



# The influence of weather conditions on bioactive compound content in sorghum grain

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Received: 12 July 2019 / Revised: 14 October 2019 / Accepted: 19 October 2019 / Published online: 5 November 2019  
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## Abstract

Sorghum is the fifth most important cereal in the world in terms of the cropped area. It is mainly grown for feeding animals and it is also used in the food industry. Sorghum grain is generally a rich source of antioxidants such as polyphenols and carotenoids. For this reason, it is considered as a good source of bioactive food components and it has health-promoting properties. Sorghum is a gluten-free cereal grown in many regions worldwide, primarily in the tropical and subtropical regions. However, new hybrids and forms of sorghum are capable to produce seeds also in temperate climate. The aim of this study was to conduct the influence of weather conditions on bioactive compound content in sorghum grain. The quantitative analysis of selected bioactive compounds, such as phenolic acids, flavonoids, carotenoids, and phytosterols, was carried out. The tested material comprised grain of two varieties: ‘Sweet Susana’ and ‘Sweet Caroline’, which have different color of grain: red and white. The research material was obtained from growing seasons 2016–2018. Quantitative analysis of free phenolic acids, total carotenoids, and total phytosterols was performed by ultra-performance liquid chromatography (UPLC) after prior basic hydrolysis followed by acid. An ultra-efficient liquid chromatograph coupled with an absorption-based detector (UPLC-PDA) was used for these analyses. The results showed the variability of the content of bioactive compounds depending on weather conditions.

**Keywords** Sorghum grain · Phenolic compounds · Flavonoids · Carotenoids · Temperate climate

## Introduction

Sorghum (*Sorghum* Moench), which belongs to the family of *Poaceae*, is an annual plant with C4 photosynthesis cycle [1, 2]. It is the fifth most important cereal in the world in terms of the cropped area [3, 4], because it is considered to be one of high nutritional value food crops [5–9]. The varieties of primary economic importance are *Sorghum bicolor* L. and *Sorghum sudanense* L., as well as numerous hybrids [10–12], which are also valuable animal fodder [13–16].

Furthermore, sorghum is used in various branches of industry [17, 18]. For example, its biomass can be useful for producing energy [19–22]. Stalks and leaves have high calorific value [23] and they are efficient solid biofuels after forming in bales [24]. Moreover, stems and grain are a feedstock for bioethanol production [23, 25–28]. In addition, biogas could also be produced from the biomass processing residue [29, 30]. Therefore, every piece of sorghum biomass can be effectively used for various purposes [23].

In Europe, various subspecies of sorghum have been grown sporadically so far, mainly for the production of animal feed or biofuels, due to the lack of adaptation of the varieties to the prevailing climate. However, intensive breeding in recent years has allowed the registration of many different hybrids and sorghum forms, which are capable of producing seeds in a temperate climate [31–33]. Continuous global warming and further development of genetic engineering make sorghum cultivation possible and profitable also in Poland, where sorghum was mainly used by the feed industry. But now, the grain can be produced successfully [23, 34].

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In terms of chemical composition, sorghum is essentially similar to maize and millet [5, 34–40]. The main ingredient of sorghum is starch, sugars, protein, and lipids [23, 34–40]. Among micro and macroelements, the largest share is potassium, phosphorus, magnesium, calcium, and small amounts of iron and other chemical elements, but it also depends on many agrotechnical factors and cultivar [4, 41, 42]. It is also a good source of bioactive nutrients, e.g., vitamins, micro- and macronutrients, as well as non-food ingredients, e.g., phenolic acids, flavonoids, carotenoids, and phytosterols [5, 8, 34], with strong antioxidant properties.

Phenolic acids are aromatic organic compounds classified to the group of polyphenols [43–45]. They can be found in many plant species [45–49]. They are distinguished based on the carbon skeleton structure into two groups: derivatives of benzoic acid and derivatives of cinnamic acid [50, 51]. Moreover, sorghum grain also contains flavonoids. They are natural pigments responsible for proper functioning of the enzymatic system in plant cells. Their structure is based on three rings, within which modifications lead to the formation of various flavonoid compounds, e.g., flavonols, flavanone, and flavones [34, 52–55]. Another group of bioactive compounds found in sorghum grain comprises carotenoids. They may be found in the acyclic, monocyclic, or bicyclic forms. Their color ranging from yellow to red is dependent on the number of double bonds. In addition to the content of a wide range of phenolic compounds, sorghum seeds are also a rich source of phytosterols. We are known about 200 types of sterols marked in plants. Plant sterols are structural and functional analogs of cholesterol synthesized by plants. These compounds are part of plant cell membranes. These are 28- or 29-carbon polycyclic alcohols [56, 57]. Antioxidative properties of bioactive compounds are primarily based on antioxidant activity, which is intensified during the stress factors on the plant. Bioactive compounds such as phenolic acids, flavonoids, carotenoids, and phytosterols in sorghum inhibit the action of free radicals protecting plant cells, particularly chloroplasts against harmful effects of reactive oxygen species (ROS) [58–62].

