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RESEARCH

Effect of manganese and nitrogen fertilization on the content of some essential micronutrients and composition of fatty acids in winter wheat grain

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ABSTRACT

Nitrogen fertilization combined with microelements is an effective way to provide nutrients to plants, which are essential for obtaining high-value crops. The aim of this study was to evaluate two N fertilizer rates (150 and 200 kg ha^{-1}) and four Mn fertilizer rates (0.0, 0.5, 1.0, and 1.5 kg Mn ha^{-1}) on the N, Mn, Zn, Cu, and Fe content and composition of fatty acids in winter wheat (*Triticum aestivum* L.) grain. An increase in the N fertilizer rate increased (12.7%) the Mn content and decreased (10%) the Cu content of wheat grain. Regardless of N fertilization, foliar application of Mn at 1.5 kg ha^{-1} contributed to the highest Zn (28.4 mg kg^{-1}) and Fe (58.4 mg kg^{-1}) content in the grain. In an analysis of lipid fractions, the highest value of the coefficient of variation was recorded for C18:0 (16.3%-low variation). Nitrogen and Mn fertilization were most strongly correlated with the Mn content of grain ($r = 0.356$, $r = 0.391$, respectively). The 200 kg N ha^{-1} treatments combined with 1.0 kg ha^{-1} Mn and 150 kg ha^{-1} N without Mn were correlated with the content of C:18:0, C18:1c11, C18:1c9, and monounsaturated fatty acids in the grain. The application of 200 kg ha^{-1} N with 1.5 kg ha^{-1} Mn was correlated with the Fe, Zn, and Mn content of the grain. The remaining fertilization treatments were correlated with the content of C18:3, C18:2, polyunsaturated fatty acids (PUFA), and the C18:2/C18:3 ratio in the grain. Results indicated that the application of 200 kg ha^{-1} N beneficially affected the PUFA content in the winter wheat grain and can therefore be used to obtain raw material with increased nutritional value. The human organism does not synthesize PUFA, so they must be taken with food (or supplements); winter wheat grain can be a good source because it contains more than 60% PUFA.

Key words: Biofortification, lipids, nitrogen content, nutritional value of grain, *Triticum aestivum*.

INTRODUCTION

The biofortification with nutrients of first-class winter wheat (*Triticum aestivum* L.) grain, which requires the selection of the optimal farming location and the most effective agronomic practices, is more complex than improving grain quality (Jaskulska et al., 2018). The micronutrients that are essential for healthy plant growth include Cu, Fe, Mo, Zn, and Mn (Lambers et al., 2015). Root uptake contributes up to 86% micronutrient accumulation in the grain (Liñero et al., 2018). The roles of vegetative tissues and specialized mechanisms responsible for micronutrient translocation to the grain have been extensively studied (Waters et al., 2009; Stepien and Wojtkowiak, 2016). Manganese is accumulated in plant cells where intense chemical reactions take place during the active growth stage (Fernando et al., 2012). Vegetative organs can be directly supplied with micronutrients through foliar fertilization alone or combined with macronutrients, mostly N. Foliar nutrition is very important because foliar nutrients penetrate the stomata or leaf cuticles and are easily absorbed by cells (Rajasekar et al., 2017). Manganese serves as a cofactor or a prosthetic group (metalloproteins) and activates enzymes responsible for breaking down carbohydrates and reducing nitrate. Manganese participates in photosynthesis, chloroplast formation, maintenance of the hormonal balance, lipid biosynthesis, and plant resistance to biotic and abiotic stresses (Socha and Guerinot, 2014). Wheat is an important source of energy, protein, and micronutrients, so that most research studies aim to improve these parameters (Zeidan et al., 2010; Wojtkowiak et al., 2018). However, the influence of agronomic factors on the fatty acid content and composition in cereal grain is less frequently investigated. Cereals are a major component of the human diet, which play important biological roles in the human body and deliver health-promoting effects due to the unique composition of fatty acids. Polyunsaturated fatty acids (PUFA), mainly linoleic acid C18:2 n-6 (LA) and linolenic acid C18:3 n-3 (ALA), produce long-chain derivatives: arachidonic acid (n-6), eicosapentaenoic acid n-3 (EPA), and docosahexaenoic acid n-3 (DHA) (Levant et al., 2010). The presence of N in the nutrient growth solution can promote root uptake, translocation of other nutrients (Kutman et al., 2011), and mineral homeostasis. Nitrogen contributes to effective nutrient transport, trafficking, and sequestration. This mechanism supplies trace amounts of nutrients to all cell types at all development stages, which is essential for grain biofortification (Pataco et al., 2017).

The aim of this study was to evaluate the effect of two rates of N fertilizer and four rates of Mn fertilizer on the content of N, Mn, Zn, Cu, and Fe, as well as the composition of fatty acids in winter wheat grain.

MATERIALS AND METHODS

From 2013 to 2016, a two-factorial field experiment with a randomized block design and three replicates was conducted at the Agricultural Experimental Station in Tomaszkowo (53°72' N; 20°42' E), Poland, which is operated by the University of Warmia and Mazury in Olsztyn. The experiment was established on slightly acidic (KCl solution with pH 5.7) gray-brown podzolic soil (classified as Haplic Cambisol according to the World Reference Base for Soil Resources, WRB 2014) with a medium silty loam granulometric composition, 10.1 g kg^{-1} C content, moderate content of available K (145 mg kg^{-1}), Mg (68.7 mg kg^{-1}), and Mn (150 mg kg^{-1}), and high P content (83.3 mg kg^{-1}). Plot size at harvest was 8.0 m^2.

