods. Flame atomic absorption spectrometry was employed for Cu, Fe, Mn, and Zn analysis, providing accurate results for these elements. Atomic emission spectrometry was utilized for Mg determination, while hydride generous atomic absorption spectrometry was used for Se analysis, showcasing the diversity of techniques applied in this study. The analysis was performed using a state-of-the-art AA Duo—AA280FS/AA280Z spectrometer equipped with a Varian hollow-cathode lamp, ensuring precision and reliability in the measurements. Calibration curves were prepared in four replicates per trace element concentration. The detection limit for the analyzed metals (in $ng \cdot kg^{-1}$) was as follows: Cu 0.18, Fe 0.11, Mn 0.005, Zn 0.06 and Se 0.21 [76].

2.2.7. Water Content Analysis

The water content of the grains was analyzed according to PN-EN ISO712:2012 [77].

2.2.8. Fat Content Analysis [78]

The fat content of the grains was analyzed according to PN-EN ISO 11085:2015-10 [78].

2.2.9. Starch Content Analysis

The analysis of the starch content was performed using the polarimetric method EN ISO 10520:1998 [79].

2.3. Statistical Analysis

In order to present the content of selected acids in both varieties (Sweet Susana and Sweet Caroline), boxplots are given. The boxplot is a chart used for the presentation of the summary of data and for the visualization of the distribution of numerical data by displaying the data quartiles and averages. Moreover, a graphical method of displaying multivariate data in the form of a two-dimensional chart of quantitative variables represented on axes starting from the middle point, the spider plot, was proposed. Curves represent the mean content of particularly acid in the two considered varieties, Sweet Susana and Sweet Caroline. The points represent the mean values of observations for each considered acid and for the two varieties. The number of vertices of the polygon corresponds to the number of acids analyzed. The blue lines represent grid lines. Moreover, in order to indicate specific relationships between acids in varieties, the linear correlation between particular acids in two varieties is calculated and presented in the form of

heatmaps. Analysis of variance (ONE WAY ANOVA) was used to indicate differences between the content of micro- and macroelements for the considered cultivars. This study was performed separately for each mentioned element. In order to provide general relationships between volatile substances in a tree-like structure, a dendrogram was used. It was the element of hierarchical clustering directly represented as a nested list where each component corresponds to a branch of the tree and that practitioners use to depict the arrangement of the clusters produced by hierarchical clustering. All calculations were performed using statistical software R (R version 3.5.2 (20 December 2018)).

3. Results and Discussion

3.1. Weather Conditions during Cultivation

Weather data for the months from May to October, which correspond to the sorghum growing season, are presented in Table 1. The data come from the weather station of the Institute of Meteorology and Water Management—National Research Institute in Słupia Wielka (52°13′02″ N 17°13′04″ E), which is next to the Experimental Station where the field experiments were carried out.

Table 1. Weather course during the sorghum growing season 2022 and the long-term average from 1960 to 2020.

Month	Temperature (°C)		Precipitation (mm)	
	Average Monthly (2022)	Long-Term Average (1960–2020)	Average Monthly (2022)	Long-Term Average (1960–2020)
May	14.7	12.7	43.0	61.0
June	19.7	15.6	73.5	53.0
July	19.4	17.4	48.5	78.0
August	21.0	16.2	28.5	60.0
September	12.9	12.6	85.0	49.0
October	12.6	8.1	31.0	41.0

Comparing the average temperature in 2022 with the average temperature over many years, it can be concluded that it is significantly higher by 3–5 °C, which significantly affects crops. This is evidence of a significant need to introduce crops that will be suitable for a warming climate, i.e., thermophilic plants. Additionally, the small amount of rainfall compared to the multi-year average in key periods of plant growth and yielding makes it important to look for plants that, in addition to high temperatures, are tolerant to water shortage. This fact is important from the point of view of Polish agriculture due to the lack of irrigation systems.

The highest average monthly temperature in 2022 was measured in August (21.0 °C). Generally, the temperature during the sorghum growing season was above the long-term average, although in September it was cooler than usual. The average temperature in June was similar to the temperature in July. September and October also showed very similar results among themselves.

The rainfall was distributed unevenly. The highest total monthly rainfall was recorded in September (much above average) and in June. The agricultural and hydrological drought occurred in July and August. The weather conditions did not bring any late spring or early autumn frost as this sorghum could develop without any obstacles both in May and October.

During cultivation, the phenological development of plants was also monitored. The observations are presented in Table 2.

Table 2. Dates of individual stages of phenological development of sorghum plants of the 'Sweet Caroline' and 'Sweet Susana' varieties.

Phenological Development	Date
Sowing	16 May 2022
Sprout	24 May 2022
One pair of leaves	28 May 2022
Two pairs of leaves	1 June 2022
Three pairs of leaves	6 June 2022
Four pairs of leaves	10 June 2022
Height—0.15 m	12 June 2022
Height—0.30 m	23 June 2022
Flowering 'Sweet Caroline'	17 July 2022
Flowering 'Sweet Susana'	19 July 2022
Height 0.8 m	19 July 2022
Height 1.0 m	25 July 2022
Harvesting 'Sweet Caroline' and 'Sweet Susana'	26 October 2022

Sorghum was sown in mid-May into moist soil. The sprouts appeared after a week. The plants grew quickly due to favorable growing conditions (fleeting rainfall, relatively high ground temperature). The flowering of the plants began in mid-July, and the plants reached their maximum height in early August. The tested varieties are generally short and reach up to 1.5 m in height. The plants were harvested later than in previous years, i.e., in the third decade of October, about 20 days later than usual, which was due to the cool and rainy weather in September. Both varieties were harvested on the same day.

3.2. Bioactive Compounds

Research carried out on two varieties of sorghum grains, i.e., Sweet Susana—red and Sweet Caroline—white, showed significant differences in the content of selected bioactive compounds. The presence of these compounds may have a significant impact on the nutritional value and potential health benefits of products derived from these sorghum varieties. Bioactive compounds, such as antioxidants, are known for their ability to neutralize free radicals in the body, which can help prevent various diseases and cellular aging. Differences in the content of these compounds may be the result of genetic differences between the Sweet Susana and Sweet Caroline varieties. This may be important for breeders who want to breed sorghum varieties with increased content of bioactive compounds. The obtained results confirm the complexity of the influence of various factors, such as the species, variety, place of growth, sunlight and harvest period, on the chemical composition of plants. This is important information for breeders whose breeding work is aimed at obtaining high-quality grain. Based on these studies, the presence of bioactive compounds was found in two tested varieties of sorghum grain (Sweet Susana—red and Sweet Caroline—white). As part of the research, the results of the level of ABTS antioxidant capacity, the content of free phenolic acids (FPAs), the content of selected phenolic acids, flavonoids, carotenoids, sterols and VOCs were obtained. Based on the results presented in Figures 1 and 2, it can be concluded that the antioxidant activity of $ABTS^{\bullet+}$ bioactive compounds was higher in the Sweet Susana sample compared to Sweet Caroline. This finding is surprising considering previous studies that showed the opposite trend [73]. The authors' previous research showed that Sweet Caroline seeds had the highest antioxidant activity in 2016 and 2018, although no significant statistical differences were observed between these years. In turn, the antioxidant activity of $ABTS^{\bullet+}$ in the Sweet Susana sample was lower by 15% and 23%, respectively. Another significant finding came from