Due to the presence of potentially valuable chemical components in sorghum grain, it can be successfully used as a substitute for conventional cereals in the production of functional food [34]. Notwithstanding, there is a need to investigate the concentration, composition, and value of its nutrients [12]. Concentrations of bioactive compounds in sorghum grain depend, e.g., on the cultivar, atmospheric conditions and on biotic stressors. Moreover, the beneficial effect of bioactive compounds on physiological functions in plant and consumer depends on their antioxidant action [63–65]. Literature reports on the contents of bioactive compounds in sorghum grain are only fragmentary [34]. In view of the above, studies were conducted on red and white grain

of sorghum grown in Poland to conduct quantitative analyses of selected bioactive compounds.

Therefore, the aim of this study was to conduct the influence of weather conditions on bioactive compound content in sorghum grain. The quantitative analysis of selected bioactive compounds: antioxidant activity applying an improved 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS<sup>•+</sup>) radical cation decolorization, total phenolic acids content, and carotenoids consisted in alkaline and acid hydrolysis, followed by analyses using an ultra-performance liquid chromatograph coupled with a photodiode array detector (UPLC-PDA) were carried out. As a result, the possibility of using sorghum grain for food purposes cultivated in temperate climate was demonstrated.

## Raw material

The research material was the grain of two cultivars of sorghum (*Sweet Susana—red* and *Sweet Caroline—white*) cultivated in 2016, 2017, and 2018. The experimental plots were located at the Sary Sielec Experimental Station in Poland (51° 39' N, 17° 10' E), which belongs to the Institute of Natural Fibers and Medicinal Plants (Instytut Włókien Naturalnych i Roślin Zielarskich). The experiments were carried out in the area with low precipitation (< 550 mm/year), on a soil of medium agricultural usefulness soil and good abundance of nutrients. Every year, the experimental plots were located next to the last position, but not in the same place to avoid the monoculture.

Sorghum was sown after triticale at the following fertilization level (in kg·ha<sup>-1</sup>): 100 N, 60 P<sub>2</sub>O<sub>5</sub>, 120 K<sub>2</sub>O, and 30 MgO. Soil cultivation from autumn to sowing seeds in first days of May was carried out in accordance with the principles of good agricultural practice. Neither before sowing nor during the growing season was any chemical plant protection products used. The weeds were removed mechanically at the initial stage of growth. Sorghum was harvested in the last days of September.

## Testing methods

The weather conditions were measured by collecting the following data: daily participation, the lowest, the highest, and medium daily temperature. The measurements were made in a covered weather station located 2 m above the ground.

Grain samples of 100 g were collected for chemical analyses. Sorghum grain was ground in a WZ-40 laboratory mill.

## Total polyphenolic contents

Samples of 50 g were collected for analyses of polyphenols. Samples were ground using a laboratory mill (WZ-1). Total phenolic compounds were extracted with 80% MeOH.

Samples of 10 g after flooding with 100 ml MeOH were placed in an ultrasound bath for 30 min; next, the precipitate was collected to distillation flasks and the extraction process was repeated three times. Next, the combined extracts were evaporated to dryness in an evaporator. Phenolic compounds were transferred quantitatively to a vial using MeOH and dried in a stream of nitrogen. The amount of 0.5 ml deionized water and 0.125 ml Folin–Ciocalteu reagent (Fluka) was added to 0.125 ml extract, and after 6 min, the mixture was supplemented with 1.25 ml 7% aqueous  $\text{Na}_2\text{CO}_3$  solution and 1 ml deionized water. After 90 min, absorbance will be read at a wavelength of 760 nm in relation to water (Helios spectrophotometer Thermo Electron Corp.). Results were expressed in mg gallic acid/kg d.m. sample [66, 67].