The first experimental factor included two rates of N fertilizer (150 and 200 kg ha^{-1}). Nitrogen fertilizer rates and fertilization term application in phenological stages are shown in Table 1. The second experimental factor included four rates of Mn fertilizer (0.0, 0.5, 1.0, and 1.5 kg Mn ha^{-1}). Foliar application of Mn occurred at the stem elongation stage (BBCH 30-31) as 0.5% aqueous $MnSO_4 \cdot 5H_2O$ solution. Mineral fertilization was supplemented with a pre-sowing application of 70 kg ha^{-1} P (triple superphosphate, 20% P) and 100 kg K ha^{-1} (potash salt, 46% K).

The experimental crop was winter bread wheat (*Triticum aestivum* L.) 'Sailor', which is characterized by high yields, resistance to lodging, and high-quality grain with a high protein and gluten content. Wheat was sown with 12 cm inter-row spacing after winter triticale (× *Triticosecale* spp.) at 550 kernels m^{-2}. Wheat was sown, cultivated, and harvested in accordance with the standard agronomic requirements for this species.

Samples of ground wheat grain were mineralized in H_2SO_4 in addition to H_2O_2 as an oxidizing agent. Nitrogen was determined by the Kjeldahl method in a distillation unit (KjelFlex K-360; Büchi Labortechnik AG, Flawil, Switzerland). The micronutrient content of grain mineralized in a mixture of HNO_3 and $HClO_4$ (4:1) was determined by flame atomic absorption spectroscopy (iCE 3000 spectrometer; Thermo-Scientific, Hemel Hempstead, Hertfordshire, UK).

Oil from dried and ground wheat grain was cold pressed (20 g) with dichloromethane/methanol (2:1 v/v) (Cequier-Sánchez et al., 2008). The extracted oil was esterified to determine its fatty acid composition according to the UNE-EN ISO 12966-1:2015 guideline (UNE, 2015). Lipid samples (100 mg) were combined with 2 M NaOH (4 mL) and heated for 10 min. The BF3 complex (4 mL) and isooctane (3 mL) were then added to the boiling mixture and heated for 1 min. Immediately after removing the flask from the heat source, 20 mL 1% NaCl solution was added. Afterward, 2 mL of the upper isooctane layer was transferred to a vial and analyzed.

Table 1. Nitrogen fertilization (type and rates of N fertilization and application at phenological stages).

	Pre-sowing	Tillering (BBCH 25-29)	Stem elongation (BBCH 30-31)	Heading (BBCH 51-52)
Fertilizer rate (kg ha^{-1})	Urea 46%	Ammonium nitrate 34%	Ammonium nitrate 34%	Urea 46% foliar application with 10% solution
150	40	70	30	10
200	40	80	60	20

Chromatographic separation was performed with a gas chromatograph (7890A, Agilent Technologies, Wilmington, Delaware, USA) with a flame-ionization detector (FID) and a capillary column (30 m × 0.32 mm) (Supelco, Bellefonte, Pennsylvania, USA). The liquid phase was Supelcowax 10 and film thickness was 0.25 µm. The separation settings were carrier gas helium (flow rate 1 mL min^{-1}), detector, and injector and column temperatures were 250, 230, and 195 °C, respectively. Fatty acids were identified by comparing retention time with known standards (Supelco).

During the 3-yr experiment, weather conditions varied considerably across growing seasons and during winter dormancy (Table 2). Spring and summer precipitation levels, which have a decisive influence on grain yield and grain quality, differed considerably across years. Total precipitation between April and July ranged from 146.8 to 285.4 mm during the experiment and with a long-term mean of 246.4 mm. In April 2014 and April 2016, total monthly precipitation was below the long-term mean. Water deficit was also observed in May and June in both 2014 and 2015. In all the years of the study, precipitation levels varied the most in July. The mean monthly temperature during the most intense wheat growth (April to August) ranged from 12.9 °C in 2015 to 14.1 °C in 2016 and 14.2 °C in 2014, and the long-term mean was 14.0 °C.

The results were statistically processed with Statistica 13.0 (StatSoft, Tulsa, Oklahoma, USA) using two-way ANOVA. Basic parameters and homogenous groups were determined by Tukey's test at $p = 0.05$. Principal component analysis (PCA) was performed.

RESULTS AND DISCUSSION

The coefficients of variation relating to the content of essential micronutrients and the fatty acid composition of winter wheat grain revealed moderate variations in Zn (34.2%) and Cu (25.2%) content. In an analysis of lipid fractions, the highest value of the coefficient of variation was recorded for C18:0 (16.3%-low variation) (Table 3). During the 3-yr study, the micronutrient content of winter wheat grain was determined in the following ranges: 1.40-2.83 mg kg^{-1} Cu, 42.8-72.1 mg kg^{-1} Fe, 14.9-40.4 mg kg^{-1} Zn, and 20.6-38.5 mg kg^{-1} Mn (Table 4).