research by Xiong and colleagues conducted in 2019 [80,81]. These researchers examined the effects of ABTS$^{\bullet+}$ and DPPH extracts and raw sorghum grains. Their results indicate that the content of total phenolic acids and other polyphenols was significantly higher in the extracts than in raw sorghum. Interestingly, despite this difference in phenolic content, the antioxidant activity remained similar. This suggests that in white sorghum, the concentration of phenolic compounds may influence antioxidant activity, but other compounds in colored sorghum may contribute to higher antioxidant activity. It is worth noting that the antioxidant activity of ABTS$^{\bullet+}$ bioactive compounds present in sorghum is higher than in wheat and triticale grains grown in temperate climates [81]. This may suggest that sorghum is a source of powerful antioxidants, which may be beneficial to human health. Moreover, the observation of higher FPAs in Sweet Caroline compared to Sweet Susana during the same atmospheric conditions suggests that the production of phenolic acids in sorghum may be dependent on the intensity of oxidative stress caused by biotic and abiotic factors. Taken together, these results highlight the importance of genetic diversity and environmental conditions in influencing the antioxidant activity of bioactive compounds in plants. This research is important for understanding the potential health benefits of eating different varieties of plants, especially those rich in antioxidants.

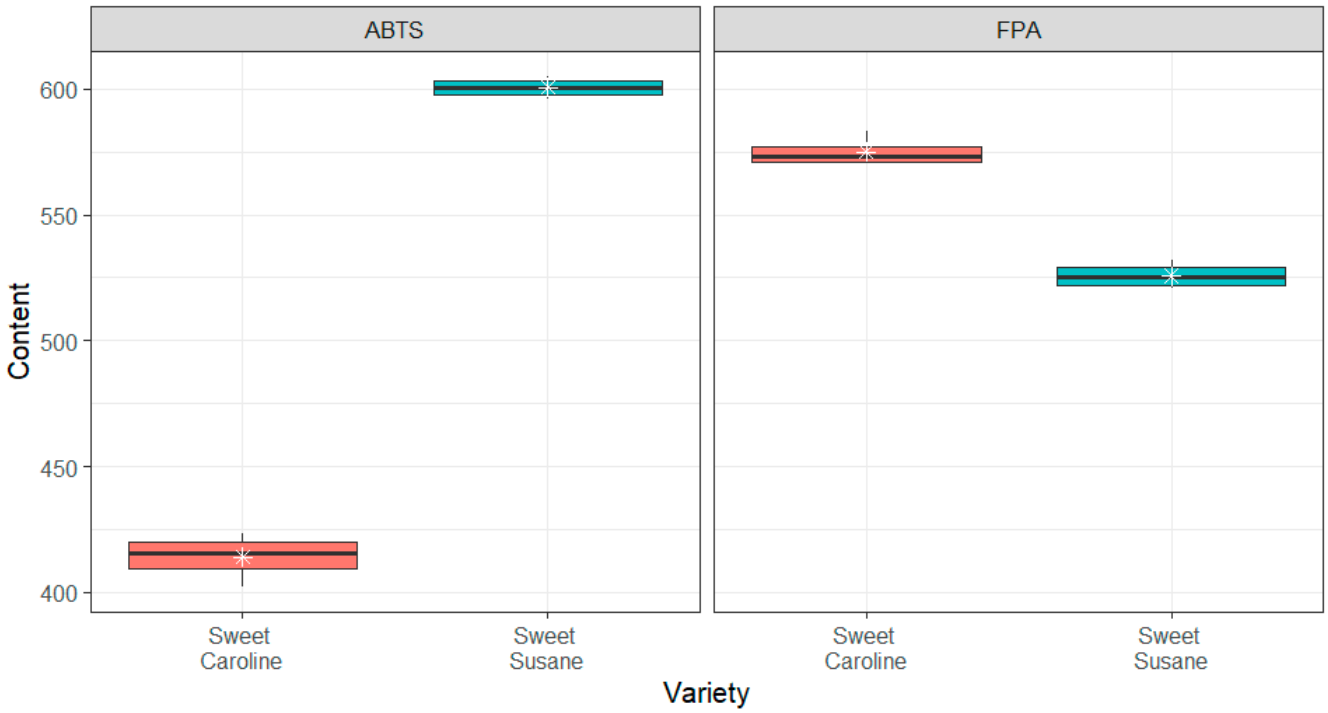


Figure 1. Comparison ABTS and FPAs in sorghum grain. ABTS$^{\bullet+}$ (µmolTROLOX/kg)—antioxidant activity applying an improved 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; FPAs (mg GAE/100 g)—free phenolic acids.

During this research, sorghum grain (Sweet Susana—red and Sweet Caroline—white) was analyzed. Based on the results presented in Figure 2, significant differences were found in the content of individual phenolic acids in white and red sorghum grains (Figure 3). Of the nine phenolic acids determined, the highest concentrations in both sorghum grain varieties were found for ferulic, p-coumaric and protocatechuic acids. The content of ferulic acid was three times higher in Sweet Caroline grains than in Sweet Susana grains. In the case of p-coumaric acid and protocatechuic acid, the concentration was twice as high in Sweet Caroline sorghum grains compared to Sweet Susana grains. A significant difference was also observed in the case of the content of caffeic and gallic acids—it was twice as high in Sweet Susana sorghum grains compared to Sweet Caroline sorghum grains. Similar results were obtained in the authors' previous studies [73]. Khoddami

et al., 2015 also determined ferulic acid in the highest concentration (26.81 mg/kg) in red sorghum [82]. According to literature reports, this acid is the most common acid found in sorghum grains and other cereals [83]. Mawouma [34] determined the content of this acid in the tested sorghum cultivars in the range of 56 µg/mL. In sorghum, ferulic acid occurs mainly in the form of esters with sugars and as a component of the cell wall. This relationship is important for both plants and humans. In plants, ferulic acid is an important component of the cell wall, helping to maintain cell structure and resist environmental stresses. In the human body, ferulic acid may act as an antioxidant, helping to protect cells from oxidative damage [84]. Phenolic compounds in sorghum often occur in the form of bound fractions; the composition and concentration of these compounds vary depending on sorghum genotypes and the production environment [85–87].

