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Bioethanol Production Efficiency from Sorghum Waste Biomass

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Abstract: The problem of global warming is still a major issue, alongside shrinking oil reserves. A great alternative to fossil fuels is offered by biofuels, such as bioethanol from lignocellulosic plants. The sorghum biomass can be effectively used in many industrial directions. It is possible to use every part of this plant; the grain can be used for food production and straw can be used for energy purposes, i.e., for bioethanol. The aim of this study was to analyze the possibilities of bioethanol production from five varieties of sorghum biomass, which is a waste product of seed harvesting. The yields of sorghum cultivars in a three-year vegetation period; the amount of cellulose, hemicellulose, and lignin in the biomass of sorghum; and the amount of ethanol obtained per hectare were evaluated. It was observed that the highest average yield for all cultivars, except GK Emese, was found in the second year of the study. The bioethanol yield per hectare from this biomass was the highest for Sweet Caroline and was 9.48 $m^3 \cdot ha^{-1}$. In addition, significant differences were found in the content of lignin and hemicellulose for the varieties tested in all years of the study and for the content of cellulose in the first and third years. The discussed results were confirmed by detailed statistical analyses, including combined matrices of Pearson correlation coefficients (cr_p) varieties and cluster analysis. In summary, the usefulness of the biomass of the studied sorghum varieties for the production of bioethanol was demonstrated.

Keywords: sorghum grain; lignocellulosic biomass; waste management; bioethanol; ethanol yield

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1. Introduction

Greenhouse gas emissions from fossil fuel combustion, one of the causes of global warming, are driving intensive research into finding an alternative and environmentally friendly biofuel. Currently, the most promising biofuel is bioethanol, which, because of its properties, can replace gasoline. According to the RED II Directive of the European Union, the share of advanced biofuels and biogas produced by lignocellulosic raw materials and other materials in the final energy consumption in the transport sector should be at least 0.2, 1, and 3.5% in 2022, 2025, and 2030, respectively [1–3]. Biofuels from plant biomass are certainly a good solution for the production of biocomponents for transport biofuels.

Sorghum (*Sorghum* Moench) belongs to *Poaceae* family. It is an annual plant with a C4 photosynthesis cycle and is one of the main cereal crops worldwide in terms of crop area [4,5]. *Sorghum bicolor* L. and Sudan grass (*Sorghum sudanense* L.), as well as numerous hybrids, are mostly grown for food purposes [6–8]. Sorghum grain is considered a high-nutritional-value crop and a rich source of bioactive compounds [9–12]. Moreover, it is useful in various branches of industry [13–15]. Sorghum biomass is also a valuable feedstock to produce solid, liquid, and gas biofuels [16–19], and each part of harvested sorghum biomass may be effectively processed into bioenergy.

The share of agricultural lignocellulosic raw materials as renewable energy sources in Poland shows a growing trend. This creates an opportunity for development in rural areas and contributes to the economic and social recovery in these areas. However, the effective technology of converting lignocellulosic raw materials into biofuels is still a problem. The methods of obtaining bioethanol from lignocellulosic biomass should contribute to the management of fallow land and the reduction of greenhouse gas emissions, as well as provide effective technological solutions and, in the future, improve fuel price stability [20].

Plant biomass contains lignocellulose, which is relatively resistant to biodegradation; it is found in the cell walls of plants and consists of cellulose, hemicellulose, and lignin. Cellulose and hemicellulose are potential substrates for effective use in fermentation processes; however, the aromatic nature and complex structure of lignin adversely affects the hydrolysis of lignocellulosic biomass. This requires the pretreatment of the biomass in order to break up the solid phase and loosen the compact structure of the lignocellulose. The next stage of bioethanol production is enzymatic hydrolysis, in which simple sugars are obtained that are metabolized by yeast in the process of ethanol fermentation. Three types of cellulases are required to break down cellulose into simple sugars: endoglucanases, cellobiohydrolases, and β-glucosidases. Enzymatic hydrolysis can be carried out separately as an independent SHF (Separate Hydrolysis and Fermentation) process preceding fermentation or simultaneously with the fermentation of the so-called SSF (Simultaneous Saccharification and Fermentation) process. In the process of combined hydrolysis and fermentation, in which the enzymes release low-molecular-weight saccharides, which are then metabolized by microorganisms, conditions should be used that allow the joint action of enzymes and microorganisms, especially a temperature in the range of 30–40 °C. However, the SSF process, which combines cellulose hydrolysis with sugar fermentation in one bioreactor, turns out to be more effective [21–23].