According to Smith et al. (2018), increased yield in response to mineral fertilization is accompanied by decreased Mn and Zn content in wheat grain. In a study by Jarecki et al. (2017), fertilization decreased Fe and increased Cu and Mn concentrations in the grain. High rates of N fertilizer can potentially promote the translocation of Fe and Zn in plants (Persson et al., 2009). In the work by Shi et al. (2010), N fertilization increased Cu, Fe, and Zn concentrations in winter wheat grain compared with an unfertilized control, but an increase in the N rate from 130 to 300 kg ha^{-1} did not induce further content changes in the above nutrients. The interactions between the minerals absorbed by plants are driven by ion antagonism and synergy (Sathiyavani et al., 2017). In our previous study (Stepien and Wojtkowiak, 2019), an increase in the N rate (from 150 to 200 kg ha^{-1}) had no effect on grain yield of the analyzed wheat cultivar. The above findings indicate that the grain micronutrient content should not be associated with grain yield. Many processes are responsible for micronutrient accumulation in cereal grains, including soil intake, root translocation, vegetative tissue remobilization to grains, each of which is controlled by many genes (Waters et al., 2009). In the present study, the higher N rate (200 kg ha^{-1}) applied with or without Mn foliar spray decreased wheat grain Cu content by 10% on average during the 3-yr experiment. Regardless of N fertilization, foliar application of Mn at 1.5 kg ha^{-1} decreased grain Cu content compared with the remaining fertilization treatments (not all differences were significant). An analysis of the interaction between N fertilization and Mn application

Table 2. Weather conditions for 2013-2016 and the multi-annual mean for 1981-2010.

Year	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep-Aug mean
							Temperature, °C						
2013-2014	11.3	8.9	5.0	2.3	-4.0	1.2	5.1	8.8	13.0	14.4	20.4	17.1	8.6
2014-2015	13.6	8.7	3.7	-0.4	0.4	0.5	4.2	6.7	11.8	15.5	17.5	19.8	8.5
2015-2016	13.5	6.1	4.8	3.4	-4.0	2.3	3.0	7.4	13.7	17.1	18.1	17.1	8.5
1981-2010	12.8	8.0	2.9	-0.9	-2.4	-1.7	1.8	7.7	13.5	16.1	18.7	17.9	7.9
							Rainfall, mm						Sep-Aug total
2013-2014	101.1	16.0	18.0	27.7	48.4	8.1	57.7	26.0	32.7	50.8	37.3	86.1	509.9
2014-2015	25.9	15.1	34.0	61.8	46.8	6.8	45.1	38.2	29.7	29.5	81.9	14.3	429.1
2015-2016	63.8	19.4	84.5	56.6	24.7	57.1	21.6	28.8	56.9	69.3	130.4	70.4	683.5
1981-2010	56.9	42.6	44.8	38.2	36.4	24.2	32.9	33.3	58.5	80.4	74.2	59.4	581.7

Table 3. Basic statistical measures of the micronutrient and fatty acid content of wheat grain.

Variables	Mean[1]	Standard deviation	Coefficient of variation (%)
Cu	2.14	0.541	25.2
Fe	51.7	7.47	14.5
Zn	22.7	7.765	34.2
Mn	28.4	4.79	16.9
C 16:0	23.0	0.574	2.50
C 18:0	0.911	0.149	16.3
C 18:1 c9	11.3	0.656	5.81
C 18:1 c11	0.946	0.064	6.80
C 18:2	57.7	0.941	1.63
C 18:3	4.29	0.082	1.90
SFA	24.8	0.609	2.46
MUFA	13.1	0.741	5.66
PUFA	62.1	0.990	1.59
C18:2/C18:3	13.5	0.246	1.83

[1]Micronutrient (mg kg^{-1}) and fatty acid (%) content; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

revealed that N applied at 200 kg ha^{-1} combined with foliar application of Mn at 1.5 kg ha^{-1} decreased Cu grain content in all the years of the study (32.4% on average). Diaz et al. (2008) suggested that when Cu is insufficient, the retention of this component by the ripening plant causes limited Cu retranslocation from the vegetative parts to the grain.

The grain Fe content decreased (4.2% on average) in the first 2 yr and increased (10.9%) in the third year of the experiment in response to the higher N fertilizer rate. Regardless of the N rate, the application of 1.5 kg ha^{-1} Mn led to the highest increase in grain Fe content in 2014 and 2015. In the third year of the study, the application of 1.5 kg ha^{-1} Mn increased grain Fe concentration, but the increase was significant only compared with the treatment without Mn foliar spraying. An increase in the N rate from 150 to 200 kg ha^{-1} in the control treatment and in the 1.0 kg ha^{-1} Mn treatment increased Fe accumulation in the wheat grain, but not all differences were significant. Grain Fe content also increased when the 200 kg ha^{-1} N rate was combined with 0.5 kg ha^{-1} Mn in 2016 and with 1.0 kg ha^{-1} Mn in 2015 and 2016. In a study conducted under similar conditions by Stepien and Wojtkowiak (2016), the foliar application of Mn at 0.5 kg ha^{-1} increased the Fe content of winter wheat grain.

