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ORIGINAL PAPER

Mineral composition and protein quality of organically grown ancient wheat under reduced tillage

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Abstract

Ancient, nutritionally valuable plant species and environmentally friendly agricultural technologies are gaining more and more interest from consumers and producers. The study objective was to compare macro- and microelements and the amino acid composition of the grain of ancient wheat species *Triticum sphaerococcum* and *T. persicum* – against common wheat, each grown both under a plowing and reduced, shallow tillage. The wheat grains came from field experiments located in three certified organic farms, in Poland. It was shown that the mineral composition of the grain depended to the greatest extent on the wheat genotype. Ancient wheat *T. sphaerococcum,* and *T. persicum*, compared to the common wheat, contained more P, as well as Fe, Zn and Cu. For both tillage systems, the total content of exogenous amino acids in the grain protein was far greater for *T. persicum* and *T. sphaerococcum* than for the common wheat; furthermore, higher content of exogenous amino acids was obtained from plowing tillage for *T. persicum* and shallow tillage for *T. sphaerococcum*. The nutritional value of the cereal protein, expressed with EAAI, was higher in *T. sphaerococcum* than in the common wheat. As compared to the contents of individual amino acids in common wheat, *T. persicum* grain protein had more Met, Ser, Pro and Tyr, whereas *T. sphaerococcum* had more Arg, Asp, Prol and Gly. The results indicate that *T. persicum* and *T. sphaerococcum* are valuable sources of macro- and microelements as well as protein, which indicates their suitability for production of food with increased nutritional value.

Keywords: nutritional value, grain, exogenous amino acids, ancient grain, Indian dwarf wheat, Persian wheat

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INTRODUCTION

Many producers and processors of foods see organic production as an opportunity to develop and fill a market niche that has been created by increasing consumer interest in high-quality, organic food (Kuś et al. 2010). It is emphasized that organic produce has greater health-promoting traits, which generally result from its higher nutritional value and much lower levels of associated pollution than conventional cultivation (Worthington 2001). Regardless of the farming system (conventional, organic), rising production costs are leading to the increasing use of shallow tillage (instead of plowing) – Beach et al. (2018), Fernandez et al. (2019*a*,*b*); such method combines aspects of environmental protection with economic and organizational benefits to farms. Grain obtained from various cultivation technologies must be of high quality, and appropriate to the type of its further use.

Due to the widespread consumption of cereal products, an important aspect of grain quality assessment is the content of macro- and especially microelements. It is known that these ingredients play an important role in metabolic processes, but it turns out that the problem of their deficiency in the diet is common. It is estimated that more than three billion people consume insufficient amounts of micronutrients, and this number is steadily increasing (Welchet al. 1997). One of the reasons for this deficit is the low content of macro and microelements in grain (Hurrel 2000), hence the direction of research on the assessment of this feature of cereal grains. Previous studies have shown higher content of macro and microelements in the primary genotypes of wheat, such as einkorn wheat (*T. monococcum)* – Rachoń et al. (2015), emmer (*T. dicoccum*) – Erba et al. (2011), spelt wheat (*T. spelta* L.) – Piergiovanni et al. (1997). However, some of these species are characterized by a very low yield, and moreover the grain is hulled, which limits their economic use. So far, primary wheat species such as *T. sphaerococcum* and *T. persicum*, with naked grain, useful for cultivation in the conventional and organic farming system (Szczepanek et al. 2020, 2023), have not been evaluated.

In addition to the genotype, an important role in shaping the mineral composition of grain is also played by environmental conditions, soil type, and agricultural technology. Previous studies indicate, among others, significant influence of farming system (Toader et al. 2019), fertilization (Bulut et al. 2022), and tillage methods (Dolijanović et al. 2019, 2022). Genetic, environmental and agrotechnical factors also affect other quality characteristics of cereal grains, including protein content and quality (Smolková et al. 2000, Gálová and Knoblochová 2001, Andruszczak 2017). Protein is the basic nutrient in cereal grains (Poutanen et al. 2021) and the quality is evaluated in terms of which amino acids it comprises and in what proportions (Harasim 2011, Crista et al. 2013, Spychaj-Fabisiak et al. 2014, Filipek, Siddiqi et al. 2020,). Some of the amino acids the human body can synthesize for itself, but others must come from the diet. Human, and animals are not able to synthesize the following nine amino acids: Lys, Thr, Met, Phe, Trp, Ile, Leu, Val, and His. Lys is the limiting amino acid in cereal grain, meaning that a low level of lysine suppresses efficient protein metabolism if no supplementary source of Lys is included in the meal (Anjum et al. 2005). Moreover, the content of the Met is not at the satisfactory level achieved by the breeding of cereals (Laze et al. 2019).

More and more demanding consumers are looking for products with high nutritional values and pro-health effects. Ancient cereals can be a valuable source of nutrients, but a comprehensive diagnosis of the parameters of the chemical composition of grain obtained from various production technologies is needed (Bavec, Bavec 2006, Arzani, Ashraf 2017).