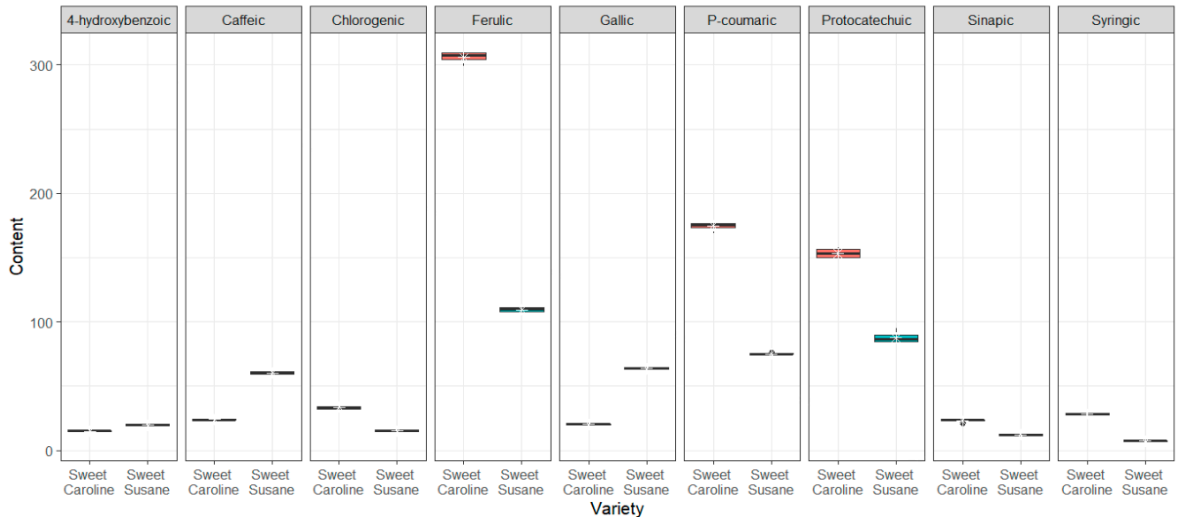


Figure 2. Contents of selected phenolic acids in sorghum grain.

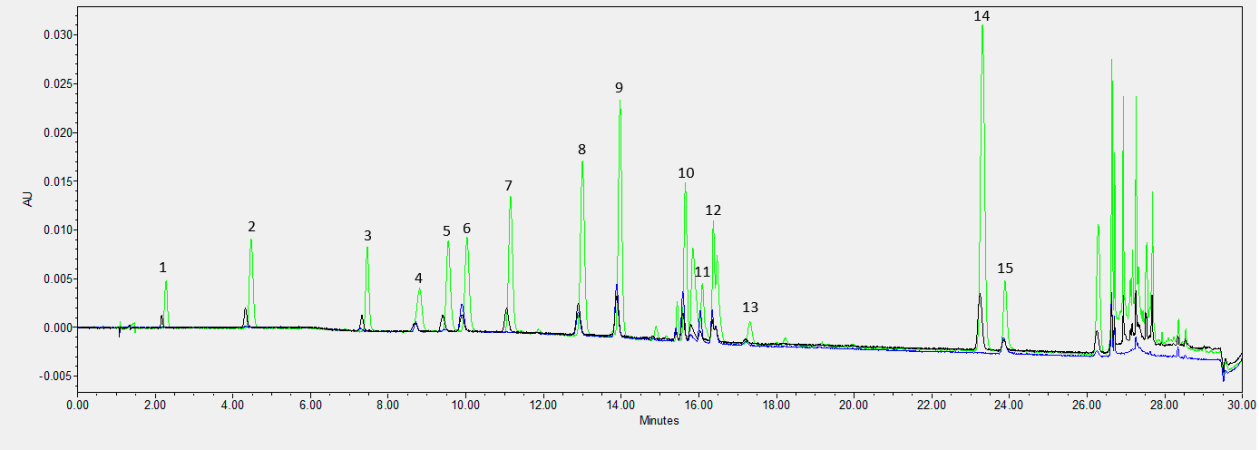


Figure 3. Comparison of chromatograms of phenolic acids and flavonoids for Sweet Susana (black line), Sweet Caroline (blue line) and Sweet Susana + addition of standards: 1—gallic acid, 2–4-dihydroxobenzoic acid, 3—caffeic acid, 4—syringic acid, 5—ferulic acid, 6—chlorogenic acid, 7—sinapic acid, 8—protocatechuic, 9—naringenin, 10—quercetin, 11—kaempferol, 12—catechin, 13—luteolin, 14—vitexin, 15—rutin.

The group of polyphenols found in sorghum grains (Sweet Susana—red and Sweet Caroline—white), apart from phenolic acids, also includes flavonoids. In this study, the content of seven flavonoids was determined in red and white sorghum grains, and the results obtained are presented in Figure 4. The highest content was found in the case of

catechins (Sweet Caroline 4.96 mg/kg and Sweet Susana 4.16 mg/kg). Concentrations of luteolin and rutin were three times higher in Sweet Caroline grains compared to Sweet Susana grains. In the case of naringenin and vitexin, the concentration was twice as high as in red sorghum grain. Similar results were obtained in the authors' previous studies [73]. The literature on the subject states that the concentration of catechin in Sweet Caroline sorghum grain is approximately 5 mg/kg and 4 mg/kg for Sweet Susana. Moreover, the content of quercetin was found to be 0.3–0.6 mg/kg and campferol (0.3–0.56 mg/kg), regardless of the variety. Additionally, Khoddami et al. 2015 determined apigenin at the level of 7.10 mg/kg, while this compound was not determined in our studies. The same authors also determined naringenin, the concentration of which was 80 times higher compared to the results presented in this study [82].