### ABTS<sup>+</sup> method

For ABTS<sup>+</sup> generation from ABTS salt, 3 mM of  $\text{K}_2\text{S}_2\text{O}_8$  was reacted with 8 mM ABTS salt in distilled, deionized water for 16 h at room temperature in the dark. The ABTS<sup>+</sup> solution was then diluted with pH 7.4 phosphate buffer solution containing 150 mM NaCl (PBS) to obtain an initial absorbance of 1.5 at 730 nm. Fresh ABTS<sup>+</sup> solution was prepared for each analysis. Reaction kinetics was determined over a 2 h period with readings every 15 min. Reactions were complete in 30 min. Samples and standards (100  $\mu\text{m}$ ) were reacted with the ABTS<sup>+</sup> solution (2900  $\mu\text{m}$ ) for 30 min. Trolox was used as a standard. Results were expressed in ABTS<sup>+</sup> ( $\mu\text{mol}$ TROLOX/kg) d.m. sample [66, 67].

### Determination of carotenoids

Carotenoid isolation and quantification were performed by Acquity UPLC (Waters, USA) in grain samples by the saponification method. Carotenoids extracts were obtained from ground beans (0.4 mg) which were triturated with a mixture of acetone and petroleum ether (1: 1). Then, after separation of the plant tissue, the acetone and the hydrophilic fraction were removed from the extract by washing with water. As a result, the ether extract was obtained with a mixture of carotenoid pigments. The extract thus prepared was concentrated in a vacuum evaporator at 35 °C until an oily residue was obtained, then digested in 2 ml of methanol (Merck), and subjected to chromatographic analysis. Total carotenoids were determined using Acquity UPLC (Waters, USA) with a Waters Acquity PDA detector (Waters, USA). Chromatographic separation was performed on a Acquity UPLC<sup>®</sup> BEH C18 column (100 mm  $\times$  2.1 mm, particle size 1.7  $\mu\text{m}$ ) (Waters, Ireland). Elution was carried out using solvent A—methanol, B—water, and tert-butyl methyl ether (TBME). A gradient was applied at a flow of 0.4 ml/min. The column and samples were thermostated, the column temperature was 30° C, and the test temperature was 10° C.

During the analysis, the solutions were degassed in a Waters device. The injection volume was 10  $\mu\text{l}$ . The registration was carried out at a wavelength  $\lambda = 445 \text{ nm}$  [67, 68].

### Chemical analysis of phytosterols

Samples containing 100 mg of ground grains were placed into 17-ml culture tubes, suspended in 2 ml of methanol, treated with 0.5 ml of 2 M aqueous sodium hydroxide, and tightly sealed. The culture tubes were then placed within 250-ml plastic bottles, tightly sealed and placed inside a microwave oven (Model AVM 401/1WH, Whirlpool, Sweden) operating at 2450 MHz and 900 W maximum output. Samples were irradiated (370 W) for 20 s and after about 5 min for an additional 20 s. After 15 min, the contents of culture tubes were neutralized with 1 M aqueous hydrochloric acid, 2 ml MeOH were added, and extraction with pentane (3  $\times$  4 ml) was carried out within the culture tubes. The combined pentane extracts were evaporated to dryness in a nitrogen stream. Before analysis, samples were dissolved in 4 ml of MeOH, filtered through 13-mm syringe filters with a 0.5 mm pore diameter (Fluoropore Membrane Filters, Millipore, Ireland), and evaporated to dryness in a  $\text{N}_2$  stream. The sample extract was dissolved in 1 ml of MeOH and 50  $\mu\text{l}$  were analyzed by Acquity H class UPLC system equipped with a Waters Acquity PDA detector (Waters, USA). Chromatographic separation was performed on a Acquity UPLC<sup>®</sup> BEH C18 column (100 mm  $\times$  2.1 mm, particle size 1.7  $\mu\text{m}$ ) (Waters, Ireland) eluted with methanol/acetonitrile/water (85:10:5) at a flow rate of 0.4 ml/min. Total sterols was detected with a Waters Acquity PDA detector (Waters, USA) set at 282 nm [69].