The research objective was to compare the mineral composition and the amino acid composition of the ancient cereal grains i.e. Indian dwarf wheat (*Triticum sphaerococcum Percival*) and Persian wheat (*Triticum persicum* Vavilov), in comparison to common wheat (*Triticum aestivum* ssp. vulgare), grown as part of an organic farming approach when each was subjected to plowing and shallow tillage (limited to shallow surface cultivation).

MATERIALS AND METHODS

Site description

The grain for the study was taken from the strict field experiments conducted in 2019 on three certified organic farms in Budziszewo (Kuyavia--Pomerania Province, 53°37′N; 19°12′E), Zblewo (Pomerania Province 53°93′N; 18°31′E) and Grabina Wielka (Greater Poland Province, 52°11′N; 18°80′E) – Figure 1. The previous crop was winter cereals (wheat or triticale), after which a catch crop with the participation of legumes was grown. In Zblewo and Grabina Wielka the soils are classified to the Haplic Luvisol, while in Budziszewo to the Cutanic Luvisol (IUSS-WRB 2015).The soil in Grabina Wielka had a slightly acid reaction (pH of KCl 5.9) and contained 64.5 mg kg^{-1} P, 182.6 mg kg^{-1} K, 57.0 mg kg^{-1} Mg (available forms), and 1.63% C_{org}. By contrast, the soils in Budziszewo and Zblewo had a neutral reaction (pH KCl: 6.8 and 6.7, respectively) and contained: 99.0 and 40.5 mg kg^{-1} P, 193.4 and 169.3 mg kg^{-1} K, 110.0 and 51.0 mg kg^{-1} Mg (available forms), and 1.92 and 0.97% C_{org}.

The content of available forms of K and P was determined using the Egner-Riehm's method (DL), available forms of Mg using Schachtschabel's method and $C_{org.}$ content – with a Vario Max CN analyzer.

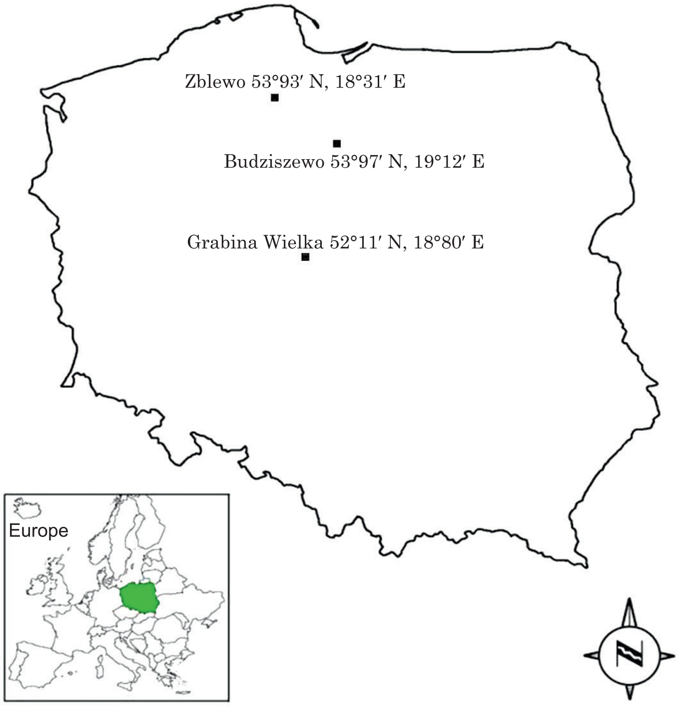

Fig. 1. Locality of field experiments (Poland)

Hydrothermal conditions

In the region where the research was conducted, the vegetation period for cereals starts at the end of March and lasts until late July or early August. Weather conditions vary quite widely from place to place during this period (Figure 2). The sum of precipitation in March and April was smallest in Budziszewo (32.1 mm), while in Grabina Wielka and Zblewo it was nearly 50% greater. From May to July, rainfall was greatest in Zblewo and slightly less in Budziszewo (187 and 170.1 mm, respectively). During this period, the sum of precipitation was smallest in Grabina Wielka (119.1 mm). Monthly average air temperatures during the growing season (from March to July) were lowest in Zblewo (March 5.8°C, April 8.6°C, May 11.9°C, June 20.0°C, July 18.0°C), while in the villages of Grabina Wielka and Budziszewo, temperatures were slightly higher (from 0.3 in March to 2.3°C in June).