The first step in the effective process of converting sorghum biomass to bioethanol is to prepare the biomass by applying physicochemical pretreatment. The physical methods of crushing and grinding help reduce the size of the substrate and reduce the degree of polymerization and crystallization of lignocellulose. By contrast, chemical processes include dilute acid treatment, alkaline, and neutral. Different changes take place in the lignocellulose complex depending on the method used. One of the most effective alkaline treatments mainly consists of delignification and partial degradation of the hemicellulose. The next step is enzymatic hydrolysis with the use of enzyme preparations, in which reducing sugars are released. The final stage is ethanol fermentation, in which simple sugars are converted into ethanol by distillery yeast, mainly *S. cerevisiae*, *S. uvarum*, *Schizosaccharomyces pombe*, *Kluyveromyces marxianus*, and *Candida utilis*. The most common microbe used for industrial ethanol production, owing to its long-time use by the food industry, is *Saccharomyces cerevisiae* [24–27].

In areas with temperate climate conditions, e.g., Central Europe, various new subspecies and forms of sorghum can be successfully cultivated because of their adaptation to the prevailing climate [15,16,28]. So far, no literature has been published on the possibility of using deseeded waste sorghum biomass cultivated in temperate conditions as a raw material in the process of obtaining bioethanol. Therefore, the aim of this paper was to study the process of obtaining bioethanol from sorghum biomass, which is a waste product of seed harvesting. The obtained results were also determined by statistical and cluster analysis.

2. Materials and Methods

2.1. Sorghum Biomass

Five varieties of sorghum cultivars, ASV KS 61B (ASV), Farmsugro 180 (FAR), GK Emese (GK), Sweet Caroline (SC), and Sweet Susana (SS) (AgriSem GmbH, Einbeck, Germany), were the feedstock used in the study. The field research was conducted on the Stary Sielec Experimental Farm (51°39′ N, 17°10′ E), which is subordinated to the Institute of Natural Fibers and Medicinal Plants—National Research Institute (Poland). The experiments were carried out in a three-year vegetation period. Each year, the experimental plots were located in a different position to avoid monoculture. The size of one plot was 30 m^2, and each variety was grown in 3 repetitions.

Sorghum seeds (10 $kg \cdot ha^{-1}$) were sown in mid-May, with the following amounts of fertilization applied before sowing (in $kg \cdot ha^{-1}$): 120 N, 100 K_2O, 60 P_2O_5, and 30 MgO. Sorghum was harvested at the end of September. During the biomass harvesting, whole sorghum biomass was collected and weighed from each plot. The dry matter content was also tested. Finally, a reference amount of straw (2 kg) was taken as mixed samples from each variety to determine the bioethanol production efficiency.

2.2. Bioethanol Production from Sorghum Biomass

2.2.1. Pretreatment

The comminuted sorghum biomass (20–40 mm) was dried for 24 h at a temperature of 50–55 °C. The material was then ground on a knife mill (Retsch SM-200, Hann, Germany) with 2 mm mesh screens. In the next stage, the chemical treatment of sorghum biomass was carried out with 1.5% sodium hydroxide at 90 °C for 5 h. The process was stopped by the filtration of the biomass suspension under reduced pressure, and the filtered biomass was washed with distilled water until it was neutral. The obtained filtrate was used as a substrate in the hydrolysis and fermentation process.

