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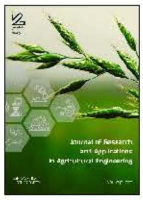

Chemical Composition of Maize Grain (*Zea Mays* L.) in Relation to the Depth of Fertilizer Application

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The article presents the results of a 2-year field study aimed at assessing the chemical composition of maize plants depending on the depth of two-component (NP) mineral fertilizer placement in the soil profile, the type of nitrogen fertilizer and the date of its application. Weather conditions, mainly thermal, in the early maize growing season, had no significant effect on the chemical composition of maize grain. The research showed the noticeable highest content of crude fiber in maize grain as a result of broadcast application of the fertilizer (0 cm), while the lowest for row fertilization at depths of 5 cm and 15 cm. The application of urea in maize cultivation and the pre-sowing application of nitrogen significantly increased the starch content in maize grain compared to ammonium nitrate and the top dressing application of nitrogen fertilizer.

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1. Introduction

Phosphorus is a nutrient playing a fundamental role in the early maize developmental stages, as well as grain formation and maturation [1, 2, 3, 4]. It stimulates root system development, indirectly increasing the plant resistance to periodic soil moisture deficiencies. Numerous literature reports indicated a large influence of soil temperature on phosphorus uptake by maize that is limited at temperatures below 12°C [5, 6]. Then, the plants exhibit clear symptoms of its deficiency in the form of red discoloration along the edges of the leaf blades and their subsequent dieback, especially in case of water deficiency, and a marked inhibition of growth and development [7]. These symptoms occur especially in cool spring years, even when phosphorus

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content in the soil is sufficient [8]. An underdeveloped root system prevents plants from absorbing sufficient amounts of phosphorus [9]. There is little information in the literature on how fertilization affects the chemical composition of maize grain. Kruczek [10] found that only varieties slightly differentiated the content of nutrients in maize ears and grains. In another article, Szulc et al. [11] reported that the chemical composition of maize grain was highly dependent on maize breeding company. The hypothesis of the experiment assumed that individual factors of the experiment, such as fertilizer application depth, nitrogen carrier type, and application date could significantly shape nutrient contents in maize grain. Therefore, the objective of this study was to determine the effect of NP fertilizer seeding depth on the chemical composition of maize grain.

2. Material and methods

2.1. Experimental field

Field trial was carried out at the Department of Agronomy of Poznań University of Life Sciences, on the fields of the Gorzyń Experimental and Educational Unit, in the years 2015–2016. It was conducted for four years in the same random block design (split-split-plot) with three factors and four field replicates. The following variables were tested: A – 1st order factor – NP fertilizer sowing depth [A1 – 0 cm (broadcast), A2 – 5 cm (in rows), A3 – 10 cm (in rows), A4 – 15 cm (in rows)]; B – 2nd order factor – type of supplementary nitrogen fertilizer [B1 – ammonium nitrate, B2 – urea]; C – 3rd order factor-date of supplementary nitrogen fertilization [C1 – before sowing, C2 – top dressing in the BBCH 15/16 stage]. The same level of mineral fertilization (100 kg N/ha, 30.8 kg P/ha and 107.9 kg K/ha) was applied in all experimental objects. Fertilization was balanced against phosphorus, which was applied at the whole required dose in the form of ammonium phosphate (18% N, 46% P_2O_5), according to the experimental design under the 1st order factor. N and K fertilization was performed before maize sowing using urea (46% N) and potassium salt (60%). The fertilizer coulters (on objects with initial fertilization) were set 5 cm aside from the seeds. Application depth of NP fertilizer was according to the 1st order factor levels. Gross plot size: 24.5 m^2 (length – 8.75 m, width – 2.8 m). The net plot area for harvesting was 12.25 m^2. Thermal and humid conditions in the growing season were favorable for the growth and development of maize. Nutrient contents (N, P, K, Mg) in the soil before the establishment of the experiment was at a medium level, while the pH ranged from 4.5 (2015) to 5.6 (2016). Organic carbon content in the study was from 0.99% C (2015) to 1.07% C (2016).

2.2. Laboratory assays

Grain samples were ground in a mill (SM 100, Retsch) to a particle size of 1 mm. The content of basic nutrients (crude ash, crude protein, crude fat and crude fiber, sugar) [12] and starch [PN-R-64785:1994] were determined in such fragmented samples [13].