Agrotechnical practice

In mid-March, the entire surface of the field was prepared for sowing using a tilling set with a cultivator and string roller. Before sowing, Bioilsa fertiliser approved for organic crops was used at 200 kg ha^{-1} (12 N, 10 P_2O_5, 26 K_2O, 4 MgO, and 20 SO_3 kg ha^{-1}). Bioilsa 6-5-13 is a multi-component NPK (MgS) fertilizer with controlled release of ingredients. Thanks to this,

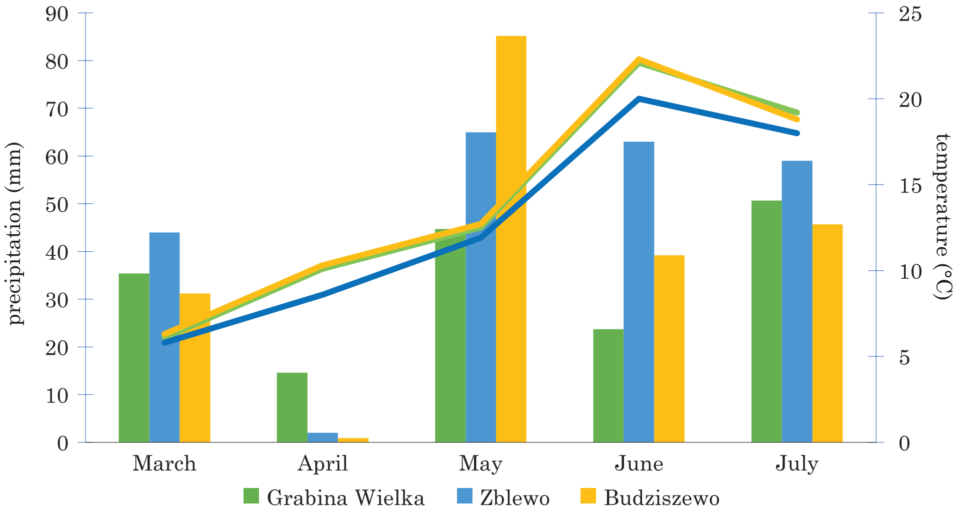

Fig. 2. Mean air temperature and precipitation at experimental sites

the components contained in the fertilizer are more effectively absorbed by plants and are subject to less losses compared to traditional fertilizers. The manufacturer is NaturalCrop. The wheat was sown in the last days of March or in the first days of April using a tine coulter (Budziszewo, Zblewo) or disc coulter (Grabina Wielka) with a narrow row spacing (10.5-11.5 cm, depending on the type of seeding machine available on the farm). The sowing density was 600 grains per sq. m. Weeding harrowing was carried out before wheat emergence and at the beginning of tillering. The grains were harvested at full grain maturity using a Wintersteiger plot combine.

Experimental treatments

The experiment was established in a split-plot design, in four replications. The whole plots consisted of tillage cultivation method: plowing or shallow tillage, and subplots consisted of three wheat species: common wheat (*Triticum aestivum* ssp. *vulgare*), Indian dwarf wheat (*Triticum sphaerococcum* Percival), and Persian wheat (*T. persicum* Vavilov).

Before winter (late November), on the part of the field, pre-winter plowing to a depth of 22-24 cm was performed, while on the other part of the field designated for shallow tillage, only shallow (10-12 cm) cultivation was performed using a cultivator. For the plowing, the topsoil was fully inverted, while for the shallow tillage system, the forecrop residue was only mixed into the topsoil.

The cultivars used in the study were: common wheat (*Triticum aestivum* ssp. *vulgare*) cv. Torridon, Indian dwarf wheat (*Triticum sphaerococcum* Percival) cv. Trispa. and Persian wheat (*Triticum persicum* Vavilov) cv. Persa (Figure 3). The Torridon cultivar is classed as quality wheat. It is characte-

Fig. 3. Persian wheat (*Triticum persicum* Vavilov) on the left, and Indian dwarf wheat (*Triticum sphaerococcum* Percival) on the right (fot. M. Szczepanek)

rized by high grain yield, a medium thousand-seed weight, and quite a high bulk density. It has a high protein content, as well as a high falling number and a high sedimentation index.

The cultivar Trispa of Indian dwarf wheat develops long, rather stiff stems. The aboveground part of the plant is covered with a wax coating (very strong on the flag leaf sheath, medium on the blade). The spikelets are two- or three-flowered, one- or two-grained. It has short awns on the ear. The grain of Indian dwarf wheat is naked, rounded, and red (Szczepanek et al. 2022). The cultivar Persa of Persian wheat has delicate, long (65-80 cm) stems, very susceptible to lodging. There is no wax coating on the leaf blades. The medium-long ear (7-8 cm) is brownish, loose, and awned (awn length approx. 5 cm). The spikelets are two- or three-flowered, one- or two-grained. Persian wheat is a naked form. Persian wheat grain is elongated, and red in color. The cultivars Trispa and Persa used in our field experiments have been bred by the Bydgoszcz University of Science and Technology in 2020. The Breeder's Right for these varieties is granted by the director of the Research Centre for Cultivar Testing and is exercisable on the territory of Poland (national Plant Breeders' Rights protection level). Previous studies have shown that the grain of Persian wheat, similarly to that of Indian dwarf wheat, is characterized by a much higher (compared to other wheat species) content of phenolic acids and alkylresorcinols, with proven pro-health effects (Skrajda-Brdak et al. 2020).