2.2.2. Simultaneous Saccharification and Fermentation (SSF)

The SSF process was carried out in 250 mL flasks, and the total volume of the prepared sorghum biomass hydrolyzate was 100 mL. The prepared hydrolyzate was subjected to pH adjustment to the desired value (pH 4.8) with the application of sulfuric acid (10%) and sodium hydroxide (10%). Then, the enzyme Flashzyme Plus 200 (AB Enzymes, Darmstadt, Germany) was added at an amount of 30 $FPU \cdot g^{-1}$. After the above-mentioned components were thoroughly mixed, non-hydrated lyophilized yeast (commercial strain Ethanol Red) was added to the hydrolyzate at a dose of 0.5 $g \cdot L^{-1}$, which corresponded to the concentration of cells after inoculation of about 1×10^7 $cfu \cdot mL^{-1}$. The flasks (plugged with stoppers with fermentation tubes) were incubated at 37 °C on a shaker (200 rpm). The process was carried out without pH adjustment for 72 h. All tests were performed in triplicate.

2.3. Analytical Methods

The content of the following chemical components in sorghum waste biomass was evaluated according to several standards: cellulose (TAPPI T17 m-55), hemicellulose (holocellulose TAPPI T9 m-54—cellulose), and lignin (TAPPI T13 m-54) [29–31].

The determination of the ethanol concentration was performed using an Elite LaChrom liquid chromatograph (HPLC, Agilent Technologies 1200, Santa Clara, CA, USA) using an RI L-2490 detector and a Rezex ROA 300 × 7.80 mm column from Phenomenex (Aschaffenburg, Germany), at a flow rate of 0.6 mL/min at 40 °C. The samples were loaded onto the column in an amount of 10 μL. The quantitative identification was made by the external standard method using the peak area (measurement and computer integration using the Ez-Chrom Elite program).

2.4. Calculations

According to Equation (1), the ethanol yield was calculated from 100 g of raw material Ys (g/100 g of raw material) [32]:

$$Ys = (Et \times 100)/M \tag{1}$$

where Et is the amount of ethanol in 1000 mL of the tested sample (g), and M is the mass of material weighed in 1000 mL of the fermentation sample (g).

Then, the amount of ethanol in L per ton of dry matter of straw ($L \cdot Mg^{-1}$) and the ethanol yield per hectare ($m^3 \cdot ha^{-1}$) were calculated.

2.5. Statistical Analysis

The diverse structure of the chemical composition and the yield of the sorghum cultivars over the years requires exploratory techniques for the analysis of multivariate data.

In order to group the varieties, taking into account the mean values of the observed variables, a cluster analysis was performed using Ward hierarchical clustering and the Euclidean distance. The number of clusters was determined in accordance with the Pseudo T-Squared grouping criterion. The analysis of the similarity between the observed variables was carried out in a similar way.

In order to compare the dry matter yield, ethanol yield, and chemical composition of the five sorghum varieties, the experiments were carried out with a completely randomized design with four independent repetitions. To test the normality, the Shapiro–Wilk W-test was used. If the hypothesis of normality was not rejected, analysis of variance (ANOVA) was performed, and Tukey's post hoc test was used. Otherwise, the nonparametric Kruskal–Wallis (K-W) rank-sum test and multiple comparisons of mean ranks were calculated [16].

To determine the relationships between the observed variables for each variety, Pearson's correlation coefficients were measured for the linear association. Moreover, Spearman's rank correlations were determined for the monotonic association. Furthermore, the strength of the correlation was described using a scale suggested by Evans [16,33].

3. Results and Discussion

3.1. Sorghum Biomass Yield

3.1.1. Straw Dry Matter Yield

The statistical analysis of the dry matter yield of sorghum revealed significant differences between the average dry matter yields of straw in all three years of the research (Table 1).