2.3. Statistical analysis

The statistical analyses, such as analysis of variance (ANOVA), Tukey's HSD test for comparisons of pairs of means, were performed through the research years according to the model of data obtained from the experiments designed as a split-split-plot in each year. All calculations were carried out using the STATISTICA 13 software package (2017) and MS Excel. Statistical significance was set at the level $\alpha = 0.05$.

3. Results

3.1. Crude ash, total protein, crude fat, crude fibre and N-free extract

The different weather conditions over the study years 2015–2016 were not reflected in the considered traits (Tab. 1–2).

Irrespective of the study year and other factors (B and C), the NP fertilizer sowing depth (A) on the crude fibre content proved to be meaningful. Significantly the highest mean crude fibre content (1.98%) was observed for the depth of 0 cm. This mean did not differ significantly from the mean (1.89%) obtained for the depth of 10 cm (Tab. 1–2).

Moreover, the tested types of nitrogen fertilizer (B) significantly influenced the crude ash content and the N-free extract content. The highest mean crude ash content (1,38%) was obtained using urea. On the other hand, the highest mean N-free extract content (82.94%) was obtained using ammonium nitrate (Tab. 1–2).

The results in Tab. 2 indicate the significant impact of date of supplementary nitrogen fertilization (C) on the total protein, the crude fat, the crude fibre and the N-free extract. The highest mean total protein content (9.52%) and the highest mean crude fibre content (1.94%) were obtained for top-dressing in the BBCH 15/16. In turn, the highest mean crude fat content (4,48%) and the highest mean N-free extract content (83.02%) were recorded for nitrogen application before sowing (Tab. 2).

Tab. 1. Results of the four-stratum (YABC) ANOVA

Source of variability	Degrees of freedom	Mean squares				
		Crude ash	**Total protein**	**Crude fat**	**Crude fibre**	**N-free extract**
Blocks	1	0.27366	0.09571	2.68755	1.95475	1.0176
Y	1	0.00220	0.00431	0.23949	1.90958	0.1131
Error 1	1	0.00024	0.00048	0.02661	0.21218	0.1160
A	3	0.00421	0.04630	0.04576	0.05122*	1.2439
Y×A	3	0.02347	0.45426**	0.01367	0.09676**	1.0176**
Error 2	6	0.00623	0.03169	0.02281	0.00793	0.1187
B	1	0.00891**	0.00296	0.04126	0.00296	0.1796**
Y×B	1	0.00844**	0.00650	0.00079	0.01978	0.0062
A×B	3	0.00423*	0.19237**	0.07851*	0.00501	0.1555**
Y×A×B	3	0.00706**	0.14162**	0.07674*	0.00182	0.0803**
Error 3	8	0.00068	0.01764	0.01065	0.00398	0.0068
C	1	0.00499	0.97886**	0.06858**	0.12382**	1.0430**
Y×C	1	0.00591	1.29533**	0.00256	0.07189**	1.6161**
A×C	3	0.00219	0.03832	0.03690**	0.04008**	0.0686
B×C	1	0.00017	0.00000	0.10041**	0.07597**	0.3706*
Y×A×C	3	0.00519*	0.12234*	0.11648**	0.06200**	0.5251**
Y×B×C	1	0.00431	0.06793	0.00383	0.01142	0.0523
A×B×C	3	0.01099**	0.16748**	0.15144**	0.04032**	0.3545**
Y×A×B×C	3	0.01168**	0.04898	0.00502	0.05144**	0.1668*
Error 4	16	0.00138	0.02503	0.00641	0.00684	0.0506