Determination of mineral composition

Grain samples were wet mineralized in concentrated sulfuric acid, and the content of total nitrogen was determined based on a modified Berthelot reaction. In brief, after dialysis against a buffer solution of pH 5.2, ammonia in the sample is chlorinated to monochloramine, which reacts with salicylate to form 5-aminosalicylate. Following oxidation and oxidative coupling, a green complex is formed. The absorption of the complex is measured at 660 nm (Skalar SANplus flow analyzer), and total phosphorus is determined with the method employing ammonium molybdate (Skalar SANplus flow analyzer). The content of K and Ca was determined by flame photometry, and that of Mg was assayed with the Atomic Absorption Spectrometry (AAS) method.

The content of Mn, Fe, Zn and Cu in grain were determined by standard atomic absorption spectrometry (ASA), following mineralization in a mixture of concentrated hydrochloric and nitric acids in a 1:3 ratio. ASA was carried out using a VARIAN AA240FS fast sequential atomic absorption spectrometer.

Assessment of amino acid contents

Grain samples for assessing amino acid content were taken in four separate repetitions for each experimental variant, 4-6 weeks after harvesting. The share of amino acids in the protein of the studied wheat cultivars was determined using an AAA-400 amino acid analyzer after prior hydrolysis with 6 M HCl at 110°C. Additionally, the sulfur-containing amino acids were hydrolysed with 6 M HCl after oxidation with a mixture of formic acid and hydrogen peroxide in a ratio of 9:1.

The established amino acid compositions of grain proteins were used in calculating the overall totals of amino acids and the total contents of exogenous and endogenous amino acids. The following were also calculated:
the integrated index of exogenous amino acids (EAAI) – FAO (2013):

$$EAAI = (c_1/c_{01} x c_2/c_{02} x \ldots x c_n/c_{0n})^{1/n};$$

the chemical score (CS) index of limiting amino acids (FAO 2013):

$$CS = (c_i/c_{0i}) \cdot 100\%,$$

where: $c_1, c_2, \ldots, c_n$ – the content of successive exogenous amino acids in the examined protein,
$c_{01}, c_{02}, \ldots, c_{0n}$ – the content of successive exogenous amino acids in the reference protein (chicken egg protein, adopted according to the model recognised by WHO/FAO/UNU (2007).

Statistical analyses

The basic statistical descriptors included mean values and standard deviation (±SD). The normality of the distribution was tested with Kolmogorov-Smirnov test, while equality of variance in different samples with the Levene test. Multifactorial analysis of variance was used to find significant differences between the means and in the case of significant differences Tukey posthoc test was employed (Stanisz 2006). The statistical calculations mentioned above were carried out with Statistica (Dell, Round Rock, TX, USA, 2019) software.

RESULTS AND DISCUSSION

Research so far shows that old wheat species are rich in macro and microelements. Golea et al. (2022) report that the ancient species einkorn wheat and spelt wheat were characterized by higher total mineral content than the common wheat. The authors pay special attention to the high amounts of Fe and Z in these ancient species. Other studies on einkorn wheat proved to be the richest in P, K, Mg, Ca, Cu, Zn, Fe and Mn as compared to modern cultivar of *T. aestivum* and *T. durum* (Rachoń et al. 2015). These widely cultivated wheats also had lower contents of Mg, P, and Zn compared to the ancient species emmer wheat (*T. dicoccum)* – Piergiovanni et al. (1997). Similarly, our research showed a significant effect of wheat genotype on the content of macronutrients P and K in grain. The ancient genotypes Persian wheat and Indian dwarf wheat, regardless of the tillage method, contained significantly more P in grain compared to common wheat, by 28.2 and 20.5%, respectively (Table 1). In addition, Persian wheat was distinguished by a higher content of K compared to Indian dwarf wheat. However, no effect of genotype on the concentration of Ca and Mg in grain was found. The content of microelements Cu, Fe and Zn, as well as some macroelements, depended on the wheat genotype (Table 2). On average, for farming system, ancient Persian wheat and Indian dwarf wheat accumulated more Cu (39.4% and 24.2%, respectively) and Fe (31.5% and 51.3%, respectively) compared to common wheat. The content of Zn, regardless

Table 1

The content of P, K, Ca, and Mg in the grain of *Triticum aestivum* cv. Torridon, *T. persicum* cv. Persa, and *T. sphaerococcum* cv. Trispa