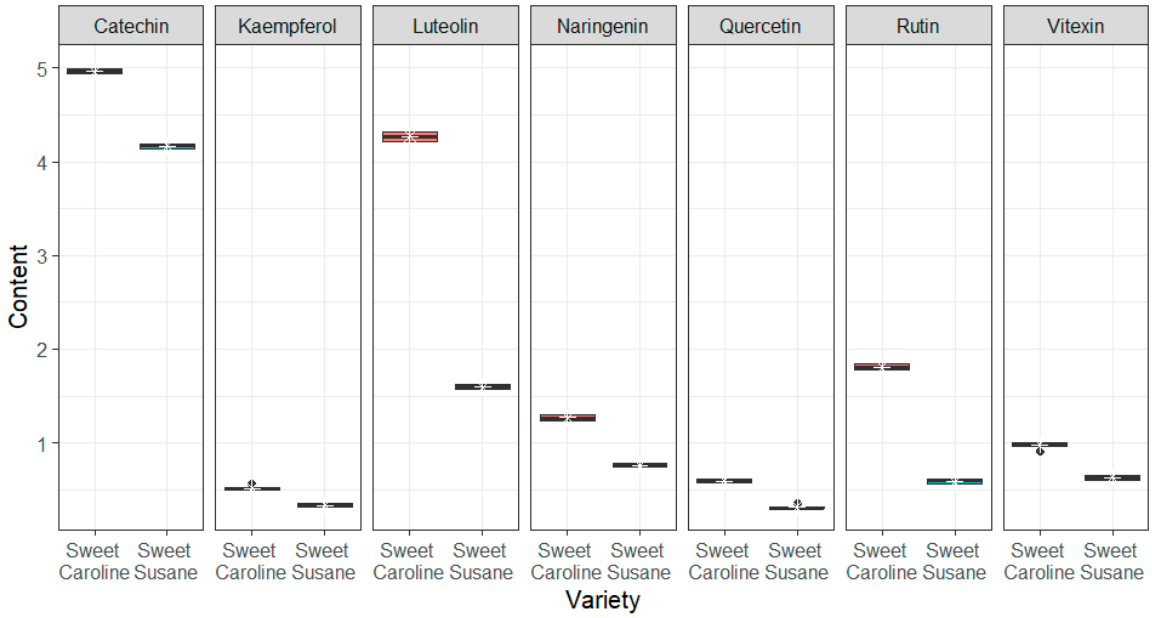


Figure 4. Contents of selected flavonoids in sorghum grain.

The content of carotenoids in sorghum grain was also analyzed (Figure 5). Based on the analyzes performed, it was found that the content of carotenoids varied in the tested forms of sorghum grain (Figure 5). In the case of white sorghum, lutein dominated, which is responsible for the light color, while its content was five times lower in the case of red sorghum (142.3 mg/kg; 31.6 mg/kg, respectively). The trend was opposite for zeaxanthin, the concentration of which was three times higher in red sorghum grains (83.8 mg/kg) compared to white sorghum grains (38.7 mg/kg). While more β-carotene was found in red sorghum grain (51.2 mg/kg) than in white sorghum grain (36.6 mg/kg). Similar observations were noted in previous studies, with the exception of β-carotene, where no statistically significant differences were found between the tested forms of sorghum [73].

Phytosterols are compounds with a structure similar to cholesterol but are produced only by plants. They play a role in plant cell membranes analogous to the role of cholesterol in animal cell membranes. In addition, phytosterols have an antioxidant effect, which is enhanced by their ability to neutralize free radicals generated in plant cells as a result of the defense reaction to oxidative stress. Understanding the importance of phytosterols is important for both plants and humans because they affect the health of the environment and the organisms that consume plant products. Research on phytosterols helps to better understand their biological functions and potential therapeutic applications. Research on phytosterols is crucial to better understand their biological functions and potential therapeutic applications. The most important phytosterols found in cereal plants include beta-sitosterol, stigmasterol, campesterol, alpha-5-avenasterol and alpha-7-avenasterol. During this study, the presence of three main phytosterols was found: beta-sitosterol,

campesterol and stigmasterol. No significant differences were found in the content of the above-mentioned sterols in the samples of the two tested varieties of sorghum grain (Figure 6). Research carried out so far shows that sorghum is a rich source of phytosterols, synthesized only by plants. Carr et al. [50,86] determined that the total phytosterol content in sorghum grain is approximately 50 mg/100 g. Similar results were obtained by Heupel et al. [87], who identified campesterol, dihydrobrasicasterol, sito-sterol and stigmasterol as the dominant free sterols in sorghum seeds. In other studies, Lee et al. [88] analyzed the content of campesterol, stigmasterol and β-sitosterol in sorghum seeds. They found a higher content of β-sitosterol compared to campesterol and stigmasterol, and an average content of campesterol (75.5 mg/kg) and stigmasterol (96.5 mg/kg). Another study by Singh et al. showed that the content of total phytosterols in sorghum grain ranges from 460 to 510 mg/kg, which is higher than in the case of corn and barley [89,90].

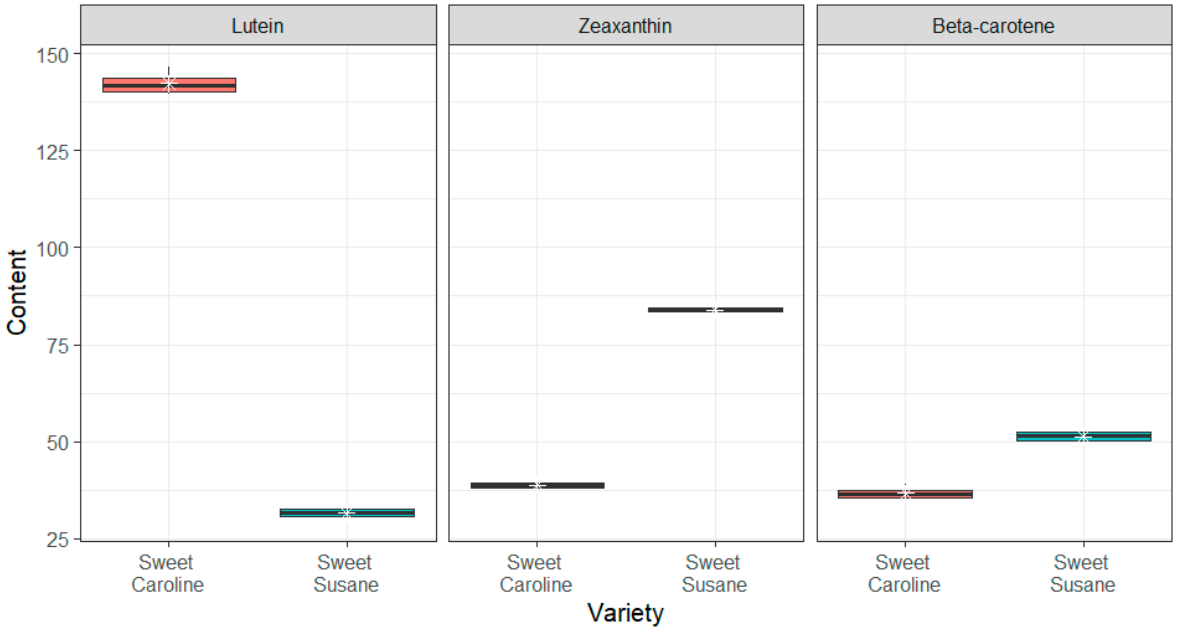


Figure 5. Contents of selected carotenoids in sorghum grain.