Table 1. Statistical analysis of sorghum straw dry matter yield ($Mg \cdot ha^{-1}$).

	Straw Dry Matter Yield		
	Year I	**Year II**	**Year III**
	General Analysis—Results		
Type of Analysis	ANOVA	ANOVA	ANOVA
df	4;10	4;10	4;10
F/χ^2	31.18	11.01	40.75
p-value	1.27×10^{-5} ***	0.00107 **	3.68×10^{-6} ***

Table 1. *Cont.*

	Straw Dry Matter Yield					
	Year I		**Year II**		**Year III**	
Mean Values for Treatments/Results of Post Hoc Tests α = 0.05						
General mean	22.24		23.71		16.19	
ASV-KS-61B	21.52	bc	25.15	a	15.25	b
Farmsugro 180	20.34	c	23.53	a	17.99	a
GK Emese	22.08	b	21.09	b	15.57	b
Sweet Caroline	23.32	a	24.30	a	14.74	b
Sweet Susana	23.94	a	24.47	a	17.41	a

*** for a significance level of 0.001; ** for a significance level of 0.01; * for a significance level of 0.05; . for a significance level of 0.1; ^{abc} means followed by a common letter are not significantly different at the 5% level of significance.

The highest average yield for all cultivars except GK was found in the second year of the study, and the lowest was found in the third year of the study. For the GK variety, the highest average yield was obtained in the first year of the research (22.08 Mg·ha^{-1}). It differed significantly from the average yield of the cultivars with the highest average yield, SS (23.94 Mg·ha^{-1}) and SC (23.32 Mg·ha^{-1}), and the cultivar with the worst yield in the first year of research, i.e., FAR (20.34 Mg·ha^{-1}). In the second year of the research, the lowest yield was found for the GK variety, with an average dry matter yield at the level of 21.09 Mg·ha^{-1}; this was significantly lower than the average yields of the other cultivars, of which the ASV cultivar yielded the highest amount of dry matter (25.15 Mg·ha^{-1}). In the second year of the investigation, SC and SS yielded at a similar level above 24 Mg·ha^{-1}, as opposed to the last year of the study, when the SS variety (17.41 Mg·ha^{-1}) gave an average yield significantly higher than that of the SC variety (14.74 Mg·ha^{-1}), the worst yielding that year. In the last year, the highest average yield was found for the FAR variety (17.99 Mg·ha^{-1}), and this was significantly higher compared with the yields of other varieties, except for the aforementioned SS.

Most sorghum yield research has been conducted in subtropical and tropical climates, where the regional comparison of factors that affect global sorghum production as well as its drought tolerance were checked [34–36]. In temperate climates, the cultivation conditions are different. So far, selected sorghum varieties have been cultivated as main and second crops. The obtained general mean for three varieties ranged from 20.15 to 35.60 Mg·ha^{-1} for the main crop and from 9.43 to 31.20 Mg·ha^{-1} for the second crop [16]. Nevertheless, the obtained results were harvested as the only crop. In the case described in this article, grain is a raw material for food production, and straw is a feedstock for energy purposes, which better refers to the principles of the circular economy.

3.1.2. Chemical Composition of Sorghum Biomass

The chemical composition of the sorghum waste biomass (cellulose, hemicellulose, and lignin) is shown in Table 2.

Table 2. Chemical composition of sorghum waste biomass (%).