Tillage method	Wheat species	P	K	Ca	Mg
		(g kg^{-1} DM)			
Plowing	*T. aestivum*	4.0±0.2 *bc*†	4.0±0.3 *abc*	0.4±0.1 *a*	1.2±0.1 *a*
	T. persicum	4.9±0.4 *a*	4.5±0.4 *a*	0.4±0.1 *a*	1.3±0.2 *a*
	T. sphaerococcum	4.8±0.2 *a*	3.8±0.5 *bc*	0.4±0.1 *a*	1.2±0.1 *a*
Shallow tillage	*T. aestivum*	3.9±0.2 *c*	3.9±0.3 *abc*	0.4±0.1 *a*	1.2±0.2 *a*
	T. persicum	5.2±0.3 *a*	4.1±0.7 *ab*	0.4±0.1 *a*	1.2±0.3 *a*
	T. sphaerococcum	4.7±1.2 *ab*	3.4±0.7 *c*	0.4±0.1 *a*	1.3±0.1 *a*
Mean	*T. aestivum*	3.9±0.2 *B*	4.0±0.3 *AB*	0.4±0.1 *A*	1.2±0.1 *A*
	T. persicum	5.0±0.4 *A*	4.3±0.6 *A*	0.4±0.1 *A*	1.3±0.2 *A*
	T. sphaerococcum	4.7±0.8 *A*	3.6±0.6 *B*	0.4±0.1 *A*	1.3±0.1 *A*
Plowing		4.6±0.2 *A*	4.1±0.3 *A*	0.4±0.1 *A*	1.2±0.2 *A*
Shallow tillage		4.6±0.2 *A*	3.8 ±0.4 *B*	0.4±0.1 *A*	1.3±0.1 *A*

† Mean values ± standard deviation (SD) in a column followed by different letters indicate significant differences between treatments at $p \leq 0.05$.

Table 2

The content of Cu, Fe, Mn and Zn in the grain of *Triticum aestivum* cv. Torridon, *T. persicum* cv. Persa, and *T. sphaerococcum* cv. Trispa

Tillage method	Wheat species	Cu	Fe	Mn	Zn
		(mg kg^{-1} DM)			
Plowing	*T. aestivum*	3.2±0.3 *b*†	77.4±31.6 *b*	27.4±12.3 *a*	22.9±1.2 d
	T. persicum	4.7±0.6 *a*	99.7±25.1 *ab*	23.8±7.4 *a*	47.1±3.3 *a*
	T. sphaerococcum	4.1±0.5 *ab*	114.7±15.8 *ab*	27.4±4.9 *a*	36.7±1.9 *c*
Shallow tillage	*T. aestivum*	3.3±0.6 *b*	80.6±34.3 *b*	30.6±13.3 *a*	24.5±1.1 d
	T. persicum	4.4±1.0 *a*	108.0±35.6 *ab*	24.7±4.2 *a*	42.5±4.5 *b*
	T. sphaerococcum	4.0±0.8 *ab*	124.4±25.2 *a*	28.1±4.6 *a*	35.2±2.3 *c*
Mean	*T. aestivum*	3.3±0.4 *B*	79.0±32.0 *B*	29.0±12.5 *A*	23.7±1.4 *C*
	T. persicum	4.6±0.8 *A*	103.9±30.2 *A*	24.3±5.8 *A*	44.8±4.5 *A*
	T. sphaerococcum	4.1±0.6 *A*	119.5±21.0 *A*	27.7±4.6 *A*	35.9±2.2 *B*
Plowing		4.0±0.5 *A*	97.2±30.0 *A*	26.2±8.0 *A*	35.6±2.2 *A*
Shallow tillage		3.9±0.7 *A*	104.3 ±35.0 *A*	27.8±10.0 *A*	34.1±3.1 *A*

† Mean values ± standard deviation (SD) in a column followed by different letters indicate significant differences between treatments at $p \leq 0.05$.

of the method of tillage, was significantly higher in Persian wheat, compared to Indian dwarf wheat and common wheat. The differences in the content of this microelement in favor of Persian wheat were 24.8% and 89.0%, respectively. It is also worth emphasizing that Persian wheat was characterized by a higher Zn content than Indian dwarf wheat grain.

Literature data show that the chemical composition of cereal grains depends on habitat conditions, genotype, and agronomic practices as well as their interaction (Erba et al. 2011, Rachoń et al. 2015, Bulut et al. 2022, Dolijanović et al. 2022). Some researchers (Toader et al. 2019) report that grain with an increased content of macro and microelements (ash) can be obtained from the organic cultivation of common wheat. However, the reactions of individual varieties on farming system (organic, conventional) may be different (Mazzoncini et al. 2015, Mitura et al. 2023). According to Atar (2019) there are two reasons for the higher micronutrient concentrations of ancient wheat: lower kernel weight and genetic potential. In turn, Piergiovanni (1997) suggests that the highest content of macro and microelements in ancient wheat may also result from their greater efficiency in their uptake. According to Golea et al. (2022) the contents of macro and microelements depend more on the yield than on whether wheat varieties belong to ancient or modern species. The wheat producing higher grain yield may have lower contents of macro and microelements because of a dilution effect.