** – significant at *p-value* < 0.01, * – significant at *p-value* < 0.05

Tab. 2. Mean values of the traits for the years and agrotechnical factors

Years and factors	The levels of the factors	Crude ash [%]	Total protein [%]	Crude fat [%]	Crude fibre [%]	N-free extract [%]
Years (Y)	2015	1.38^{ns}	9.41^{ns}	4.38^{ns}	2.07^{ns}	82.76^{ns}
	2016	1.37^{ns}	9.39^{ns}	4.51^{ns}	1.72^{ns}	83.02^{ns}
NP fertilizer sowing depth (A)	0 cm (broadcast)	1.39^{ns}	9.44^{ns}	4.39^{ns}	1.98^{a}	82.81^{ns}
	5 cm	1.35^{ns}	9.37^{ns}	4.47^{ns}	1.86^{b}	82.95^{ns}
	10 cm	1.38^{ns}	9.34^{ns}	4.42^{ns}	1.89^{ab}	82.98^{ns}
	15 cm	1.36^{ns}	9.45^{ns}	4.51^{ns}	1.85^{b}	82.83^{ns}
Type of nitrogen fertilizer (B)	ammonium nitrate	1.36^{b}	9.39^{ns}	4.42^{ns}	1.89^{ns}	82.94^{a}
	urea	1.38^{a}	9.41^{ns}	4.47^{ns}	1.90^{ns}	82.84^{b}
Date of nitrogen ertilization (C)	before sowing	1.38^{ns}	9.28^{b}	4.48^{a}	1.85^{b}	83.02^{a}
	top dressing in the BBCH 15/16 stage	1.36^{ns}	9.52^{a}	4.41^{b}	1.94^{a}	82.76^{b}

Values in columns marked with at least the same letter do not differ significantly; *ns* – not significant.

It was observed (Tab. 1 and 3) that the tested NP fertilizer sowing depth (A) and the tested dates of nitrogen application (C) reacted differently to the changing conditions over the study years with respect to the total protein, the crude fibre and the N-free extract.

Greatly the highest mean total protein content (9.64%) was obtained in 2015 for the depth of 0 cm. This mean did not differ noticeably from the means obtained in the same year for the depths of 5 cm and 10 cm. In turn, the highest mean total protein content (9.66%) was recorded in 2016 for the depth of 15 cm. This mean did not differ extensively from the means obtained in the same year for the depths of 5 cm and 10 cm.

Significantly the highest mean crude fibre content (2.22%) was recorded in 2015 for the depth of 0 cm. This mean did not differ significantly from the mean obtained in the same year for the depth of 10 cm. While the lowest means were obtained for all depths in 2016.

Significantly the highest means N-free extract were obtained in 2016 for the depths of 0 cm and 10 cm. These means did not differ significantly from the means obtained in the same year for the depths of 5 cm and 15 cm. In turn, significantly the lowest mean N-free extract content (82.37) was obtained in 2015 for the depth of 0 cm. This mean did not differ significantly from the means obtained in the same year for other depths.

As far as the examination of the total protein content is concerned, the only significant difference occurred in 2015, when top-dressing in the BBCH 15/16 obtained significantly higher mean total protein (9.67%) than nitrogen application before sowing (9.14%).

Significantly the highest mean crude fibre content (2.15%) was recorded in 2015 for top-dressing in the BBCH 15/16. While the lowest means were obtained for both dates of nitrogen application in 2016.

Moreover, significantly the highest means N-free extract were observed in 2016 for both dates of nitrogen application. These means did not differ significantly from the mean obtained in 2015 for the top-dressing in the BBCH 15/16.

It was noticed (Tab. 1 and 3) that the tested types of nitrogen fertilizer (B) reacted differently to the changing conditions over the study years, but only for crude ash content. For the remaining traits, no significant interactions between years (Y) and the type of supplementary nitrogen fertilizer (B) were found.

Significantly the highest mean crude ash content (1.39%) was observed in 2016 for urea. In turn, significantly the lowest mean crude ash content (1.34%) was observed in the same year for ammonium nitrate.

Tab. 3. Mean values for the combinations years (Y) × A factor, years (Y) × B factor and years (Y) × C factor