	Cellulose			**Hemicellulose**			**Lignin**		
	Year I	**Year II**	**Year III**	**Year I**	**Year II**	**Year III**	**Year I**	**Year II**	**Year III**
General Analysis—Results									
Type of Analysis	ANOVA	ANOVA	ANOVA	ANOVA	ANOVA	ANOVA	K-W	K-W	K-W
df	4;10	4;10	4;10	4;10	4;10	4;10	4	4	4
F/χ2	6.457	0.25	7.086	13.66	20.40	72.33	12.233	12.9	13.50
p-value	0.0078 **	0.903	0.0057 **	0.0005 ***	8.46×10^{-5} ***	2.43×10^{-7} ***	0.0157	0.01177	0.00907

Table 2. *Cont.*

	Cellulose			**Hemicellulose**			**Lignin**		
	Year I	**Year II**	**Year III**	**Year I**	**Year II**	**Year III**	**Year I**	**Year II**	**Year III**
Mean Values for Treatments/Results of Post Hoc Tests $\alpha = 0.05$									
General mean	37.74	37.80	37.83	31.33	31.04	31.39	16.93	16.70	16.43
ASV-KS-61B	37.26 ^{b}	37.74 ^{a}	37.89 ^{ab}	29.29 ^{c}	29.12 ^{c}	30.10 ^{d}	18.43 ^{a}	18.21 ^{a}	17.52 ^{b}
Farmsugro 180	37.30 ^{b}	37.72 ^{a}	37.93 ^{ab}	33.13 ^{a}	32.75 ^{a}	32.79 ^{a}	17.86 ^{b}	17.24 ^{b}	16.80 ^{c}
GK Emese	38.27 ^{a}	37.86 ^{a}	37.47 ^{b}	31.24 ^{b}	31.27 ^{b}	31.46 ^{b}	17.97 ^{ab}	18.08 ^{a}	17.84 ^{a}
Sweet Caroline	37.93 ^{ab}	37.91 ^{a}	38.19 ^{a}	31.75 ^{ab}	31.64 ^{ab}	31.72 ^{b}	15.78 ^{c}	15.77 ^{c}	16.04 ^{d}
Sweet Susana	37.93 ^{ab}	37.75 ^{a}	37.66 ^{b}	31.22 ^{b}	30.42 ^{bc}	30.91 ^{c}	14.62 ^{c}	14.20 ^{d}	13.95 ^{e}

*** for a significance level of 0.001; ** for a significance level of 0.01; * for a significance level of 0.05; . for a significance level of 0.1; ^{abc} means followed by a common letter are not significantly different at the 5% level of significance.

Significant differences were found in the content of lignin and hemicellulose for the varieties tested in all years of the study and for the content of cellulose in the first and third years of the study. In the first year of research, the highest cellulose content was found for cultivar GK (38.27%), and it was significantly higher compared with the averages for cultivars FAR (37.30%) and ASV (37.26%). In the second year of the investigation, no relevant differences were found in the cellulose content; however, the highest mean value was observed for SC (37.91%), similar to that of the previous year (38.19%). In the third research year, the cellulose content for SC was significantly higher than that for the SS (37.66%) and GK (37.47%) varieties. The ranking of varieties in terms of hemicellulose content was the same in the first two years of research. The highest content was found for the FAR variety (33.13% and 32.72% in the first and second vegetation periods, respectively), and it did not differ significantly from the hemicellulose content in SC (31.75% and 31.64%). The lowest mean hemicellulose content in all research years was found for the ASV variety and, except for the second year, it differed significantly from the average for the other varieties. The highest average lignin content in the first two years of the study was found for the ASV variety (18.43% and 18.21%), and the highest average lignin content in the last year of the study was found for the GK variety (17.84%). On the other hand, the lowest content in all three years was found for the SS variety. The average overall chemical composition of sorghum was similar in all years of research. The moisture of the samples of all analyzed sorghum varieties was 8%, which is comparable with other obtained results described in the literature [16].

As is known, the content of cellulose, hemicellulose, and lignin in sorghum biomass is a key factor in bioconversion. Cellulose and hemicellulose are potential substrates for effective use in fermentation processes; however, the aromatic nature and complex structure of lignin adversely affect the hydrolysis of sorghum biomass. Increased cellulose content and partial degradation of hemicellulose contribute to effective bioethanol production [37].