In our research, tillage methods generally did not differentiate the content of mineral components in the grain of the examined wheat species, with the exception of potassium. The amount of this component was significantly higher in grain from the plowing system compared to shallow tillage. This method of cultivation probably was conducive to better development of the root system and uptake of this component from soil organic matter. On the contrary, Stankowski et. al. (2016) state that the grain of spelt (*Triticum spelta* L.) cultivated according to the simplified tillage system was marked by an increased content of mineral elements (potassium and manganese). Similarly, Dolijanovic et al. (2019, 2023) claim that contents of the macro and microelements in the wheat grain are significantly higher under reduced tillage (chisel plowing). However, in this tillage system, low nitrogen doses were applied. As a consequence, it had a better effect on the increase of the content of Ca, Cu, Fe, K, Mg, Mn, P, and Zn in the wheat grain than the conventional tillage (moldboard plowing) which applied higher nitrogen rates.

In our study on the amino acid composition of the grain protein of three wheat species from organic cultivation, a significant effect of the wheat species/cultivar and its interaction with soil tillage methods on the content of exogenous amino acids was shown (Table 3, 4). Based on averaged contents across both tillage methods, the Persian wheat had significantly more Met, Leu, and Phe than the common wheat (by 17.8, 9.83, and 13.0%, respectively). Similarly, the Phe content in grain protein of Indian dwarf wheat was significantly higher (by 10.4%) than in the common wheat. In turn, the differences in the content of Met and Leu in Indian dwarf wheat and common wheat although quite large (amounting to 10.3% and 7.6%, respectively) were not statistically proven.

Table 3

The content of Met, Ile, Leu, and Phe in the grain protein of *Triticum aestivum* cv. Torridon, *T. persicum* cv. Persa, and *T. sphaerococcum* cv. Trispa

Tillage method	Wheat species	Met	Ile	Leu	Phe
		(g kg^{-1} DM)			
Plowing	*T. aestivum*	10.7±0.4 *b*†	24.4±2.4 *a*	56.6±3.3 *b*	34.7±1.6 *b*
	T. persicum	12.7±1.9 *a*	25.5±2.4 *a*	63.3±8.6 *a*	41.5±2.7 *a*
	T. sphaerococcum	12.1±0.6 *a*	24.5±1.3 *a*	55.6±1.4 *b*	36.0±2.9 *b*
Shallow tillage	*T. aestivum*	10.7±0.3 *a*	24.3±1.7 *a*	54.1±1.8 *b*	34.6±1.1 *b*
	T. persicum	12.6±2.2 *a*	27.2±3.2 *a*	57.3±4.9 *ab*	36.9±2.5 *b*
	T. sphaerococcum	11.5±1.9 *a*	26.7±4.9 *a*	61.5±8.4 *a*	40.6±4.2 *a*
Mean	*T. aestivum*	10.7±0.4 *B*	24.4±2.0 *A*	54.9±1.8 *B*	34.7±1.3 *B*
	T. persicum	12.6±2.0 *A*	26.4±2.9 *A*	60.3±7.5 *A*	39.2±3.5 *A*
	T. sphaerococcum	11.8±1.4 *AB*	25.6±3.7 *A*	59.1±6.7 *AB*	38.3±4.3 *A*
Plowing		11.8±1.4 *A*	24.8±2.1 *A*	58.5±6.2 *A*	37.4±3.8 *A*
Shallow tillage		11.6 ±1.8 *A*	26.1 ±3.6 *A*	57.6±6.3 *A*	37.4±3.8 *A*

† Mean values ± standard deviation (SD) in a column followed by different letters indicate significant differences between treatments at $p \leq 0.05$.

Table 4

The content of His, Lys, Thr, Val and Arg in the grain protein of *T. Triticum aestivum* cv. Torridon, *T. persicum* cv. Persa, and *T. sphaerococcum* cv. Trispa

Tillage method	Wheat species	His	Lys	Thr	Val	Arg
		(g kg^{-1} DM)				
	T. aestivum	19.6±2.4 *a*†	21.5±0.8 *a*	24.7±2.7 *a*	32.8±4.7 *a*	37.1±0.6 *b*
Plowing	*T. persicum*	18.8±3.3 *a*	23.7±6.5 *a*	24.1±2.3 *a*	32.8±3.5 *a*	42.0±6.6 *a*
	T. sphaerococcum	18.2±5.1 *a*	19.4±2.9 *a*	25.6±2.7 *a*	31.1±3.5 *a*	42.0±0.9 *a*
Shallow tillage	*T. aestivum*	19.7±0.9 *a*	21.4±1.6 *ab*	24.9±1.9 *a*	32.4±2.5 *a*	36.2±1.1 *b*
	T. persicum	17.8±3.7 *a*	20.1±1.6 *b*	23.6±2.5 *a*	33.3±3.3 *a*	41.2±4.7 *ab*
	T. sphaerococcum	18.5±2.1 *a*	22.0±1.0 *a*	25.7±2.8 *a*	32.8±3.3 *a*	46.3±8.5 *a*
Mean	*T. aestivum*	19.6±1.8 *A*	21.4±1.2 *A*	24.8±2.3 *A*	32.6±3.6 *A*	36.6±1.0 *B*
	T. persicum	18.3±3.4 *A*	21.9±5.0 *A*	23.9±2.3 *A*	33.1±3.3 *A*	41.6±5.6 *A*
	T. sphaerococcum	18.3±3.8 *A*	20.7±2.5 *A*	25.7±2.6 *A*	32.0±2.8 *A*	44.1±6.3 *A*
Plowing		18.9±3.7 *A*	21.6±4.4 *A*	24.8±2.6 *A*	32.2±3.5 *A*	40.3±4.4 *A*
Shallow tillage		18.7 ±2.5 A	18.7 ±2.5 *A*	21.1 ±1.6 *A*	24.7 ±2.5 *A*	32.9 ±3.0 *A*