Years (Y)	NP fertilizer sowing depth (A)	Crude ash [%]	Total protein [%]	Crude fibre [%]	N-free extract [%]
2015	0 cm (broadcast)	1.43[ns]	9.64[a]	2.22[a]	82.37[b]
	5 cm	1.39[ns]	9.42[ab]	1.97[b]	82.80[ab]
	10 cm	1.36[ns]	9.34[ab]	2.12[ab]	82.82[ab]
	15 cm	1.33[ns]	9.24[b]	1.96[b]	83.06[ab]
2016	0 cm (broadcast)	1.35[ns]	9.24[b]	1.73[c]	83.25[a]
	5 cm	1.32[ns]	9.33[ab]	1.76[c]	83.10[ab]
	10 cm	1.40[ns]	9.34[ab]	1.65[c]	83.13[a]
	15 cm	1.40[ns]	9.66[a]	1.75[c]	82.59[ab]
Years (Y)	**Type of nitrogen fertilizer (B)**	**Crude ash [%]**	**Total protein [%]**	**Crude fibre [%]**	**N-free extract [%]**
2015	ammonium nitrate	1.38[ab]	9.39[ns]	2.04[ns]	82.83[ns]
	urea	1.38[ab]	9.42[ns]	2.09[ns]	82.70[ns]
2016	ammonium nitrate	1.34[b]	9.39[ns]	1.73[ns]	83.06[ns]
	urea	1.39[a]	9.39[ns]	1.71[ns]	82.97[ns]
Years (Y)	**Date of nitrogen fertilization (C)**	**Crude ash [%]**	**Total protein [%]**	**Crude fibre [%]**	**N-free extract [%]**
2015	before sowing	1.40[ns]	9.14[c]	1.99[b]	83.05[a]
	top dressing in the BBCH 15/16 stage	1.36[ns]	9.67[a]	2.15[a]	82.48[b]
2016	before sowing	1.36[ns]	9.41[b]	1.71[c]	82.98[a]
	top dressing in the BBCH 15/16 stage	1.37[ns]	9.37[b]	1.73[c]	83.05[a]

Values in columns marked with at least the same letter do not differ significantly, *ns* – not significant.

The analysis in Tab. 1 showed a significant interaction effect of the NP fertilizer sowing depth (A) and the type of supplementary nitrogen fertilizer (B) for four traits. For the crude fibre content, no significant interaction between factors A and B was found.

A detailed analysis (Tab. 4) showed:

1. Significantly the highest mean crude ash content (1.41%) was obtained using urea at the depth of 10 cm. On the other hand, significantly the lowest means crude ash were obtained using ammonium nitrate at the depths of 5 cm, 10 cm, 15 cm and using urea at the depth of 5 cm.
2. Significantly the highest mean total protein content (9.58%) was observed using ammonium nitrate at the depth of 15 cm. In turn, significantly the lowest means total protein were obtained using urea at the depth of 15 cm and using ammonium nitrate at the depth of 0 cm.
3. Significantly the highest mean crude fat content (4.55%) was recorded using urea at the depth of 5 cm. In turn, significantly the lowest means crude fat were recorded using urea at the depth of 0 cm and using ammonium nitrate at the depth of 10 cm.
4. Significantly the highest mean N-free extract content (83.10%) was obtained using ammonium nitrate at the depth of 5 cm. On the other hand, significantly the lowest means N-free extract were obtained using ammonium nitrate at the depth of 15 cm and using urea at the depths of 0 cm and 5 cm.

A significant interaction was found for the NP fertilizer sowing depth (A) and the date of supplementary nitrogen fertilization (C) on the crude fat content and the crude fibre content (Tab. 3). For the remaining traits, no significant interactions between factors A and C were found.

A detailed analysis (Table 4) showed:

1. Significantly the highest mean crude fat content (4.54%) was recorded using top-dressing in the BBCH 15/16 at the depth of 15 cm. In turn, significantly the lowest mean crude fat content (4.34%) was obtained using top-dressing in the BBCH 15/16 at the depth of 10 cm.
2. Significantly the highest means crude fibre were observed using nitrogen application before sowing at the depth of 0 cm and using top-dressing in the BBCH 15/16 at the depths of 0 cm and 15 cm. On the other hand, significantly the lowest mean crude fibre content (1.74%) was obtained using nitrogen application before sowing at the depth of 15 cm.

A significant interaction was, also, found for the type of supplementary nitrogen fertilizer (B) and the date of supplementary nitrogen fertilization (C) on the crude fat, the crude fibre and the N-free extract (Tab. 3). For the remaining traits, no significant interactions between factors B and C were found.

A detailed analysis (Tab. 4) showed:

1. For ammonium nitrate, the date of supplementary nitrogen fertilization did not differentiate the mean values of crude fibre and the N-free extract.
2. Significantly the highest mean crude fibre content (1.98%) was observed on the application of urea by top-dressing in the BBCH 15/16.
3. Significantly the highest mean N-free extract content (83.04%) was observed on the application of urea by nitrogen application before sowing.

Significantly the lowest mean crude fat content (4.35%) was recorded on the application of ammonium nitrate by top-dressing in the BBCH 15/16. For the remaining combinations of types of supplementary nitrogen fertilization and dates of their application, there were no significant differences among the means crude fat.