### Statistical analysis

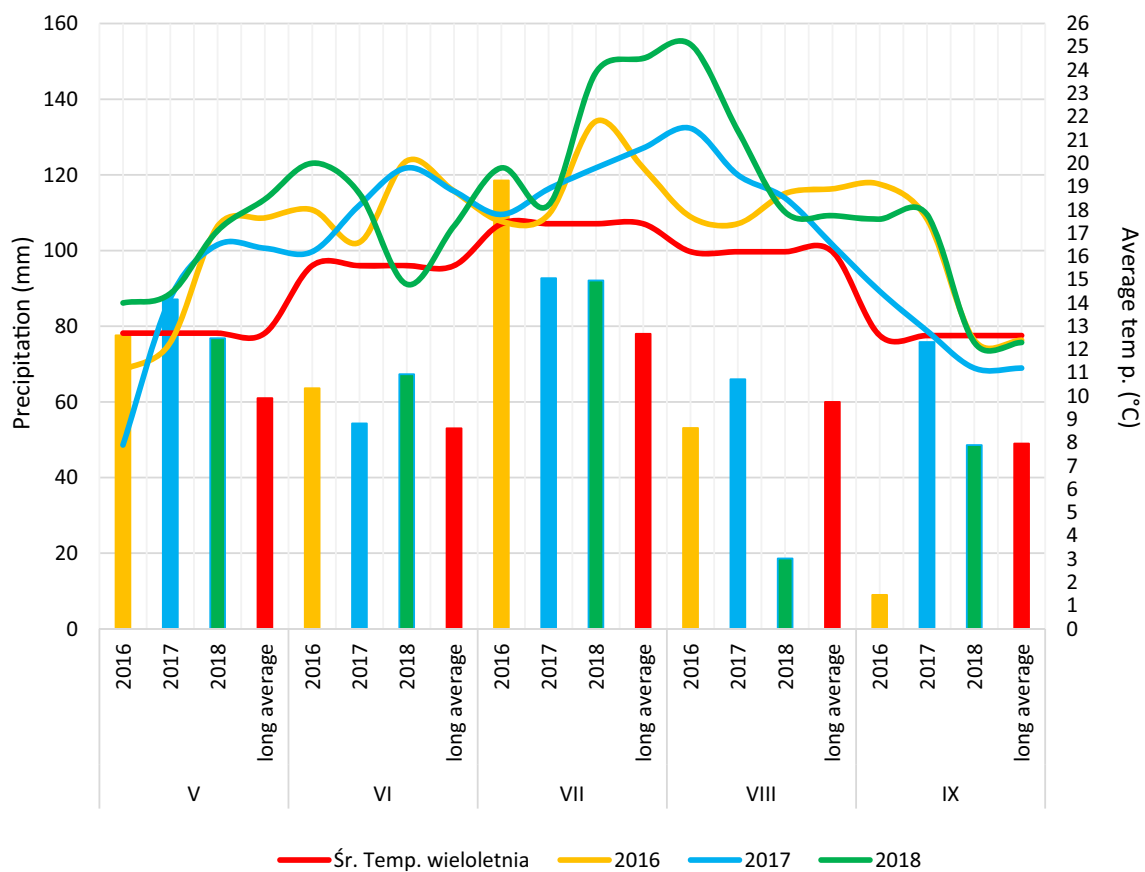
The results of the chemical analyses were analyzed statistically by means of the STATISTICA v 8.0 software. Tukey's multiple comparison procedure was used to compare the contents of individual metabolites in the samples. Identical letters in rows or columns denote the lack of differences at a significance level  $\alpha = 0.05$ .

### Results and discussion

Due to the fact that sorghum was sown in the first days of May and collected in the last days of September (in the period without risk of frost); 5 months were included in the climate data (Table 1 and Fig. 1). Compared to the long-term average temperature (1960–2000) for this region, the monthly temperatures in the spring and summer 2016–2018 were generally higher. Nevertheless, average temperatures within 10 days fluctuated significantly,

**Table 1** Climate data in the decades of the months May–September in the triennium 2016–2018

Month	Decade	2016		2017		2018	
		Average temp. (°C)	Precipitation (mm)	Average temp. (°C)	Precipitation (mm)	Average temp. (°C)	Precipitation (mm)
May	I	11.1	25.7	7.9	19.1	14.0	14.7
	II	12.3	3.2	14.2	0.0	14.4	60.9
	III	17.3	48.7	16.5	15.8	17.1	1.2
June	I	18.0	28.1	16.2	10.4	20.0	13.5
	II	16.6	23.3	18.2	15.1	18.7	3.3
	III	20.1	12.2	19.8	28.8	14.8	50.5
July	I	17.5	15.5	17.8	21.8	19.8	0.0
	II	17.8	101.0	18.9	41.4	18.2	92.0
	III	21.8	2.0	19.8	29.5	23.9	0.1
August	I	17.7	41.3	21.5	29.3	25.1	0.0
	II	17.4	0.8	19.5	36.5	21.4	5.0
	III	18.7	11.0	18.5	0.2	17.9	13.6
September	I	19.1	4.0	14.5	44.3	17.6	3.1
	II	17.6	4.8	12.8	8.8	17.8	8.5
	III	12.4	0.2	11.2	22.7	12.3	37.0