3.2. Bioethanol Yield

The average yield of bioethanol differed significantly for the studied varieties in all three years of the study (Table 3).

Table 3. Bioethanol yield per hectare from waste sorghum biomass ($m^3 \cdot ha^{-1}$).

	Bioethanol Yield		
	Year I	**Year II**	**Year III**
	General Analysis—Results		
Type of Analysis	ANOVA	K-W	ANOVA
df	4;10	4	4;10
F/χ2	133.3	11.63333	39.87
p-value	1.25×10^{-8} ***	0.0203 *	4.08×10^{-6} ***
Mean Values for Treatments/Results of Post Hoc Tests α = 0.05			
General mean	6.40	7.95	3.42
ASV-KS-61B	5.66 ^{d}	7.99 ^{b}	2.89 ^{d}
Farmsugro 180	5.44 ^{d}	7.05 ^{c}	3.49 ^{bc}
GK Emese	6.56 ^{c}	7.51 ^{b}	3.26 ^{c}
Sweet Caroline	7.41 ^{a}	9.48 ^{a}	3.55 ^{b}
Sweet Susana	6.94 ^{b}	7.71 ^{b}	3.88 ^{a}

*** for a significance level of 0.001; ** for a significance level of 0.01; * for a significance level of 0.05; . for a significance level of 0.1; ^{abc} means followed by a common letter are not significantly different at the 5% level of significance.

In the first and second years of the research, the highest average bioethanol yield was found for SC (7.4 $m^3 \cdot ha^{-1}$ and 9.48 $m^3 \cdot ha^{-1}$, respectively) and in each year, the value was higher than that of the other cultivars. In turn, the lowest average bioethanol yield was found in the FAR variety, both in the first and second years of the study. In the first year of the study, the bioethanol yield for cultivar FAR (5.44 $m^3 \cdot ha^{-1}$) did not differ significantly from the average yield for ASV (5.66 $m^3 \cdot ha^{-1}$) and was significantly lower than the mean for the other cultivars. On the other hand, in the second year of the study, no significant differences were found in the bioethanol yield for the cultivars GK (7.51 $m^3 \cdot ha^{-1}$), ASV (7.99 $m^3 \cdot ha^{-1}$), or SS (7.71 $m^3 \cdot ha^{-1}$). In the third year of research, the ranking of varieties in terms of bioethanol yield was different from that in the previous years. The highest value was found for the SS variety (3.88 $m^3 \cdot ha^{-1}$), which was over 50% lower than the general average in the second year of the study. The lowest mean bioethanol yield was obtained for cultivar ASV (2.89 $m^3 \cdot ha^{-1}$), and it was significantly lower than the average yield of the other cultivars in the third year of the study.

Researchers from Ukraine also conducted research on the potential of sweet sorghum biomass to obtain bioethanol. Their results showed that sweet sorghum is characterized by high ethanol productivity, and the highest potential bioethanol productivity from various parts of sorghum can reach a total of 11.4 $m^3 \cdot ha^{-1}$ [38]. Research on bioethanol from sorghum biomass was conducted by López-Sandin et al. [39]. Scientists found that sweet sorghum is a promising crop for bioethanol production, with a high biomass yield and high sugar content, with the highest ethanol yield of 2.1 $m^3 \cdot ha^{-1}$. In addition, Aydinsakir et al. dealt with determining the effect of different irrigation levels on the yield and productivity of sorghum bioethanol (*Sorghum bicolor* L.). Within two years, they observed a bioethanol yield from sorghum of 1.4 to 2.3 $m^3 \cdot ha^{-1}$ [40].

3.3. Bioethanol Yield, Chemical Composition, and Sorghum Biomass Yield—Relationship

For all cultivars, a very strong positive correlation was found between the straw yield and the bioethanol yield (Figure 1).