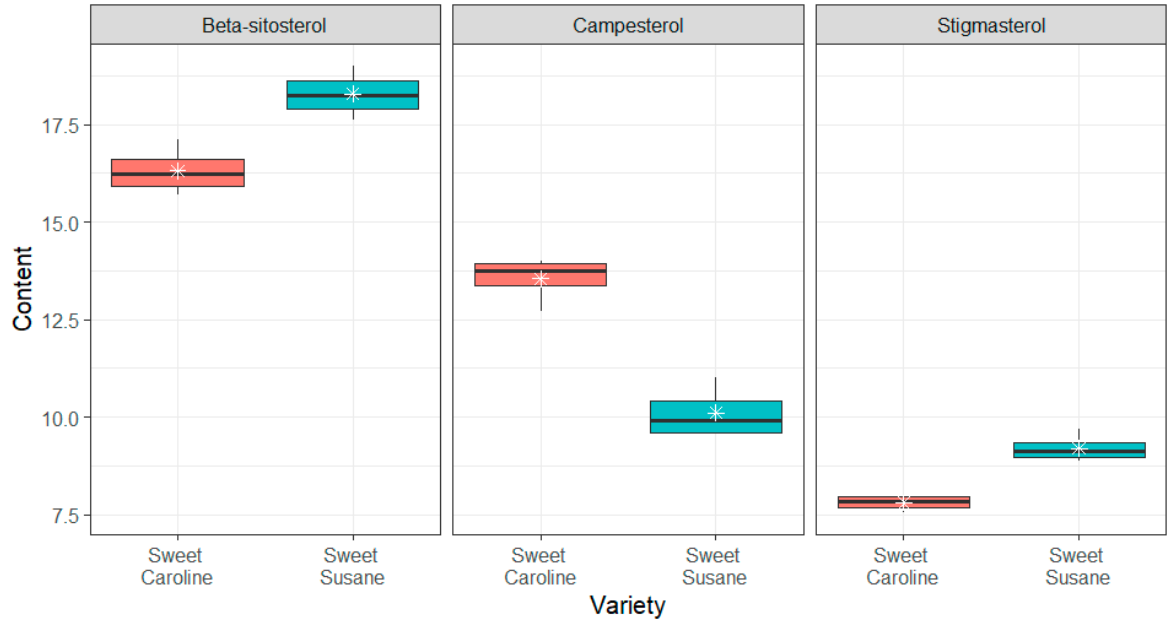


Figure 6. Contents of selected phytosterols in sorghum grain.

These results suggest that sorghum may be used as a valuable source of phytosterols in the human diet, especially compared to other cereals. Phytosterols are known for their beneficial effects on human health, especially by lowering blood cholesterol levels. Using sorghum as a source of phytosterols may provide health benefits, especially for people with cholesterol problems. Additionally, research into the phytosterol content of various food sources is key to understanding how diet can impact our health and how we can improve our diet to support overall health.

3.3. Content of Major and Trace Elements

The presence and concentrations of eight elements were assessed using optical emission spectrometry using the inductively coupled plasma technique (ICP-OES), which allows for the simultaneous determination of a large number of elements present in samples (Table 3). Eight elements were quantified. Comparison of the content of four macro- (Ca, Mg, Na, P) and four micro- and trace elements (Fe, Mn, Zn, Cu) indicates the existence of differences in the total content of minerals between sorghum varieties and significant differences in the amounts of various elements. Namely, the multi-element composition may vary depending on the grain cultivation conditions and the genetic variety. Table 3 shows the content of trace elements (mg $kg^{-1} \pm$ SD) in the tested sorghum grains. Taking into account the results obtained, the most abundant element was P, the concentration of which, depending on the variety, ranged from 425 to 485 mg/100g, which was much higher than the previously reported data [91–93]. The high P concentration in the samples is consistent with the study by Soetan et al. [94], who noted that high P concentrations are important for the structure of carbohydrates and proteins in plants, but also for general metabolic activity, as this element is a component of adenosine triphosphate. Zinc and iron deficiencies are major problems worldwide [95–98]. In this study, the Fe and Zn contents in Sweet Susana sorghum grain are 4.55 mg/100 g and 4.28 mg/100 g, and in Sweet Caroline sorghum grain, they are 4.28 mg/100 g and 4.33 mg/100 g, respectively. Taking into account the obtained results, the amount of these two elements is consistent with the available data [99]. Magnesium plays an important role as a coenzyme in many enzymes and therefore in human metabolism. The Mg content in the tested samples was generally lower compared to the literature on the subject [91]. During the present research, the Mg content in Sweet Susana sorghum grain was found to be 285 mg/100 g, while in Sweet Caroline grain, there was less magnesium, 211 mg/100 g. Microelements, although required in the human body in small amounts, play a significant role in the proper functioning of the body. The presented screening of valuable microelements showed that sorghum grain has the potential to provide a significant concentration of minerals in the human diet.

Table 3. Content of major and trace elements (mg/100 g).

	Content of Major and Trace Elements (mg/100 g)								Content of Fat (g/100 g)	Content of Water (%)
	Ca	**Fe**	**Mn**	**Mg**	**P**	**Cu**	**Na**	**Zn**		
Sweet Susana	19.54 b	4.55 a	2.55 a	225 b	425 a	20.75 a	3.28 a	4.28 a	0.92 a	11.3 a
Sweet Caroline	17.52 a	4.28 a	2.67 a	211 a	485 b	20.49 a	3.41 a	4.33 a	0.91 a	12.3 b

a, b—the same letters in column mean no significant differences $p \geq 0.05$.

Based on the analysis, it was found that the fat content in sorghum grain is approximately 0.9 g/100 g of dry matter, which is less than that described in the literature: 1.4–10.5%. In turn, the water content in sorghum grain was determined to be 11.3% in Sweet Susana and 12.3% in Sweet Caroline, while other researchers found a higher water content of 14–19% [100,101].

3.4. Starch Content Analysis

As with other cereals, starch is the most important component of grain. The dry matter of sorghum grain contains 56–73% starch. During this research, the starch content in

sorghum grain was also analyzed. It was found that the average starch content in Sweet Susana sorghum grain was 27 g/100 g of the sample, while in Sweet Caroline grain, it was 40.7 g/100 g of the sample. The average starch content for the Sweet Susana variety was 27 g/100 g of the sample and was almost 33% lower compared to the Sweet Caroline variety; these values are lower compared to the literature [100,101].

3.5. Statistical Analysis of the Results

In Figure 7, spider (radar) charts for the content of different acids in two varieties, Caroline (red) and Susana (black), are given. Curves represent the mean content of particular phenolic acids in the two considered varieties, Sweet Caroline and Sweet Susana. The points represent the mean values of observations for each acid and variety. The number of vertices of the polygon corresponds to the number of acids analyzed. In the carotenoid group, a higher lutein content was observed in the Sweet Caroline variety, and in the beta-sitosterols group, Sweet Caroline was characterized by a higher campesterol content. For phenolic acids, it is not possible to distinguish clearly for which variety the content of all acids is higher. The situation is different for flavonoids. The content of all flavonoids in the plants of the Sweet Caroline variety is higher than in the other variety analyzed. In the group of phytosterols, the content of campesterol is higher in plants of the Sweet Caroline variety.