**Fig. 1** The temperature and precipitation during sorghum vegetation period

which is typical of central Poland. The inflow of humid and relatively cool masses of air from the Atlantic Ocean intertwines with the continental wind, which promises sunny and hot weather in the summer. The hottest part of the year in Poland is above all the turn of July and August. During these days, the daytime temperature exceeds 30° C and fluctuates around 20 at dawn. After a few days of heat, the weather changes suddenly and the temperature drops. Rainfall is not evenly distributed and is mainly caused by local storms. From time to time, as in the second decade of July 2016 and 2018, there were several days of intense rainfall. This situation undoubtedly influenced the growth of sorghum, because the puddles lasted for a long time on the surface of the plot. However, Wielkopolska, where the Experimental Station is located, is known as an area with water shortages. In summer, there are periods of drought each year. Weather conditions in the summer season do not have such a significant impact on bioactive compounds in sorghum grains, such as insolation and temperature in September, when the kernels ripen. The harvest time is usually much colder than the plant growth time. The most important thing is to do it before the beginning of even a light night frost, which immediately damages sorghum. On the basis of this study, unstable atmospheric conditions were observed during the growing season of sorghum. This could affect the quality and quantity of crops. Therefore, during these experiments, attention was paid to the influence of stress factors on the yield quality. The determinant of the severity of symptoms of oxidative stress was the concentration of selected bioactive compounds and the measurement of their antioxidant activity. Comparing the data from Table 1 with Table 2, it was found that both the air temperature and the amount of rainfall are important during the vegetation period of the plant. Sorghum is a thermophilic plant; therefore, the vegetation period of this plant in a temperate climate was from May to the end of September. It is important that at this time, optimal conditions for the correct yielding of the plant are met. The present study was conducted in a 3-year cycle, in which significant differences in the intensity of oxidative stress (biotic and abiotic) were noted. In the defense reaction to oxidative stress in plant cells, antioxidant compounds are formed that capture free radicals. The concentration of bioactive compounds was different for the two tested sorghum varieties, in individual years. In 2016, the average air temperature throughout the growing season was even, and the amount of rainfall was associated with sudden downpours. In 2017, the temperature and precipitation were quite stable. Another period is 2018. While high temperatures in the commune were dominated by long-lasting drought and rare rains. Each of the vegetation periods discussed was specific, but the sorghum grew under all experimental conditions. In this research,

**Table 2** Contents of bioactive compounds in sorghum grain [mg/kg]

Chemical compound	Vegetation period	Sorghum grain	
		Sweet Susana—red	Sweet Caroline—white
ABTS <sup>+</sup> ( $\mu$ molTROLOX/kg)	2016	789a	921b
	2017	659a	893b
	2018	703a	916b
TPC (mg GAE/100 g)	2016	549a	822b
	2017	496a	913b
	2018	548a	879b
Carotenoids (mg/kg)	2016	124a	428b
	2017	116a	571b
	2018	132a	499a
Phytosterols (mg/kg)	2016	26a	29a
	2017	27a	30a
	2018	23a	41a

ABTS<sup>+</sup>( $\mu$ molTROLOX/kg): antioxidant activity applying an improved 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; TPC (mg GAE/100 g): total phenolic compounds

a, b: These same letters in rows mean no significant statistical differences,  $p \leq 0.05$

temperature and rainfall were not connected with level of bioactive compound in sorghum. This is new information about these sorghum varieties grown in Poland.

As part of the present study, 18 samples of white sorghum grains (Sweet Susana) and 18 samples of white sorghum grain (Sweet Caroline) were analyzed. Based on the obtained results, the presence of selected bioactive compounds was found in all samples and significant differences in the content of these compounds were found. The amount and type of antioxidants in plants depends on the species, variety, place of growth, insolation, and the period in which the crops were harvested [3, 21]. The plants rich in bioactive compounds include fruits, vegetables, herbs, grain, as well as sorghum [70]. As part of this study, the results of free phenolic acid content, total carotenoid concentration as well as antioxidant activity were obtained. Based on the results presented in Table 2, it was found that the antioxidant activity of ABTS<sup>+</sup> bioactive compounds was a higher sample of sweet Caroline compared to sweet Susana. The highest antioxidant activity was found in Sweet Caroline seeds in 2016 and 2018, but there were not significant statistical differences between years. ABTS<sup>+</sup>'s antioxidant activity was lower by 15 and 23%, respectively, in Sweet Susana. Other studies were conducted by Xiong et al. [71]; they tested the activity of ABTS<sup>+</sup> and DPPH extracts and raw sorghum grains. Studies have shown that the content of total phenolic acids and other polyphenols was significantly higher in extracts than in raw sorghum. However, the antioxidant activity has not changed, suggesting that the concentration of



phenolic compounds in white sorghum may affect the level of antioxidant activity. In contrast, in colored sorghum, other compounds may contribute to higher antioxidant activity. It is also worth mentioning that the antioxidant activity of ABTS<sup>+</sup> bioactive compounds in sorghum is higher compared to ABTS<sup>+</sup> grains of wheat and triticale cultivated in temperate climates [72].