The 200 kg ha^{-1} N rate decreased wheat grain Zn content in 2015 (11.1% on average) and increased wheat grain Zn content in 2016 (18.7% on average) compared with the 150 kg ha^{-1} N rate. According to Erenoglu et al. (2011), a higher N rate not only significantly increases Zn uptake and its translocation to vegetative organs, but also contributes to the movement of Zn from the flag leaf to the grain. In all the years of the study, wheat grain Zn content was the highest in treatments in which there was foliar application of Mn at 1.5 kg ha^{-1}, regardless of N fertilization. An analysis of the interaction between N and Mg fertilization revealed that the highest accumulation of Zn in the grain occurred when foliar application of Mn at 1.5 kg ha^{-1} was combined with 150 or 200 kg ha^{-1} N in 2014, with 150 kg ha^{-1} N in 2015 and 200 kg ha^{-1} N in 2016.

An increase in the N rate from 150 to 200 kg ha^{-1} increased the Mn content of wheat grain in all the years of the study (12.7% on average). Not all treatments with foliar application of Mn responded alike to an increase in the N rate. Nitrogen fertilization induced a clear increase in the Mn grain content only in the control treatment in 2014, control treatment and 0.5 kg ha^{-1} Mn treatment in 2015, and in all Mn treatments in 2016.

In a study by Pahlavan-Rad and Pessarakli (2009), the foliar application of 0.5% $MnSO_4$ solution increased the Mn concentration in the wheat grain, but did not affect the Fe and Zn grain content. In the work by Narwal et al. (2012), the highest accumulation of Fe and Mn in the grain was reported when Fe and Mn sulfate salts were applied as 0.5% foliar solution. These results indicate that Fe and Mn are more effectively absorbed by wheat leaves, as confirmed by their translocation from leaves to grain. An increase in the Cu and Fe grain content under the influence of Mn fertilization was also reported by Zeidan et al. (2010) and Stepien and Wojtkowiak (2016). Micronutrient concentrations in plant tissues are influenced by the mode of fertilizer application (to soil or leaves). The effectiveness of nutrient transport to the grain is also determined by phloem mobility (Fernández and Brown, 2013). Increasing or decreasing micronutrient content in plants can result from antagonistic or synergistic interactions between the forms of N supplied in fertilizers and microelements at the plant uptake stage (Diatta and Grzebisz, 2006). Page and Feller (2015) observed low remobilization

Table 4. Micronutrient content (Cu, Fe, Zn, Mn) of wheat grain under different integrated treatments from 2014 to 2016.

Year	N rate	Mn rate (kg ha^{-1})				Mean
		0.0	0.5	1.0	1.5	
	kg ha^{-1}	Cu (mg kg^{-1})				
2014	150	2.54a	2.65a	2.55a	2.65a	2.60a
	200	2.45a	2.57a	2.68a	1.67b	2.34b
	Mean	2.50a	2.61a	2.61a	2.16b	-
2015	150	2.54a	2.57a	2.43a	2.63a	2.54a
	200	2.25ab	2.37ab	2.83a	1.80b	2.31b
	Mean	2.40ab	2.47ab	2.63a	2.22b	-
2016	150	1.60b	1.43b	1.43b	2.00a	1.62a
	200	1.53b	1.40b	1.40b	1.43b	1.44b
	Mean	1.57a	1.42a	1.42a	1.72a	-
		Fe (mg kg^{-1})				
2014	150	45.6b	47.3a	46.0b	64.8a	50.9a
	200	46.8a	45.3b	49.6b	57.8a	49.9b
	Mean	46.2b	46.3b	47.8b	61.3a	-
2015	150	44.3d	45.8d	45.5d	72.1a	51.9a
	200	56.4b	45.8d	49.4c	42.8d	48.6b
	Mean	50.3b	45.8c	47.5c	57.5a	-
2016	150	49.8ef	53.6de	47.0f	56.0bcd	51.6b
	200	52.0de	58.1ab	62.0a	56.8bc	57.2a
	Mean	50.9b	55.8a	54.5a	56.4a	-
		Zn (mg kg^{-1})				
2014	150	15.4c	14.9c	19.6b	29.1a	19.8a
	200	15.3c	16.3bc	17.4bc	27.0a	19.0a
	Mean	15.4c	15.6c	18.5b	28.1a	-
2015	150	14.9c	15.4c	18.4b	26.8a	18.9a
	200	19.3b	16.0c	15.6c	16.4c	16.8b
	Mean	17.1b	15.7c	17.0b	21.6a	-
2016	150	26.8a	28.8a	27.0a	30.6a	28.3b
	200	28.6a	32.4a	32.4a	40.4a	33.6a
	Mean	27.7c	30.9ab	29.7ab	35.5a	-
		Mn (mg kg^{-1})				
2014	150	20.6d	23.6cd	26.0bc	29.5ab	24.9b
	200	26.2bc	26.9bc	29.5ab	33.8a	29.1a
	Mean	23.4c	25.3bc	27.8b	31.6a	-
2015	150	22.6de	21.8e	25.6abc	25.8abc	24.0b
	200	27.5a	24.8bc	27.0ab	24.4cd	26.0a
	Mean	25.1a	23.3b	26.3a	25.1a	-
2016	150	30.2d	29.0d	31.0cd	34.4bc	31.2b
	200	30.8cd	35.0ab	36.4ab	38.5a	35.2a
	Mean	30.5c	32.0bc	33.7b	36.5a	-

Means in two rows (same year) followed by the same letter are nonsignificant ($\alpha < 0.05$).

of Fe and Mn from leaves to grain. In a study by Curtin et al. (2008), foliar application of Mn increased Mn concentration in vegetative tissues, but induced only a minor increase in Mn grain content.