Tab. 4. Mean values for the combinations A factor × B factor, A factor × C factor and B factor × C factor

NP fertilizer sowing depth (A)	Type of nitrogen fertilizer (B)	Crude ash [%]	Total protein [%]	Crude fat [%]	Crude fibre [%]	N-free extract [%]
0 cm (broadcast)	ammonium nitrate	1.39^{ab}	9.31^{b}	4.46^{ab}	1.97^{ns}	82.87^{bc}
	urea	1.39^{ab}	9.56^{ab}	4.32^{b}	1.98^{ns}	82.75^{c}
5 cm	ammonium nitrate	1.35^{b}	9.32^{ab}	4.38^{ab}	1.85^{ns}	83.10^{a}
	urea	1.36^{b}	9.42^{ab}	4.55^{a}	1.88^{ns}	82.79^{c}
10 cm	ammonium nitrate	1.35^{b}	9.35^{ab}	4.34^{b}	1.90^{ns}	83.06^{ab}
	urea	1.41^{a}	9.32^{ab}	4.49^{ab}	1.87^{ns}	82.90^{abc}
15 cm	ammonium nitrate	1.35^{b}	9.58^{a}	4.50^{ab}	1.83^{ns}	82.74^{c}
	urea	1.38^{ab}	9.32^{b}	4.52^{ab}	1.88^{ns}	82.91^{abc}

NP fertilizer sowing depth (A)	Date of nitrogen fertilization (C)	Crude ash [%]	Total protein [%]	Crude fat [%]	Crude fibre [%]	N-free extract [%]
0 cm (broadcast)	before sowing	1.41^{ns}	9.29^{ns}	4.42^{abc}	1.93^{a}	82.96^{ns}
	top dressing in the BBCH 15/16 stage	1.37^{ns}	9.59^{ns}	4.36^{bc}	2.03^{a}	82.66^{ns}
5 cm	before sowing	1.35^{ns}	9.20^{ns}	4.52^{ab}	1.87^{ab}	83.07^{ns}
	top dressing in the BBCH 15/16 stage	1.35^{ns}	9.55^{ns}	4.42^{abc}	1.86^{ab}	82.82^{ns}
10 cm	before sowing	1.38^{ns}	9.23^{ns}	4.50^{abc}	1.87^{ab}	83.02^{ns}
	top dressing in the BBCH 15/16 stage	1.38^{ns}	9.44^{ns}	4.34^{c}	1.90^{ab}	82.94^{ns}
15 cm	before sowing	1.38^{ns}	9.39^{ns}	4.48^{abc}	1.74^{b}	83.02^{ns}
	top dressing in the BBCH 15/16 stage	1.35^{ns}	9.52^{ns}	4.54^{a}	1.96^{a}	82.63^{ns}
Type of nitrogen fertilizer (B)	**Date of nitrogen fertilization (C)**	**Crude ash [%]**	**Total protein [%]**	**Crude fat [%]**	**Crude fibre [%]**	**N-free extract [%]**
ammonium nitrate	before sowing	1.37^{ns}	9.27^{ns}	4.49^{a}	1.88^{ab}	82.99^{a}
	top dressing in the BBCH 15/16 stage	1.35^{ns}	9.52^{ns}	4.35^{b}	1.90^{ab}	82.89^{ab}
urea	before sowing	1.39^{ns}	9.28^{ns}	4.46^{a}	1.82^{b}	83.04^{a}
	top dressing in the BBCH 15/16 stage	1.38^{ns}	9.53^{ns}	4.48^{a}	1.98^{a}	82.63^{b}

Values in columns marked with at least the same letter do not differ significantly, *ns* – not significant.

3.2. Starch and sugar

The different weather conditions over the study years 2015–2016 were not reflected in the considered traits (Tab. 5–6).

Irrespective of the study year and other factors (B and C), the NP fertilizer sowing depth (A) on the sugar content proved to be significant. Significantly the highest mean sugar content (1.987%) was observed for the depth of 10 cm. This mean did not differ significantly from the mean (1.976%) obtained for the depth of 0 cm (Tab. 5–6).

The results in Tab. 5 indicate the significant impact of type of supplementary nitrogen fertilizer (B) on the starch content. The highest mean starch content (71.08%) was obtained using urea (Tab. 6).