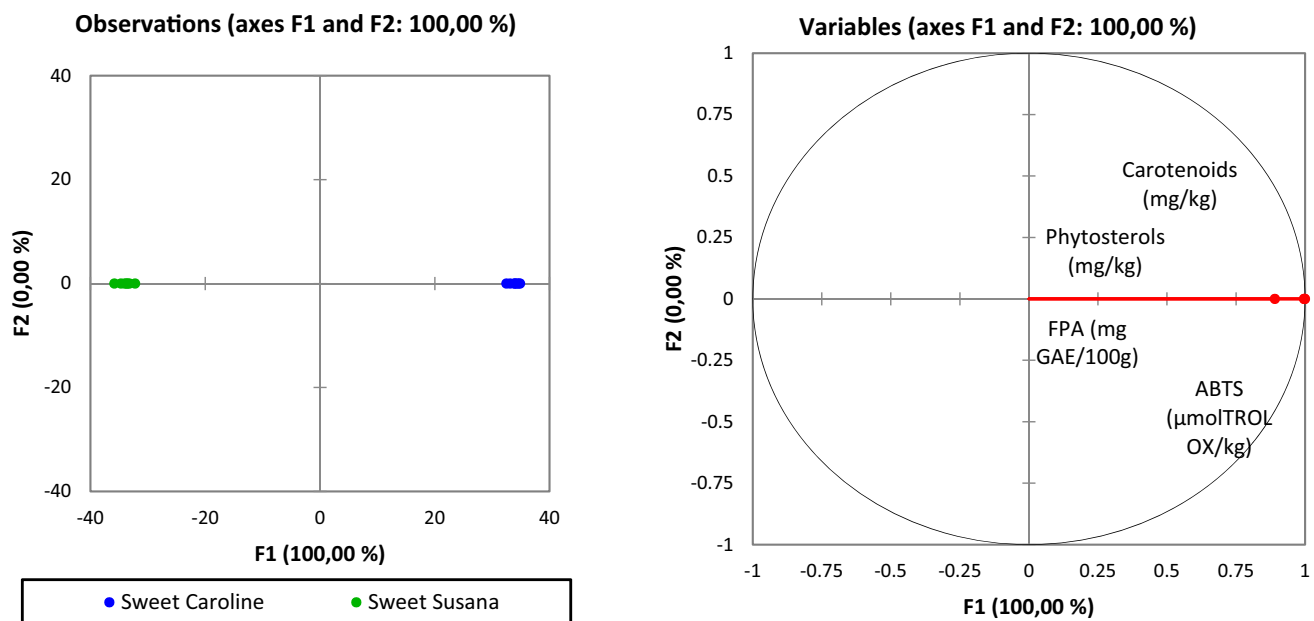
In this work, the concentration of TPC in two types of sorghum was also determined. Phenolic acids are responsible for preventing reactions of oxidation, cell degradation, and plant death. The production of phenolic acids depends on the intensity of oxidative stress caused by biotic and abiotic factors. Based on the experiments carried out under the same atmospheric conditions, a higher concentration of TPC in Sweet Caroline was found compared to Sweet Susana in each crop year. The average TPC concentrations varied, respectively, in 2016 by 33%, in 2017, by 45%, and in 2018 by 37%. In addition to TPC, other bioactive compounds, such as carotenoids, are also found in sorghum grains. Carotenoids perform very important functions in the plant world. They are responsible for the stability of lipid membranes; they participate in the accumulation of light in the process of photosynthesis, as well as protection against the process of photooxidation induced by reactive oxygen species formed during the chlorophyll excitation in the process of photosynthesis. The antioxidant activity of carotenoids on lipid membranes depends on their orientation, location, and organization in membranes. The antiradical activity of carotenoids is based on two mechanisms, such as: the first involves electron transfer and the second on the formation

of adducts with peroxide radicals. During this study, it was found that Sweet Caroline is a richer source of carotenoids than Sweet Susana. At Sweet Caroline in 2016, 71% more carotenoids were determined compared to the second tested variety. In 2017, the difference was 80%, and in 2018, it was 73%.