A strong positive linear correlation was demonstrated between the straw yield and lignin content for the ASV and SS cultivars ($cr_p = 0.68$ and $cr_s = 0.5$ for ASV; $cr_p = 0.7$ and $cr_s = 0.55$ for SS) and a moderate correlation was demonstrated for the GK cultivar ($cr_p = 0.56$ and $cr_s = 0.29$). The content of lignin was very strong negatively correlated with the content of hemicellulose for the ASV variety ($cr_p = -0.85$ and $cr_s = -0.82$) and moderate for SC ($cr_p = -0.48$ and $cr_s = -0.49$) GK ($cr_p = -0.40$ and $cr_s = -0.36$). For the

FAR variant, a very strong negative correlation between the content of lignin and cellulose was found ($cr_p = -0.81$ and $cr_s = -0.75$) and a strong positive linear correlation was found between the content of lignin and hemicellulose ($cr_p = 0.68$ and $cr_s = 0.59$). For all varieties except SC, the lignin content is positively correlated with the bioethanol yield, with a strong correlation for GK, ASV, and SS variants, and a moderate correlation for FAR.

ASV-KS-61B

	SY	BY	C	HC	L
SY	-	0.97 ***	−0.45	−0.78 *	0.50
BY	0.99 ***	-	−0.43	−0.80 *	0.60 .
C	−0.37	−0.27	-	0.62 .	−0.68 .
HC	−0.77 *	−0.73 *	0.41	-	−0.82 *
L	0.68 *	0.63 .	−0.62 .	−0.85 **	-

Sweet Caroline

	SY	BY	C	HC	L
SY	-	0.95 ***	−0.63 .	−0.17	−0.28
BY	0.97 ***	-	−0.45	−0.02	−0.29
C	−0.53	−0.47	-	0.43	0.06
HC	−0.06	−0.09	0.42	-	−0.49
L	−0.32	−0.31	0.12	−0.48	-

Farmsugro 180

	SY	BY	C	HC	L
SY	-	0.93 ***	−0.12	−0.05	0.47
BY	0.98 ***	-	−0.22	−0.05	0.50
C	−0.22	−0.29	-	−0.65 .	−0.75 *
HC	−0.09	−0.03	−0.72 *	-	0.59 .
L	0.33	0.46	−0.81 **	0.68 *	-

Sweet Susana

	SY	BY	C	HC	L
SY	-	0.87 **	0.43	−0.13	0.55
BY	0.99 ***	-	0.18	−0.05	0.47
C	0.36	0.30	-	0.27	0.13
HC	−0.07	−0.12	0.21	-	0.18
L	0.70 *	0.62 .	0.24	0.25	-

GK Emese

	SY	BY	C	HC	L
SY	-	0.62 .	0.75 *	0.07	0.29
BY	0.93 ***	-	0.43	0.02	0.64 .
C	0.67 *	0.56	-	−0.40	0.57
HC	−0.13	−0.17	−0.60 .	-	−0.36
L	0.56	0.73 *	0.55	−0.40	-

LEGEND:

SY -	Straw yield [Mg/ha]
BY -	Bioethanol yield [m^3/ha]
C -	Cellulose (%)
HC -	Hemicellulose (%)
L -	Lignin (%)

Figure 1. Combined matrices of Pearson correlation coefficients cr_p (lower left) and Spearman rank correlation coefficients cr_s (upper right) for the chemical composition and yield of tested varieties. Significance codes: *** for a significance level of 0.001; ** for a significance level of 0.01; * for a significance level of 0.05; . for a significance level of 0.1.

3.4. Cluster Analysis

Figure 2 presents the heat map extended with the results of the cluster analysis.