According to Ciolek et al. (2012) and Escarnot et al. (2012), the lipid content of wheat grain ranges from 1% to 3%. This is a very stable trait that is genetically conditioned (Konopka et al., 2017). In the present study, the lipid content of wheat grain was similar, ranging from 1.19% to 1.36%. Agronomic factors (N fertilization and Mn application) led to minor differences in the lipid content of wheat grain (data not shown).

The extracted oil from the wheat grain was characterized by a typical composition of fatty acids, with a predominance (~58%) of ω-6 linoleic acid (C18:2) (Table 5); this corroborates findings by Ciolek et al. (2012) and Konopka et al. (2017). The remaining fatty acids included oleic acid (C18:1c9+c11; ~12%), α-linolenic acid (C18:3, ω-3; > 4%), and palmitic acid (C16:0; 23%). In a study by Wojtkowiak et al. (2018), increasing N fertilization levels had nonsignificant effect on the fatty acid profile of wheat grain.

Table 5. Fatty acid profile (%) in grain under different integrated treatments from 2014 to 2016.

Year	N rate	Mn rate (kg ha^{-1})				Mean
		0.0	0.5	1.0	1.5	
	kg ha^{-1}	C16:0				
2014	150	23.4a	23.1a	23.4a	22.9a	23.2a
	200	22.6a	23.6a	23.5a	23.4a	23.3a
	Mean	23.0a	23.4a	23.5a	23.2a	-
2015	150	23.0a	23.1a	22.7a	22.8a	22.9a
	200	23.4a	22.9a	22.9a	23.0a	23.1a
	Mean	23.2a	23.0a	22.8a	22.9a	-
2016	150	23.2a	22.7a	22.8a	22.3a	22.8a
	200	22.9a	22.5a	22.7a	22.4a	22.6a
	Mean	23.1a	22.6a	22.8a	22.4a	-
		C18:0				
2014	150	0.697g	0.736ef	0.764d	0.719f	0.729b
	200	0.752de	0.920a	0.869b	0.818c	0.840a
	Mean	0.724d	0.827a	0.816c	0.768b	-
2015	150	0.951cd	0.941d	0.972bc	1.013a	0.969b
	200	0.985b	0.976b	1.025a	0.981b	0.992a
	Mean	0.968b	0.959b	0.999a	0.997a	-
2016	150	1.453a	1.005b	0.889c	0.891c	1.059a
	200	0.895c	0.893c	0.883c	0.857c	0.882b
	Mean	1.174a	0.949b	0.886c	0.874c	-
		C18:1 c9+c11				
2014	150	11.7a	11.7a	11.9a	12.0a	11.82a
	200	11.9a	12.0a	11.9a	11.6a	11.86a
	Mean	11.8a	11.9a	11.9a	11.8a	-
2015	150	12.4a	12.1a	12.4a	12.2a	12.30a
	200	11.9a	12.0a	12.5a	12.0a	12.10a
	Mean	12.2a	12.1a	12.5a	12.1a	-
2016	150	14.4a	12.6b	12.2b	12.6b	13.00a
	200	12.3b	12.3b	12.4b	12.5b	12.40b
	Mean	13.4a	12.5ab	12.3b	12.6ab	-
		C18:2				
2014	150	57.8a	58.3a	57.7a	58.0a	58.00a
	200	58.6a	57.6a	57.5a	58.2a	57.93a
	Mean	58.2a	58.0a	57.6a	58.1a	-
2015	150	57.5a	57.8a	57.9a	58.0a	57.80a
	200	57.4a	58.1a	57.3a	57.8a	57.70a
	Mean	57.5a	58.0a	57.6a	57.9a	-
2016	150	54.5b	57.4a	57.9a	57.9a	56.90a
	200	57.7a	58.0a	58.0a	58.0a	57.90b
	Mean	56.1b	57.7a	58.0a	58.0a	-
		C18:3 n-3+n-6				
2014	150	4.66a	4.33a	4.32a	4.29a	4.33a
	200	4.35a	4.25a	4.41a	4.35a	4.34a
	Mean	4.36a	4.29a	4.37a	4.32a	-
2015	150	4.29ab	4.34ab	4.38a	4.32ab	4.33a
	200	4.28ab	4.32ab	4.21b	4.28ab	4.27b
	Mean	4.29a	4.33a	4.30a	4.30a	-
2016	150	4.16a	4.14a	4.27a	4.29a	4.22a
	200	4.21a	4.30a	4.21a	4.27a	4.25a
	Mean	4.19a	4.22a	4.24a	4.28a	-
		Other fat fraction				
2014	150	1.66a	1.55ab	1.57ab	1.59ab	1.59a
	200	1.56ab	1.37c	1.56ab	1.51b	1.50b
	Mean	1.61a	1.46b	1.56a	1.55a	-
2015	150	1.57b	1.41cd	1.38d	1.35d	1.43b
	200	1.59b	1.49bc	1.71a	1.53b	1.58a
	Mean	1.58a	1.45b	1.54a	1.44b	-
2016	150	1.79a	1.64bc	1.65bc	1.71ab	1.70a
	200	1.63c	1.60c	1.46d	1.63c	1.58b
	Mean	1.71a	1.62b	1.56c	1.67a	-

Mean in two rows (same year) followed by the same letter are nonsignificant ($\alpha < 0.05$).