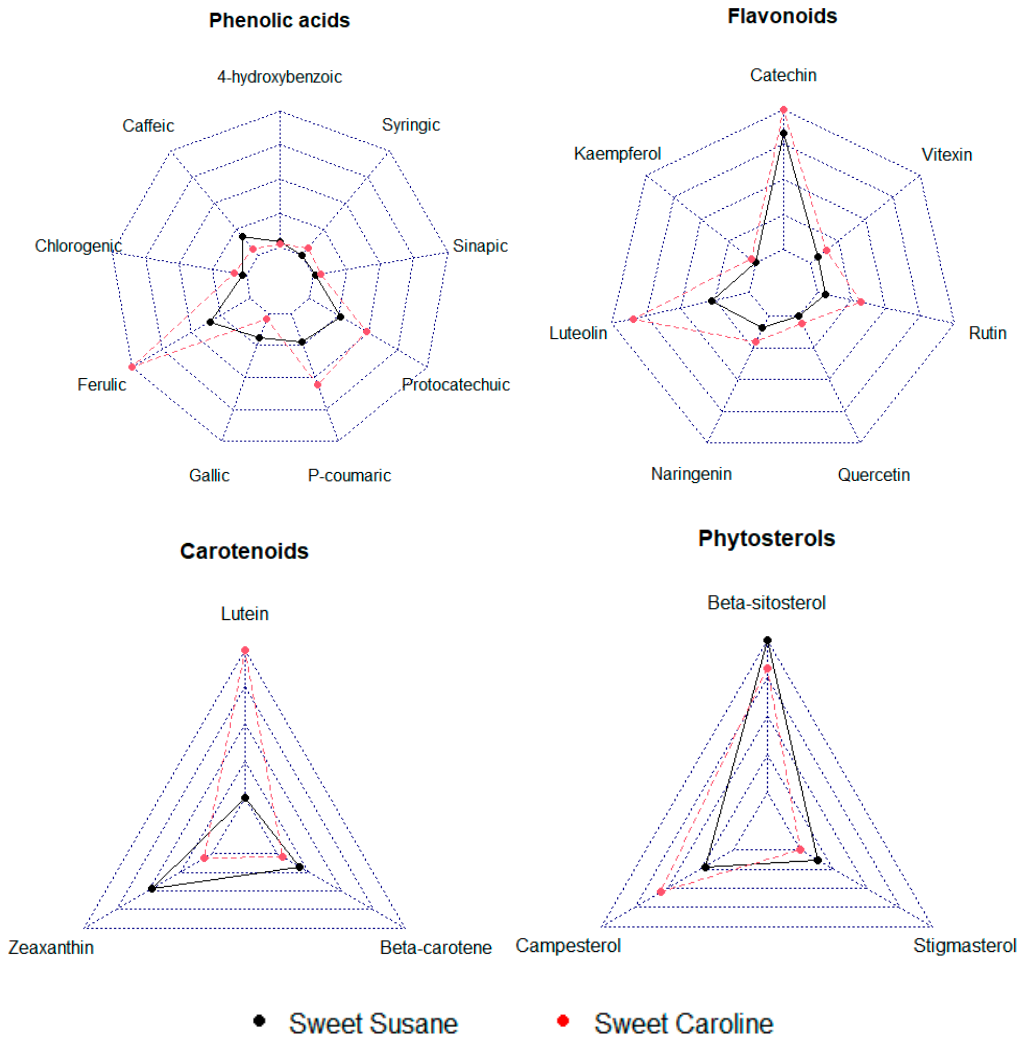
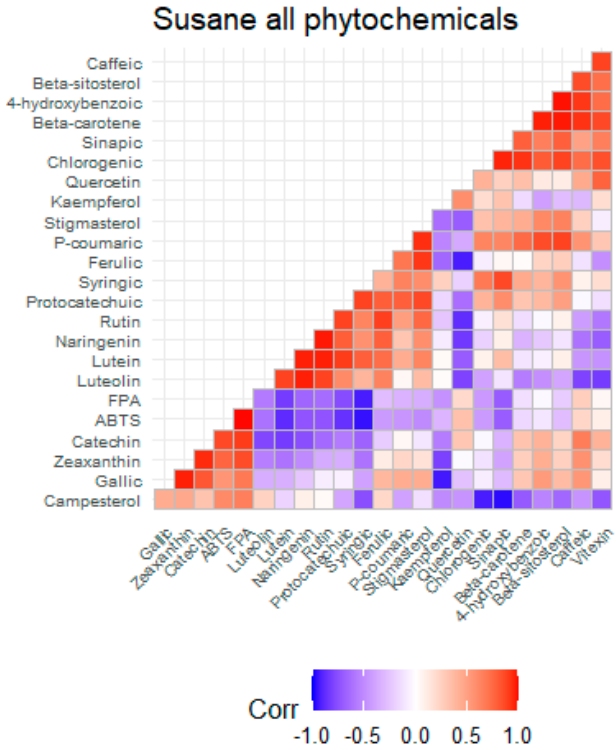
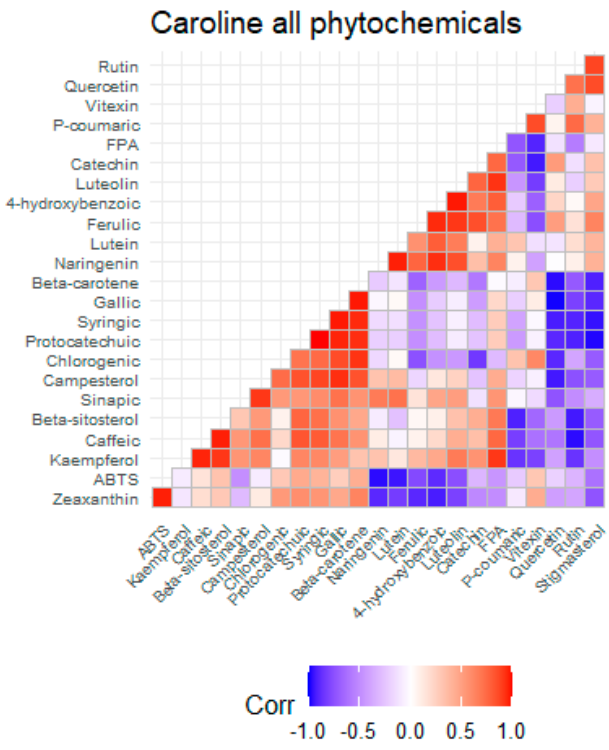


Figure 7. Spider plots of the content of considered acids in the varieties Sweet Caroline and Sweet Susana.

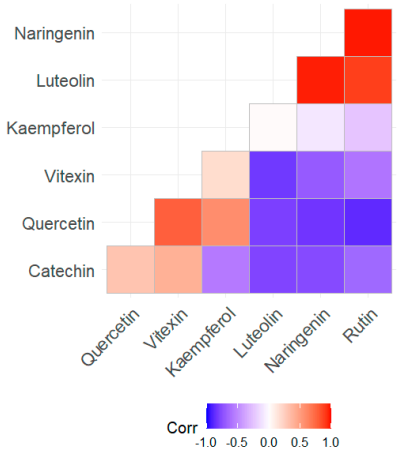
Sweet Caroline is characterized by a significant relationship between ABTS and FPA content. As the content of one of these acids increases, the content of the other increases at the same time. In the case of Sweet Caroline, the opposite trend was noted (Figure 8).