Statistical analyses show that there was no correlation between years. John et al., based on their studies, noted a higher concentration of carotenoids, especially in brown beans. They also found that the main bioactive compounds in sorghum beans are phenolic acids, flavonoids, and tannins [73, 74]. Similar studies were conducted by Przybylska-Balcerek et al. in 2019 [68], and they found the presence of many phenolic acids, flavonoids, and carotenoids. The presence of bioactive compounds, including polyphenols and their antioxidant activity, was also touched by Morais Cardoso et al., Dykes and Rooney, as well as Stefoska-Needham et al., the assertion that polyphenols are present in sorghum grain [8, 74, 75].

### Discriminant analysis

The discriminant analysis was used to identify among the analyzed bioactive compounds, those which content characterizes sorghum red and white (Figs. 2, 3, 4). On this basis, it was found all identified bioactive compounds are characterized by the highest discriminatory power for the entire population of analyzed samples. In the case of sorghum Sweet Caroline, it was found more concentration analyzed compounds.



**Fig. 2** Principal component analysis (PCA) and discriminant analysis (DA) of various types of sorghum-bioactive compounds—2016

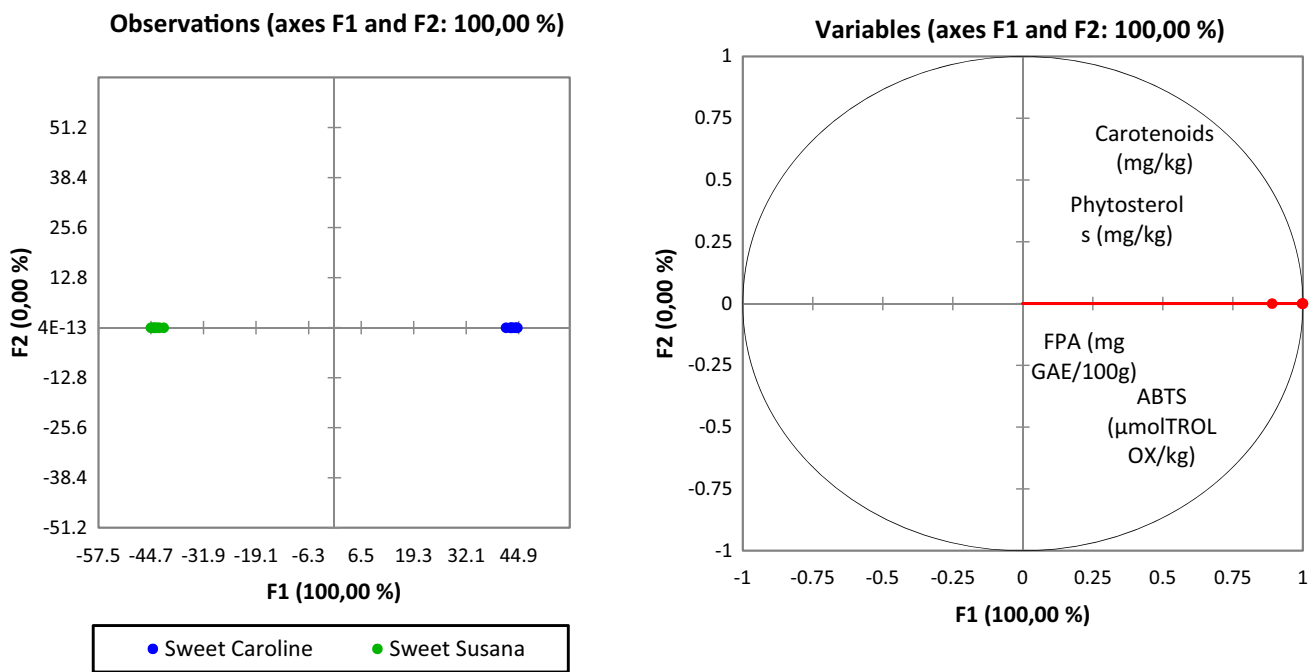


Fig. 3 Principal component analysis (PCA) and discriminant analysis (DA) of various types of sorghum-bioactive compounds—2017