An increase in the N rate (from 150 to 200 kg ha^{-1}) increased C18:0 acid content in 2014 and 2015, and increased the total concentrations of the remaining fatty acids (C12:0, C14:0, C15:0, C16:1, C17:0, C17:1, C20:0, C20:1, C20:2, and C22:0) in 2015. The higher N rate decreased C18:0, C18:1c9+c11, and C18:2 content as well as the total concentrations of the remaining fatty acids in 2016; it decreased the content of C18:3 n-3+n-6 in 2015 and decreased the total concentrations of the remaining fatty acids in 2014.

The C18:2 content , which had the highest share of the total fatty acid pool, increased significantly in response to foliar application of Mn only in 2016. The foliar application of Mn and the interaction between Mn and N fertilization exerted varied effects on the content of the remaining fatty acid groups. The role of Mn in lipid metabolism is more complex. It increases the content of typical thylakoid membrane constituents such as glycolipids and PUFA (Salama et al., 2015). The extracted oil contained on average 62.3% PUFA, 24.5% saturated fatty acids (SFA), and 13.2% monounsaturated fatty acids (MUFA) (Table 6). The C18:2 (ω-6) to C18:3 n-3+n-6 (ω-3) ratio was 13.5:1. The recommended dietary ratio of n-6

Table 6. Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), % content, and C18:2/C18:3 ratio in wheat grain from 2014 to 2016.

		Mn rate (kg ha^{-1})				
Year	N rate	0.0	0.5	1.0	1.5	Mean
	kg ha^{-1}	Saturated fatty acids				
2014	150	24.8a	24.4a	24.8a	24.2a	24.5a
	200	23.9a	24.9a	24.9a	24.8a	24.6a
	Mean	24.3a	24.7a	24.8a	24.5a	-
2015	150	24.5a	24.5a	24.1a	24.2a	24.3a
	200	25.0a	24.4a	24.5a	24.5a	24.6a
	Mean	24.8a	24.5a	24.3a	24.4a	-
2016	150	25.3a	24.3a	24.3a	23.8a	24.4a
	200	24.4a	24.0a	24.1a	23.8a	24.1a
	Mean	24.8a	24.1ab	24.2ab	23.8b	-
		Monounsaturated fatty acids				
2014	150	12.5a	12.5a	12.7a	12.9a	12.6a
	200	12.8a	12.8a	12.8a	12.4a	13.0a
	Mean	12.6a	12.7a	12.8a	12.6a	-
2015	150	13.2a	12.9a	13.2a	12.0a	13.1a
	200	12.7a	12.8a	13.5a	12.8a	13.0a
	Mean	13.0a	12.8a	13.4a	12.9a	-
2016	150	15.5a	13.6b	13.1b	13.5b	13.9a
	200	13.2b	13.2b	13.2b	13.4b	13.3b
	Mean	14.3a	13.4b	13.2b	13.5ab	-
		Polyunsaturated fatty acids				
2014	150	62.4a	62.8a	62.1a	62.7a	62.5a
	200	62.1a	62.0a	62.2a	62.5a	62.4a
	Mean	62.7a	62.4a	62.1a	62.6a	-
2015	150	61.9a	62.3a	62.4a	62.5a	62.3a
	200	61.8a	62.6a	61.7a	62.2a	62.1a
	Mean	61.1a	62.4a	62.1a	62.4a	-
2016	150	58.8b	61.7a	62.3a	62.4a	61.3b
	200	62.1a	62.5a	62.3a	62.4a	62.3a
	Mean	60.4b	62.1a	62.3a	62.4a	-
		C18:2/C18:3 n-3+n-6				
2014	150	13.3bc	13.5ab	13.4ab	13.7a	13.4a
	200	13.5ab	13.6ab	13.0c	13.3abc	13.3a
	Mean	13.4ab	13.5a	13.2b	13.5a	-
2015	150	13.4a	13.3a	13.2a	13.4a	13.3a
	200	13.4a	13.5a	13.6a	13.5a	13.5a
	Mean	13.4a	13.4a	13.4a	13.5a	-
2016	150	13.1b	13.9a	13.6a	13.5a	13.5a
	200	13.7a	13.5a	13.8a	13.6a	13.6a
	Mean	13.4a	13.7a	13.7a	13.5a	-

Means in two rows (same year) followed by the same letter are nonsignificant (α < 0.05).

to n-3 PUFA is 4-5:1, which should not exceed 10:1 (Candela et al., 2011). Ciolek et al. (2012) confirmed that the high C18:2 (ω-6) to C18:3 (ω-3) ratio in cereal grain is genetically conditioned. The higher N rate in 2016 and application of Mn increased the proportion of PUFA and decreased the proportion of MUFA in wheat grain. The interaction between N and Mn fertilization only slightly modified fatty acid composition and the C18:2 to C18:3 n-3 + n-6 ratio in wheat grain; the observed differences were nonsignificant.

In the group of the evaluated experimental factors and variables, N and Mn fertilization were most strongly correlated with the Mn content of wheat grain (Table 7). The foliar application of Mn was also correlated with the Fe, Zn, C18:2, and PUFA concentrations in the grain. There was a negative correlation between Mn fertilization and the SFA grain content.