Regardless of the year when the study was carried out and regardless of the remaining factors (A and B), a significant difference was noted between the means of the studied traits for both dates of supplementary nitrogen fertilization (C) (Tab. 5–6). The highest mean starch content (71.18%) and the highest mean sugar content (1.981%) were recorded for nitrogen application before sowing.

Tab. 5. Results of the four-stratum (YABC) ANOVA

Source of variability	Degrees of freedom	Mean squares	
		Starch	Sugar
Blocks	1	2.29712	0.039502
Y	1	36.24793	0.005077
Error 1	1	4.02755	0.000564
A	3	2.49075	0.001015**
Y×A	3	2.46058	0.000605*
Error 2	6	0.73562	0.000099
B	1	1.27549*	0.000156
Y×B	1	0.00914	0.000000
A×B	3	5.15758**	0.001264*
Y×A×B	3	2.36863**	0.000534
Error 3	8	0.23860	0.000169

Source of variability	Degrees of freedom	Mean squares	
		Starch	Sugar
C	1	3.71767**	0.001600**
Y×C	1	1.82757*	0.000056
A×C	3	1.76650**	0.000576**
B×C	1	3.08222**	0.000352
Y×A×C	3	0.90218	0.000197
Y×B×C	1	0.15064	0.000014
A×B×C	3	4.66212**	0.001292**
Y×A×B×C	3	4.34800**	0.000923**
Error 4	16	0.30860	0.000084

** – significant at *p-value* < 0.01, * – significant at *p-value* < 0.05

Tab. 6. Mean values of the traits for the years and agrotechnical factors

Years and factors	The levels of the factors	Starch [%]	Sugar [%]
Years (Y)	2015	71.69[ns]	1.985[ns]
	2016	70.18[ns]	1.967[ns]
NP fertilizer sowing depth (A)	0 cm (broadcast)	70.95[ns]	1.976[ab]
	5 cm	70.43[ns]	1.968[b]
	10 cm	71.39[ns]	1.987[a]
	15 cm	70.97[ns]	1.974[b]
Type of nitrogen fertilizer (B)	ammonium nitrate	70.79[b]	1.975[ns]
	urea	71.08[a]	1.978[ns]
Date of nitrogen fertilization (C)	before sowing	71.18[a]	1.981[a]
	top dressing in the BBCH 15/16 stage	70.69[b]	1.971[b]

Values in columns marked with at least the same letter do not differ significantly, *ns* – not significant.

It was observed (Tab.e 5 and 7) that the tested NP fertilizer sowing depth (A) reacted differently to the changing conditions over the study years with respect to the sugar content. Significantly the highest mean sugar content (1.996%) was obtained in 2015 for the depth of 10 cm. This mean did not differ significantly from the means were obtained in the same year for the remaining depths and from the means were obtained in 2016 for the depths of 0 cm and 10 cm. While the lowest mean (1.952%) was obtained in 2016 for the depth of 5 cm.

No significant interaction was found between type of supplementary nitrogen fertilizer (B) and the year of research for both traits (Tab. 5).

It was noticed (Tab. 5 and 7) that the tested dates of supplementary nitrogen fertilization (C) reacted differently to the changing conditions over the study years, but only for starch content. Significantly the highest means starch contents were recorded in 2015 for both dates of nitrogen fertilization (C1-71.76%, C2-71.62%). In turn, significantly the lowest mean starch content (69.77%) was recorded in 2016 for the top-dressing in the BBCH 15/16.

Tab. 7. Mean values for the combinations years (Y) × A factor and years (Y) × C factor

Years (Y)	NP fertilizer sowing depth (A)	Starch [%]	Sugar [%]
2015	0 cm (broadcast)	71.35[ns]	1.977[ab]
	5 cm	71.74[ns]	1.984[ab]
	10 cm	72.14[ns]	1.996[a]
	15 cm	71.53[ns]	1.983[ab]

Years (Y)	NP fertilizer sowing depth (A)	Starch [%]	Sugar [%]
2016	0 cm	70.54^{ns}	1.975^{ab}
	5 cm	69.13^{ns}	1.952^{c}
	10 cm	70.65^{ns}	1.978^{ab}
	15 cm	70.41^{ns}	1.964^{bc}
Years (Y)	**Date of nitrogen fertilization (C)**	**Starch [%]**	**Sugar [%]**
2015	before sowing	71.76^{a}	1.989^{ns}
	top-dressing in the BBCH 15/16	71.62^{a}	1.981^{ns}
2016	before sowing	70.59^{b}	1.973^{ns}
	top-dressing in the BBCH 15/16	69.77^{c}	1.961^{ns}

Values in columns marked with at least the same letter do not differ significantly, *ns* – not significant.