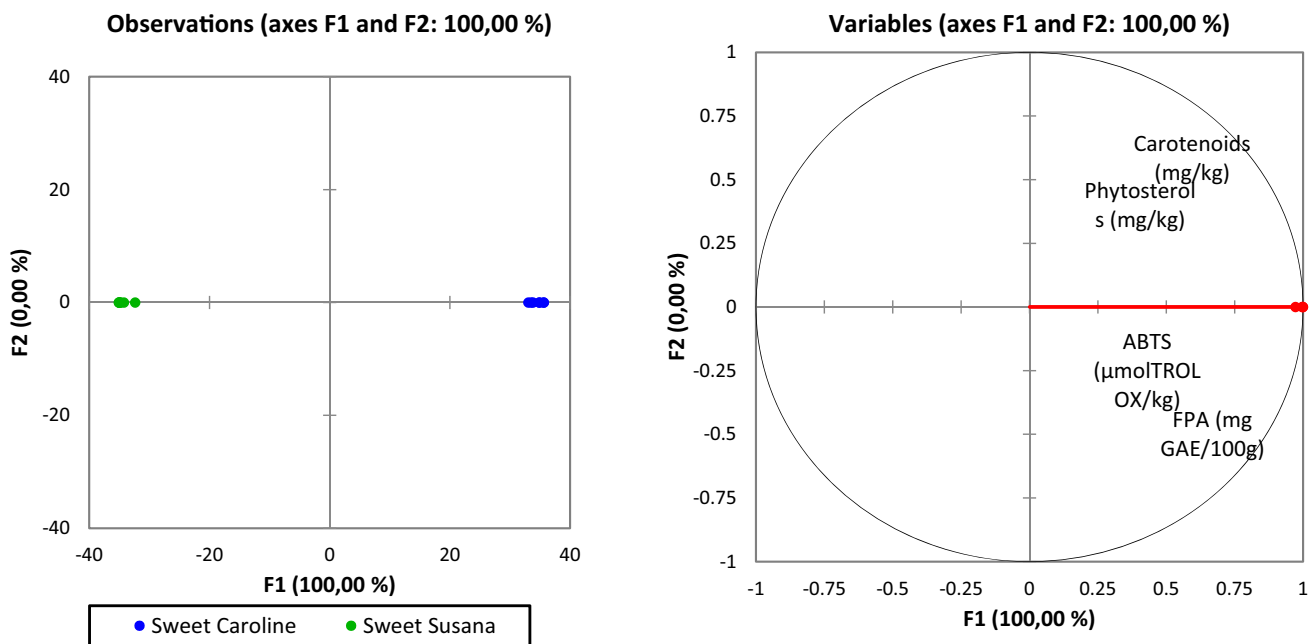


Fig. 4 Principal component analysis (PCA) and discriminant analysis (DA) of various types of sorghum-bioactive compounds—2018

Based on the results of chemical analyses and their statistical analysis, it was stated that contents of bioactive compounds are a factor differentiating individual forms sorghum.

Our research shows that Sweet Carolina sorghum is a more rich source of bioactive compounds compared to Sweet Susana. It was a surprise that in the Sweet Carioline variety,

the level of carotenoids responsible for the yellow–brown color of the grain was much higher than in the brown Sweet Susane variety. Similar studies have been carried out by Choi et al. who, during their research, also found the presence of bioactive compounds in sorghum and divided them into five groups depending on the color of the grain [76]. Choi et al.

found that the color of the grain was associated with the content of TPC, but not with the content of anthocyanins. In addition, the antioxidant activity in the ABTS test was similar to the TPC content. On the basis of the tests, it was found that the amount of antioxidants differs from sorghum. The current literature on sorghum is quite extensive. There are many scientific reports on the cultivation of sorghum in the equatorial and tropical climate zone. In South America and in Africa, sorghum is widely used for food, feed, and energy. Our experiments prove that in conditions of a moderate climate, it is also possible to grow sorghum. Based on the results obtained, it was found that during 3 years of sorghum cultivation in Poland, the results are reproducible. Antioxidant activity and the presence of bioactive compounds are characteristic and stable for a given variety.

## Concluding remarks

The study showed confirmed that the content of bioactive compounds in sorghum grain depends on the variety and fluctuate naturally as weather conditions change during the vegetation period. It was also investigated that white sorghum grain of *Sweet Caroline* is a richer source of phenolic acids and carotenoids. The color of the thin seed coat of caryopsis does not reflect the wealth of carotenoid compounds contained within. Moreover, the analysis of antioxidant activity applying an improved ABTS radical cation decolorization showed that for this variety, the capacity is also higher. Notwithstanding the foregoing, the content of bioactive compounds was confirmed in both tested sorghum varieties. Therefore, they may be a good source of grain considered as a functional food, since it combines nutritive and medicinal properties. On the basis of these results, the content of bioactive compounds was confirmed in sorghum grain cultivated in temperate climate.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Compliance with ethics requirement** This article does not contain any studies with human or animal subjects.

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