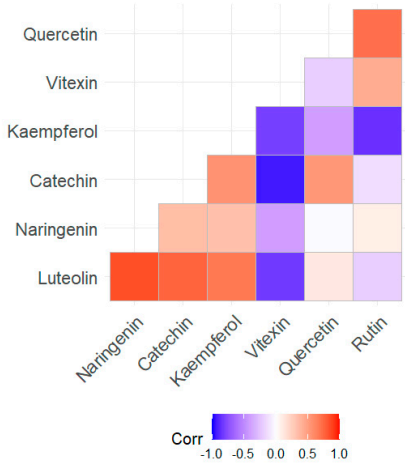
Flavonoids in Sweet Susana



Flavonoids in Sweet Caroline



Phenolic acids in Sweet Susana



Phenolic acids in Sweet Caroline

Figure 8. *Cont.*

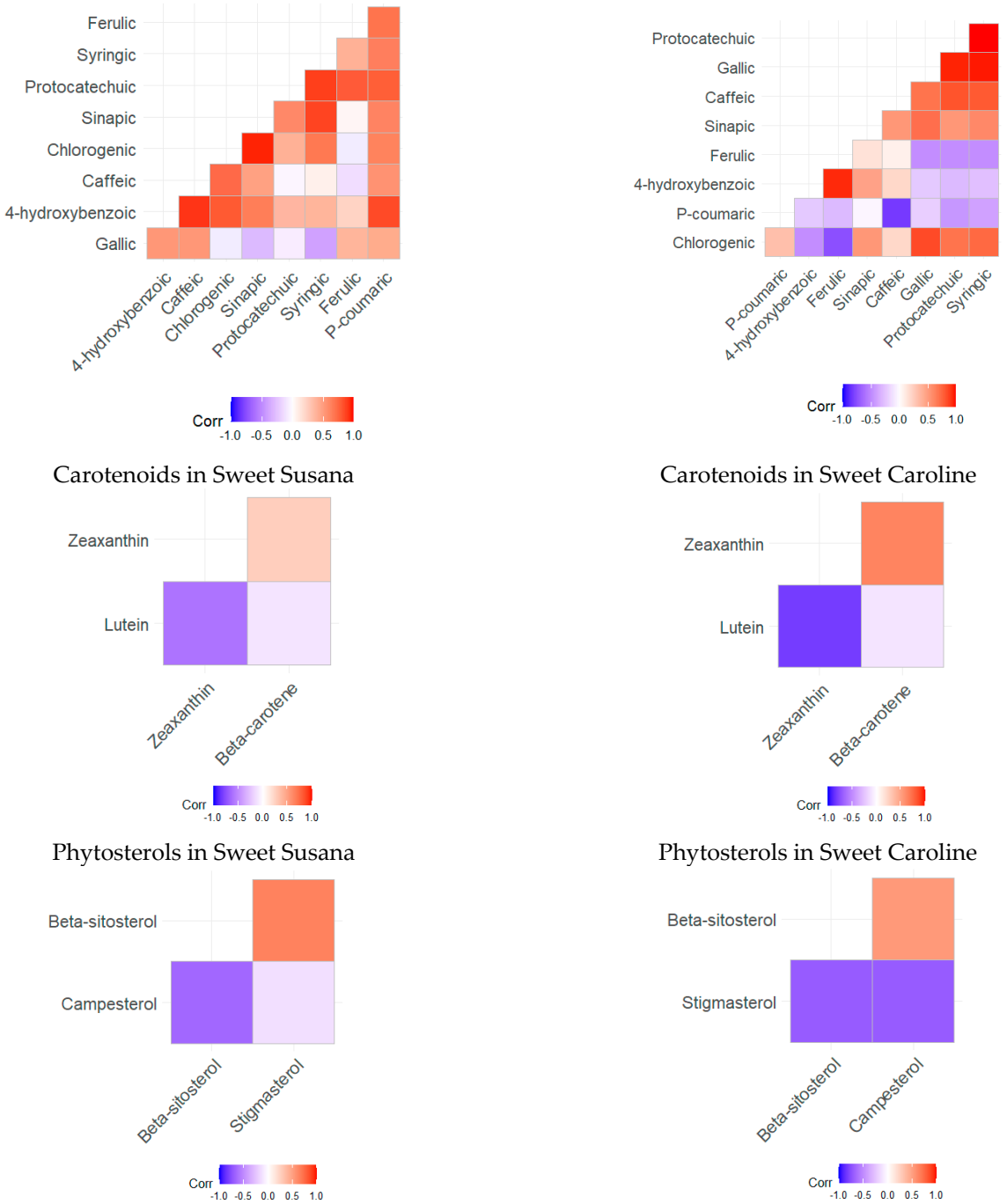


Figure 8. The correlation of particular acids in two varieties: Sweet Susana and Sweet Caroline.

The analysis of correlations between individual bioactive compounds for each variety separately showed that the direction of biosynthesis of individual bioactive compounds is a varietal feature. Growing in the same agrotechnical and weather conditions (subjected to the same abiotic stresses), both varieties responded in different ways. The direction of biosynthesis of individual flavonoids varied. In the case of flavonoids, Sweet Caroline increased the biosynthesis of flavonoids with higher antioxidant potential, while Sweet Susana directed its metabolism towards phenolic acids. The result is significant correlations between individual compounds within the variety.

In the flavonoid group, no significant correlations were observed between their contents for the Sweet Caroline variety. In plants of the Sweet Susana cultivar, the rutin and luteolin contents increased significantly with increasing naringenin content (Figure 8).

Due to the varieties, the correlations between acid contents within the phenols were arranged inversely compared to the flavonoid group. Within the phenolic group, no significant correlations were observed between their contents for the Sweet Susana cultivar. In plants of the Sweet Caroline cultivar, the gallic and syringic contents increased significantly with increasing protocatechuic content (Figure 8).

In the carotenoid and phytosterol groups, no significant correlations were observed between their contents in question for the two varieties (Figure 8).

3.6. Organic Volatile Compounds

In this study, an analysis of the VOCs in sorghum grain was carried out. It was found that, regardless of the variety, each sample contained approximately 180 VOCs. However, those that most characterize sorghum grain are presented in Table 4. A dendrogram was used to present general relationships between volatile substances with a woody structure. A dendrogram is an element of hierarchical clustering, represented directly as a nested list in which each component corresponds to a branch of the tree, and which practitioners use to visualize the distribution of clusters formed by hierarchical clustering. The dendrogram (Figure 9) shows the hierarchical clustering of 182 volatile observations. In this case, the dendrogram shows us that there is a big difference between cluster A and D.

Table 4. Organic volatile compounds (VOC) in sorghum grain.