The analysis in Tab. 5 showed a significant interaction effect of the NP fertilizer sowing depth (A) and the type of supplementary nitrogen fertilizer (B) for both traits.

A detailed analysis (Tab. 8) showed:

1. For the depth at most 10 cm, the type of supplementary nitrogen fertilizer did not differentiate the mean values of starch.
2. For the depth of 15 cm, the highest mean starch content (71.65%) was obtained using urea.
3. Significantly the lowest mean sugar content (1.956%) was obtained using urea at the depth of 5 cm. This mean did not differ significantly from the mean was obtained using ammonium nitrate for the same depth, from the mean was obtained using ammonium nitrate at the depth of 0 cm and from the means were obtained using both ammonium nitrate and urea at the depth of 15 cm.

The analysis in Tab. 5 showed a significant interaction effect of the NP fertilizer sowing depth (A) and the date of supplementary nitrogen fertilization (C) for both traits.

A detailed analysis (Tab. 8) showed:

1. For the nitrogen application before sowing, the NP fertilizer sowing depth did not differentiate the mean values of starch and sugar.
2. For the top-dressing in the BBCH 15/16, significantly the highest the mean values of starch (71.58%) and sugar (1.991%) were recorded for the depth of 10 cm. In turn, significantly the lowest the mean values of starch (69.87%) and sugar (1.958%) were recorded for the depth of 5 cm.

A significant interaction was, also, found for the type of supplementary nitrogen fertilizer (B) and the date of supplementary nitrogen fertilization (C) on the starch (Tab. 5 and 8). For urea, the date of nitrogen fertilization did not differentiate the mean values of starch. On the other hand, for ammonium nitrate, the highest the mean values of starch (71.25%) was recorded for nitrogen application before sowing.

Tab. 8. Mean values for the combinations A factor × B factor, A factor × C factor and B factor × C factor

NP fertilizer sowing depth (A)	Type of nitrogen fertilizer (B)	Starch [%]	Sugar [%]
0 cm (broadcast)	ammonium nitrate	70.84^{ab}	1.969^{ab}
	urea	71.05^{ab}	1.983^{a}
5 cm	ammonium nitrate	71.07^{ab}	1.979^{ab}
	urea	69.80^{b}	1.956^{b}
10 cm	ammonium nitrate	70.98^{ab}	1.983^{a}
	urea	71.81^{a}	1.991^{a}
15 cm	ammonium nitrate	70.29^{b}	1.967^{ab}
	urea	71.65^{a}	1.981^{ab}

Date of nitrogen fertilization (C)	NP fertilizer sowing depth (A)	Starch [%]	Sugar [%]
before sowing	0 cm (broadcast)	71.10^{a}	1.984^{ab}
	5 cm	70.99^{ab}	1.978^{abc}
	10 cm	71.21^{a}	1.983^{ab}
	15 cm	71.41^{a}	1.980^{ab}
top-dressing in the BBCH 15/16	0 cm (broadcast)	70.79^{ab}	1.968^{bc}
	5 cm	69.87^{b}	1.958^{c}
	10 cm	71.58^{a}	1.991^{a}
	15 cm	70.53^{ab}	1.968^{bc}
Type of nitrogen fertilizer (B)	**Date of nitrogen fertilization (C)**	**Starch [%]**	**Sugar [%]**
ammonium nitrate	before sowing	71.25^{a}	1.982^{ns}
	top-dressing in the BBCH 15/16	70.33^{b}	1.967^{ns}
urea	before sowing	71.10^{a}	1.980^{ns}
	top-dressing in the BBCH 15/16	71.06^{ab}	1.975^{ns}

Values in columns marked with at least the same letter do not differ significantly, *ns* – not significant.

The results of the study also showed a significant interaction between the NP fertilizer sowing depth (A), the type of nitrogen fertilizer (B) and year of research (Tab. 5 and 9). During 2015, the impact of the combination of NP fertilizer sowing depth (0 cm, 5 cm, 10 cm and 15 cm) and type of nitrogen fertilizer (ammonium nitrate or urea) on the starch content was not significant. On the other hand, the year 2016 significantly affected the starch content. The highest means starch content were obtained using urea at the depths of 0 cm and 5 cm and using ammonium nitrate at the depth of 0 cm. In turn, the lowest means starch content were obtained using ammonium nitrate at the depths of 5 cm and 15 cm and using urea at the 5 cm.