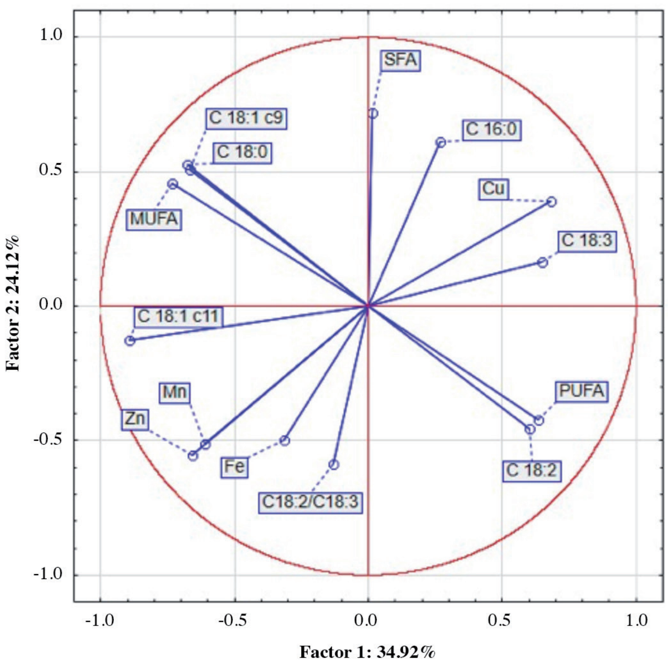
The correlations between the input variables and the principal components are graphically illustrated in Figure 1. Two groups of variables formed direction vectors. The first group consisted of C18:1c9, C18:0, MUFA, C18:1c11, Mn, Zn,

Table 7. Analysis of correlation results.

Variables	N rate	Mn rate
Cu	-0.205	-0.064
Fe	0.029	0.426*
Zn	0.054	0.378*
Mn	0.356*	0.391*
C 16:0	0.029	-0.164
C 18:0	-0.049	-0.181
C 18:1 c9	-0.185	-0.123
C 18:1 c11	-0.007	-0.059
C 18:2	0.138	0.245*
C 18:3	-0.026	0.131
Saturated fatty acids (SFA)	0.009	-0.248*
Monounsaturated fatty acids (MUFA)	-0.168	-0.115
Polyunsaturated fatty acids (PUFA)	0.124	0.246*
C18:2/C18:3 ratio	0.154	0.086

*Correlation coefficients are significant at $p < 0.05$.

Figure 1. Variable plot. Location of load vectors to two principal components.



SFA: Saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Fe, and C18:2/C18:3. The second group consisted of SFA, C18:0, Cu, C18:3, C18:2, and PUFA. The vectors designating the Zn and Mn grain content were bound by a strong positive correlation. A positive correlation was also found between the Cu and C18:3 content and between the Fe content and the C18:2/C18:3 ratio. Negative correlations were observed between the Cu content *vs*. Mn and Zn content (strong negative correlations) and Fe content (negative correlation). This indicates the antagonistic effect of Cu with Fe, Zn, and Mn, but only at a high disproportion between Cu and Zn ion contents (Rietra et al., 2017). The SFA direction vectors C16:0 and C18:3 were negatively correlated with the Mn and Zn wheat grain content.

The application of 200 kg ha^{-1} N combined with 1.0 kg ha^{-1} Mn exerted varied effects on fatty acid composition and micronutrient content in wheat grain compared with the remaining fertilizer treatments (Figure 2). The clusters formed by different fertilizer treatments were evaluated based on the distribution of objects in two-dimensional component space. Nitrogen applied at 150 kg ha^{-1} without Mn and N applied at 150 kg ha^{-1} combined with 1.0 kg ha^{-1} Mn had a similar influence on the analyzed parameters. The remaining fertilizer treatments had a similar position and exerted a similar influence on the evaluated quality attributes of wheat grain.

The projection of variables and objects onto the plane formed by the first two principal components is displayed in Figure 3. The application of 200 kg ha^{-1} N combined with 1.0 kg ha^{-1} Mn and the application of 150 kg ha^{-1} N alone were correlated with the C:18:0, C18:1c11, C18:1c9, and MUFA content in the grain. The application of 200 kg ha^{-1} N combined with 1.5 kg ha^{-1} Mn was correlated with the Fe, Zn, and Mn grain content. The remaining fertilization treatments were correlated with the C18:3, C18:2, and PUFA content and the C18:2/C18:3 ratio in grain.

Figure 2. Fertilization variants in the first two main components.