Sorghum Variety	VOC	TR	IR	Number on Dendrogram (Figure 6) and Clusters	
Sweet Caroline	Nonanoic acid	12.03	1269	36	D
	2,4-Di-tert-butylphenol	15.29	1518	85	A
	trans-Calamenene	15.54	1545	96	C
	Actinidiolide, dihydro-	15.72	1564	101	C
	Spathulenol	16.07	1602	112	B
	Caryophyllene oxide	16.14	1613	113	B
	Humulene-1,2-epoxide	16.34	1644	120	C
	Humulenol-II	16.49	1667	125	D
	Azulene, 1,4-dimethyl-7-(1-methylethyl)-	16.7	1698	132	D
	Oplopanone	17.07	1770	153	D
	Tricyclo [5.4.3.0(1,8)]tetradecan-6-one, 4-ethenyl-3-hydroxy-2,4,7,14-tetramethyl	18.03		179	D
Sweet Susana	2-Pinen-4-one	11.42	1225	30	D
	Nonanoic acid	12.03	1268	36	D
	Copaene	13.31	1360	55	D
	β-Elemen	13.89	1402	70	C
	Cyclobuta [1,2:3,4]dicyclooctene, hexadecahydro	14.12	1421	73	C
	Caryophyllene	14.38	1442	74	D
	Humulene	14.84	1478	76	C
	2,4-Di-tert-butylphenol	15.29	1518	85	A
	Cadine-3,9-diene	15.49	1539	94	D
	Actinidiolide, dihydro-	15.73	1565	101	C

Table 4. *Cont.*

Sorghum Variety	VOC	TR	IR	Number on Dendrogram (Figure 6) and Clusters	
Sweet Susana	Spathulenol	16.08	1603	112	B
	Caryophyllene oxide	16.14	1613	113	B
	Humulene-1,2-epoxide	16.34	1644	120	C
	Azulene, 1,4-dimethyl-7-(1-methylethyl)-	16.7	1698	132	D

TR—time retention; IR—index retention.

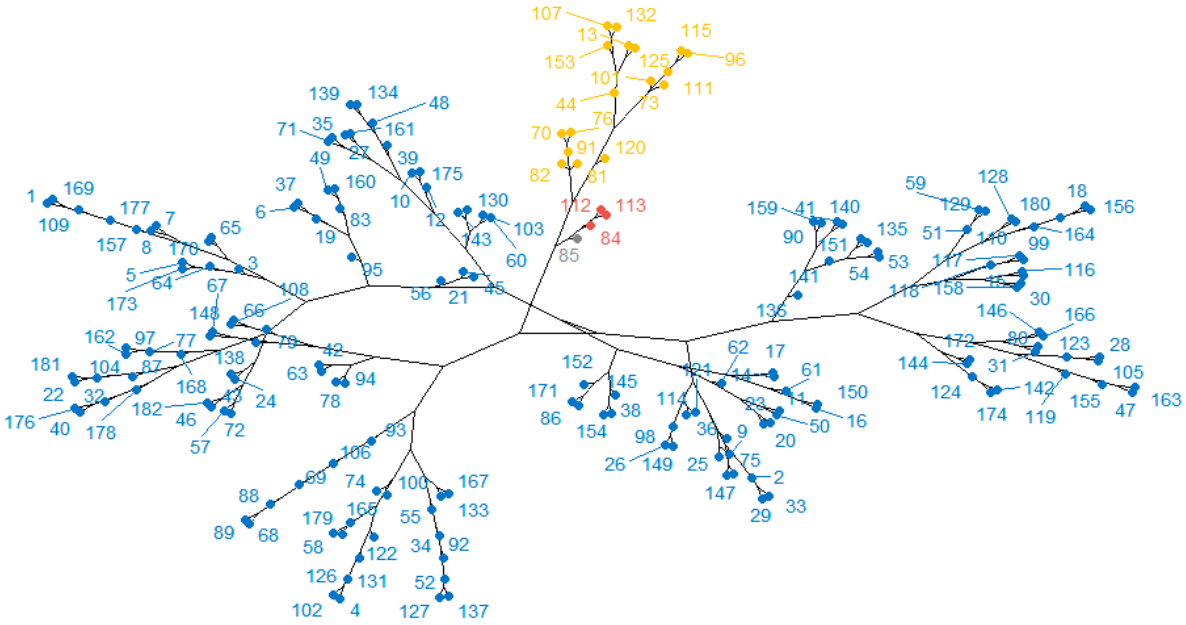


Figure 9. Dendrogram for volatile substances in Sweet Susana and Sweet Caroline.

The study carried out an analysis of VOCs in sorghum grain. It was found that, regardless of the variety, each sample contained approximately 180 VOCs. However, those that most characterize sorghum grain are presented in Table 4. The dendrogram in Figure 9 presents the hierarchical clustering of 180 observations of VOCs in sorghum grain observations (Table 4). The dendrogram is an element of hierarchical clustering, represented directly as a nested list in which each component corresponds to a branch of the tree. Four clusters are indicated on the graph: first cluster "A": 85; second cluster "B": 84, 112, 113; third cluster "C": 70, 73, 81, 82, 91, 96, 101, 111, 115, 120; fourth cluster "D": the remaining. It can be noted that the contents of volatile compounds in clusters A, B, and C versus the content of volatile compounds in cluster D differ greatly. The content of 85 (2,4-Di-tert-butylphenol) differs significantly from the content of other substances. However, the contents of substances in particular groups are more similar, i.e., the contents of volatile compounds 84, 112 and 113 are similar to the contents of volatile compounds in cluster C.

4. Conclusions

Based on the conducted research, it was found that sorghum (Sweet Susana and Sweet Caroline) is a cereal plant that is gaining recognition due to its resistance to drought, low soil requirements and richness in bioactive compounds. The most important limitations in growing sorghum in a temperate climate are the need to grow sorghum varieties that are resistant to relatively low temperatures and temperature fluctuations, as well as a short growing season. Nevertheless, due to climate change and intensive plant breeding, Polish farmers are looking for alternatives to existing cereal crops, introducing sorghum

into cultivation. Its ability to grow in conditions with limited water availability makes it attractive to Polish farmers, especially in drought-affected regions.

Sorghum is rich in bioactive compounds such as phenolic acids (dominant: Sweet Caroline: ferulic and p-coumaric acid; Sweet Susana: ferulic acid and protocatechuic acid), flavonoids (dominant: Sweet Caroline: catechins, luteolin; Sweet Susana: catechins) and carotenoids (dominant: Sweet Caroline: lutein; Sweet Susana: zeaxanthin). Is a valuable source of phytosterols. These substances not only give the plant its antioxidant properties but also make it a valuable component of the human diet. Its grain can be used in the production of functional foods, dietary supplements and natural food dyes. This research and the presented information resulting from the quantitative and qualitative analysis of chemical compounds may contribute to the expansion of the possibilities of using sorghum grain in various industrial fields. This opens the door to innovative solutions that integrate agriculture, industry and environmental protection.

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