Tab. 9. Mean values of the starch for the combinations years (Y) × A factor × B factor

NP fertilizer sowing depth (A)	Type of nitrogen fertilizer (B)	Years	
		2015	2016
0 cm (broadcast)	ammonium nitrate	71.19^{abc}	70.50^{abc}
	urea	71.52^{ab}	70.58^{abc}
5 cm	ammonium nitrate	71.96^{ab}	70.17^{bcd}
	urea	71.51^{ab}	68.08^{d}
10 cm	ammonium nitrate	71.72^{ab}	70.24^{bc}
	urea	72.55^{a}	71.07^{abc}
15 cm	ammonium nitrate	71.37^{ab}	69.21^{cd}
	urea	71.69^{ab}	71.61^{ab}

Values in columns marked with at least the same letter do not differ significantly.

4. Discussion

Starch is the basic component of maize grain. In the present study, the average starch content for the experimental factors constituted 70.93% dry weight. Idikut et al. [14] and Podkówka et al. [15] reported similar levels, while Li et al. [16] and Radosavljević et al. [17] higher concentrations of starch in maize grain. The analysis of individual experimental factors showed that maize fertilized with urea was characterized by a significantly higher content of starch in the grain compared to ammonium nitrate. Application of the entire dose of nitrogen before sowing was also a significant predictor of a higher content of this component compared to pre-sowing treatment supplemented with top dressing. Proteins are the second largest group of compounds present in maize grain [18]. Maize protein contains prolamines, globulins, albumin and glutelins. Among these groups, the most abundant are globulins and prolamines. Significant quantities of globulins are mainly found in the embryo, while prolamins predominate strongly in the endosperm, i.e. the main protein storage site in the grain, and are therefore the dominant protein fraction within the whole grain. Prolamines include zein, a protein specific to maize, from which it takes its name. Zein constitutes 40–50% maize grain proteins, and its amino acid composition does not contain lysine and tryptophan, while glycine is present in small amounts, which significantly affects the biological value of the total maize protein [19]. In the present study, the average concentration of total protein in grain for all examined experimental factors was 9.4%. Podkówka et al. [15] found higher content of total protein in grain dry weight. The low biological value, with the indication of tryptophan and lysine as limiting amino acids, was also confirmed by numerous publications [20, 21]. The development of plant breeding techniques has made it possible to obtain the so-called QPM varieties, i.e. quality protein maize. In studies comparing the amount of protein among cereals, maize contained it in the range of 9–10 g protein$\cdot$100^{-1} g dry matter. This result places maize at a lower level than wheat, rye, buckwheat or rice. QPM varieties contain approximately two times more lysine and tryptophan, making their proteins much more similar to the amino acid composition of casein, and thus they have a significantly higher nutritional value [20]. The fat in maize grain is mainly present in the maize germ. Maize fat contains 11–14% saturated fatty acids, 20–30% monounsaturated fatty acids and as much as 56–65% polyunsaturated fatty acids [22]. Among them, linoleic acid is the most abundant, and along with linolenic acid, is part of the essential unsaturated fatty acids, important for the proper functioning of the body. The current study has shown that the fat content in maize grain is significantly higher in the case of pre-sowing nitrogen application compared to the pre-sowing supplemented with top dressing treatment.

5. Conclusions

1. The significantly highest content of crude fiber in maize grain was found as a result of NP fertilizer application at a depth of 0 cm, while the lowest for the depths of 5 and 15 cm.
2. The highest content of crude ash was found in maize grain fertilized with urea compared to ammonium nitrate. The effect of the tested nitrogen fertilizers was opposite for N-free extract compounds.
3. Significantly higher total protein content in maize grain was found for top dressing nitrogen fertilization as compared to pre-sowing. For such traits as crude fat and N-free extract compounds, the effect of the tested fertilizers was opposite.
4. The application of urea in maize cultivation increases the starch content in maize grain compared to ammonium nitrate.
5. Pre-sowing nitrogen application in maize cultivation increases the starch content in maize grain compared to top dressing application.

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