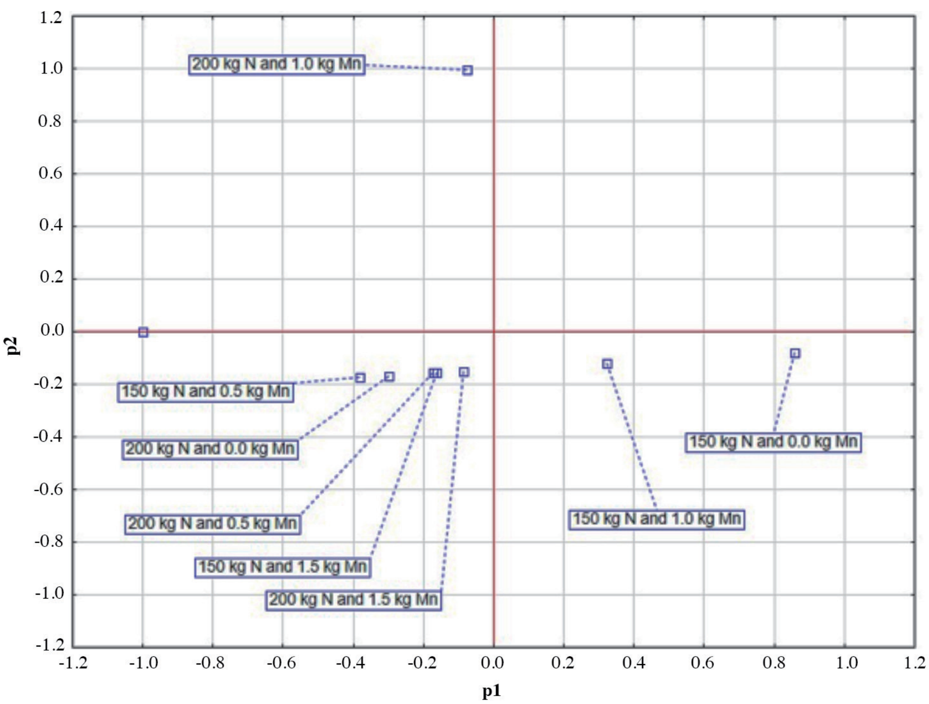
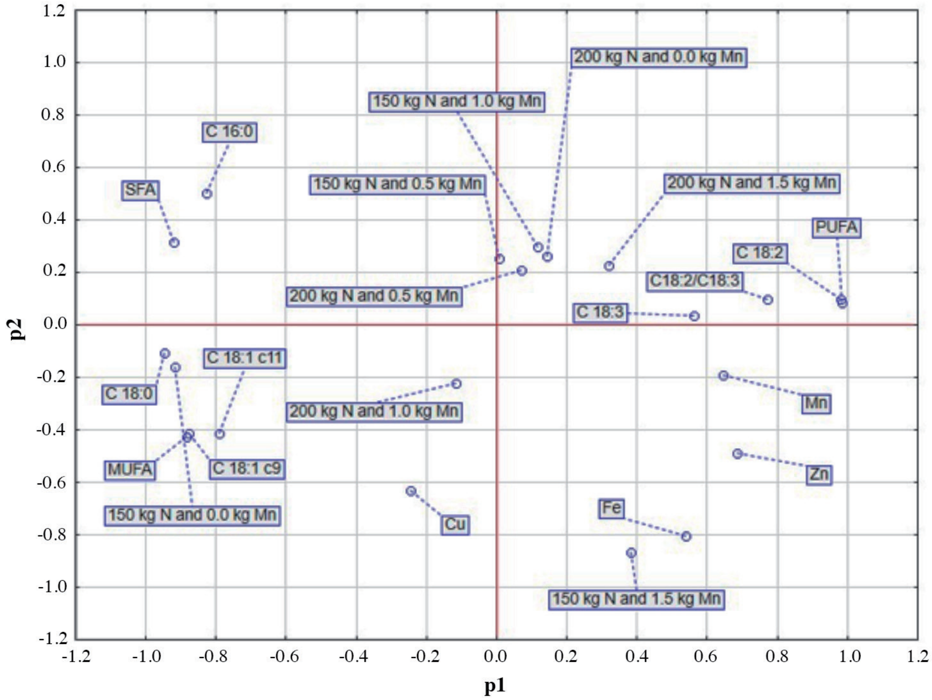


Figure 3. Projection of variables and objects on the plane of the first two main components.



SFA: Saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

CONCLUSIONS

Weather conditions have a large impact on the effectiveness of N and Mn fertilization used and on the evaluated variables. An increase in the N fertilizer rate from 150 to 200 kg ha^{-1} increased Mn content. Regardless of N fertilization, foliar application of Mn at 1.5 kg ha^{-1} contributed to the greatest increase in the Zn and Fe grain content. The position of vectors of such properties as the Zn and Mn content in the grain is strong and positively correlated with each other. Negative correlations were found between the Cu content and Mn content as well as Zn and Fe in wheat grain. There is a positive correlation between the Cu content with C18: 3 and between the Fe content and the C18:2/C18:3 acid ratio. Vector variables of saturated fatty acids (SFA) and C16: 0 and C18: 3 acids are negatively correlated with the content of Mn and Zn and Fe in the wheat grain. Increased Fe, Zn, and Mn content can be expected when using 150 kg ha^{-1} N and 1.5 kg ha^{-1} Mn, and Cu using 150 kg ha^{-1} N (without Mn) and 200 kg ha^{-1} N and 1.0 kg ha^{-1} Mn. Results indicated that the N fertilizer application at 200 kg ha^{-1} used in the present study beneficially affected polyunsaturated fatty acid (PUFA) content in the winter wheat grain and can therefore be used to obtain raw material with increased nutritional value. The human organism does not synthesize PUFA, and they must be taken with food (or supplements) and plant origin food such as winter wheat grain that can also be a good source because it contains more than 60% PUFA.

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