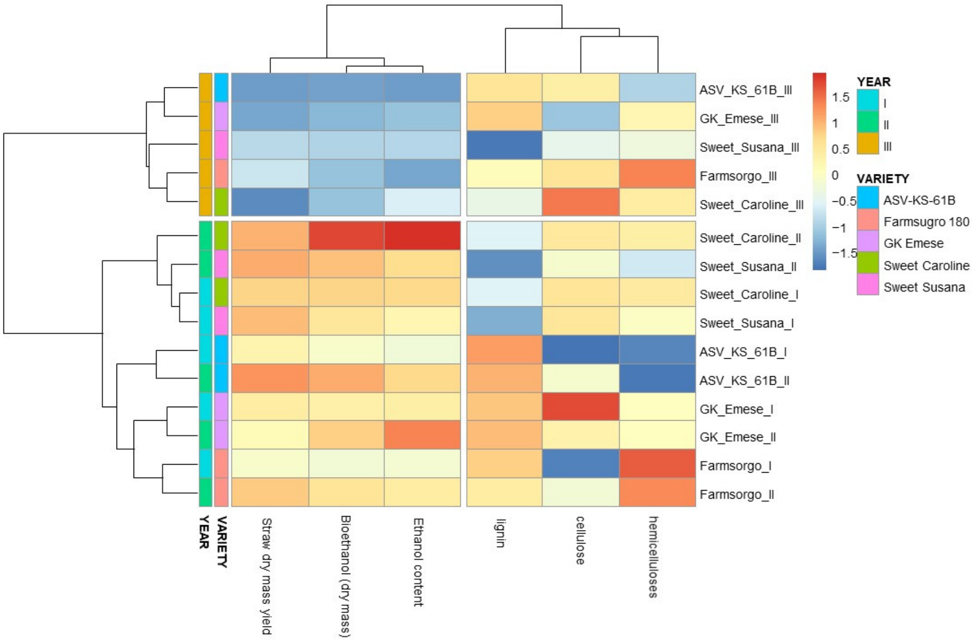

Figure 2. Heat map extended with the results of the cluster analysis. A cluster analysis was performed using Ward hierarchical clustering and the Euclidean distance. The number of clusters was determined in accordance with the Pseudo T-Squared grouping criterion.

Because of the differences in the measurement values for the observed variables, data scaling (z-score) was performed, the results of which showed that the mean for all variables was 0, and the unit of difference was one standard deviation. A cluster analysis was carried out in two directions: to compare the samples in terms of the mean values of the examined features and to determine the relationship between the observed variables. On the basis of the Pseudo T-Squared criterion, two main clusters for the studied cultivars were found. The first included all sorghum varieties from the third year of research. It should be noted that they were characterized by lower average dry matter yield values, content, and yield of bioethanol than in the first and second years.

The greatest similarity within this cluster was shown by the ASV and GK varieties as well as FAR and SC, all of them with a total lignin content similar to the average. In the third year of the study, for the ASV and GK varieties, the content of cellulose and hemicellulose was similar to or lower than the average. The second cluster was related to the varieties in the first and second years of the research, all with average or above-average mean yields and content and yield of bioethanol. In this cluster, it is possible to note the similarities between SS in the first and second years and SC in the first year of research. The different distance structure in both clusters indicates the necessity to carry out detailed analysis over the years.

4. Conclusions

The results of the study showed that the highest average yield for all cultivars except GK Emese was obtained in the second year of the study, and the lowest was obtained in the third year. The bioethanol yield per hectare from waste sorghum biomass was the highest for Sweet Caroline and the lowest for Farmsugro 180. In addition, significant differences were found in the content of lignin and hemicellulose for the varieties tested in all years of the study and in the content of cellulose in the first and third years of the study. The chemical composition and yield undoubtedly influenced bioethanol yield.

In summary, the analyzed sorghum varieties, capable of producing seeds in a temperate climate, can be successfully cultivated in a temperate climate. The biomass, which is a waste product after deseeding, can be effectively used for bioethanol production; therefore, there is a possibility to use each part of the plant for various economic purposes—the grain can be used for food and the straw can be used for energy purposes. Therefore, the rational management of raw materials and compliance with the principles of sustainable development and bioeconomy are enabled.

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