† Mean values ± standard deviation (SD) in a column followed by different letters indicate significant differences between treatments at $p \leq 0.05$.

According to earlier research results, the amino acid composition of wheat proteins depends on species, ploidality, and genome. In the studies of Pepo and Gyori (2007), 1.4 times greater content of Met was found in *T. spelta* than in *T. aestivum*, with the highest content of Met and Arg being found in *T. beoticum*. Different results were obtained by Galova and Knoblochowa (2001)] and by Smolkowa et al. (2000). In their studies, the ranges of values for amino acids were similar between spelt and common wheat. In turn, studies of ten organically grown soft wheat genotypes showed that some amino acids, including Arg and Met, differed significantly between cultivars (Laze et al. 2019). Conversely, research on winter spelt (*T. spelta*) found that amino acid contents did not differ statistically significantly between cultivars (Smolková 2000).

In our studies, under plowing tillage, contents of Leu, Phe, and Arg in grain protein were higher for Persian wheat than for common wheat. In turn, under shallow tillage, Leu, Phe, and Lys contents were higher in Indian dwarf wheat than in common wheat. However, neither tillage method nor species was found to influence the contents of Ile, His, Thr, or Val in the grain protein of the examined wheat species. On average, for both tillage system, the total content of exogenous amino acids in the protein was far greater for the Persian wheat and Indian dwarf wheat than for the common wheat; furthermore, higher exogenous protein contents were achieved with plowing tillage for the Persian wheat, and shallow tillage for the Indian dwarf wheat (Table 5). When performing a qualitative assessment of wheat grain protein, the impact of fertilization on amino acid composition is usually

Table 5

Total amino acids sum and exogenous and endogenous amino acids sum in the grain protein of *Triticum aestivum* cv. Torridon, *T. persicum* cv. Persa, and *T. sphaerococcum* cv. Trispa

Tillage method	Wheat species	Total sum of amino acids	Sum of exogenous amino acids	Endogenous amino acids sum
		(g kg^{-1} DM)		
Plowing	*T. aestivum*	819.1±59.1 *a*†	261.1±11.4 *b*	557.9±48.2 *a*
	T. persicum	855.2±12.4 *a*	281.6±15.0 *a*	573.6±19.4 *a*
	T. sphaerococcum	840.5±44.0 *a*	265.5±12.4 *b*	574.9±37.6 *a*
Shallow tillage	*T. aestivum*	825.7±27.9 *a*	258.2±9.3 *b*	567.5±19.5 *a*
	T. persicum	844.7±9.3 *a*	273.3±8.2 *ab*	574.7±13.2 *a*
	T. sphaerococcum	848.0±23.9 *a*	284.3±20.2 *a*	563.8±30.8 *a*
Mean	*T. aestivum*	822.4±45.0 *B*	259.7±10.2 *B*	562.7±36.0 *A*
	T. persicum	849.9±11.9 *A*	277.4±12.5 *A*	574.2±16.1 *A*
	T. sphaerococcum	844.3±34.6 *AB*	274.9±18.9 *A*	569.4±33.8 *A*
Plowing		838.2±44.1 *A*	269.4±15.4 *A*	568.8±36.4 *A*
Shallow tillage		839.5 ±23.3 *A*	271.9 ±17.1 *A*	568.7 ±22.0 *A*

† Mean values ± standard deviation (SD) in a column followed by different letters indicate significant differences between treatments at $p \leq 0.05$.

analyzed (Weber et al. 2008, Filipek, Harasim 2011, Crista et al. 2013). The influence of the tillage method on the content of individual amino acids has not been widely investigated, and for ancient wheat species is limited to research on spelt (Andruszczak 2017). Eight winter spelt cultivars were compared in two tillage systems (plowing and reduced tillage). In the plowing tillage system, skimming and harrowing were done after the harvest of the previous crop. Pre-sowing plowing with harrowing was carried out three weeks before sowing. In the reduced tillage treatment, cultivating and harrowing were done after the harvest of the previous crop and before sowing. That study did not show any differences in amino acid content as affected by the soil tillage system.

In our studies, both the wheat species and its interaction with the tillage method have been proven to significantly influence the content of endogenous amino acids in the grain protein (Tables 6, 7). Based on averaged contents across both tillage methods, Persian wheat had more Ser, Pro, and Tyr than common wheat, while Indian dwarf wheat had more Asp, Pro, and Gly than common wheat. Comparing the concentrations of the above-mentioned amino acids between seventeen species of the genus Triticum (WHO/FAO/UNU), the diversity between species is greatest for Pro.

The content of Tyr was greater in Indian dwarf wheat grain protein than in common wheat under shallow tillage only. The comparison of Persian wheat against Indian dwarf wheat showed that, under plowing, the content

Table 6

The content of Asp, Glu, Ser, and Pro in the grain protein of *Triticum aestivum* cv. Torridon, *T. persicum* cv. Persa, and *T. sphaerococcum* cv. Trispa

Tillage method	Wheat species	Asp	Glu	Ser	Pro
		(g kg^{-1} DM)			
Plowing	*T. aestivum*	42.8±3.4 *b*†	318.6±44.3 *a*	37.1±3.4 *a*	79.8±6.5 *b*
	T. persicum	44.7±3.3 *ab*	313.7±14.0 *a*	39.9±5.3 *a*	91.4±5.1 *a*
	T. sphaerococcum	47.0±0.6 *a*	316.2±28.4 *a*	37.5±6.6 *a*	90.9±3.1 *a*
Shallow tillage	*T. aestivum*	43.4±3.4 *a*	322.5±22.4 *a*	38.1±3.4 *ab*	89.0±3,9 *a*
	T. persicum	44.9±3.5 *a*	315.0±9.0 *a*	41.4±4.0 *a*	90.8±6.7 *a*
	T. sphaerococcum	45.8±3.8 *a*	312.0±28.9 *a*	35.6±4.1 *b*	83.0±9.4 *a*
Mean	*T. aestivum*	43.1±3.3 *B*	320.6±34.1 *A*	37.6±3.4 *AB*	81.4±8.0 *B*
	T. persicum	44.8±3.3 *AB*	314.4±11.4 *A*	40.7±4.6 *A*	91.1±5.8 *A*
	T. sphaerococcum	46.4±2.7 *A*	314.1±27.9 *A*	36.6±5.4 *B*	90.0±3.6 *A*
Plowing		44.8±3.2 *A*	316.2±30.3 *A*	38.2±5.2 *A*	87.4±7.3 *A*
Shallow tillage		44.7 ±3.5 *A*	316.5 ±21.4 *A*	38.4 ±4.4 *A*	87.6 ±7.6 *A*

† Mean values ± standard deviation (SD) in a column followed by different letters indicate significant differences between treatments at $p \leq 0.05$.

Table 7

The content of Gly, Ala and Tyr in the grain protein of *T. Triticum aestivum* cv. Torridon, *T. persicum* cv. Persa, and *T. sphaerococcum* cv. Trispa

Tillage method	Wheat species	Gly	Ala	Tyr
		(g kg^{-1} DM)		
	T. aestivum	32.4±4.1 *a*†	31.3±4.3 *a*	18.4±2.3 *b*
Plowing	*T. persicum*	33.4±3.9 *a*	30.2±2.3 *a*	21.6±2.1 *a*
	T. sphaerococcum	36.4±4.2 *a*	31.5±4.1 *a*	18.8±2.2 *b*
	T. aestivum	32.8±2.8 *b*	31.2±4.5 *a*	19.1±1.1 *b*
Shallow tillage	*T. persicum*	32.9±0.8 *b*	31.0±1.4 *a*	20.4±2.4 *ab*
	T. sphaerococcum	36.1±3.2 *a*	31.6±3.1 *a*	22.1±1.2 *a*
	T. aestivum	32.6±3.4 *B*	31.2±4.3 *A*	18.8±1.8 *B*
Mean	*T. persicum*	33.2±2.8 *B*	30.6±1.9 *A*	21.0±2.3 *A*
	T. sphaerococcum	36.3±3.6 *A*	31.5±3.5 *A*	20.4±2.4 *AB*
Plowing		34.1±4.3 *A*	31.0±3.6 *A*	19.6±2.6 *A*
Shallow tillage		33.9 ±2.9 *A*	31.3 ±3.2 *A*	20.5 ±2.0 *A*

† Mean values ± standard deviation (SD) in a column followed by different letters indicate significant differences between treatments at $p \leq 0.05$.

of Leu, Phe, and Tyr was significantly greater in the protein of Persian wheat than Indian dwarf wheat. On the other hand, under shallow tillage, the content of Phe, Lys, and Gly in grain protein was greater for Indian wheat than Persian wheat (Tables 3, 4, and 7). The average total amount of endogenous amino acid for all the tested grain was 568.8 g kg^{-1} (Table 5). A similar value was found for six cultivars of common wheat (609.1) – Weber et al. (2008). Neither wheat species nor tillage method was found to influence either Glu or Ala content or the sum of endogenous amino acids.

A protein's nutritional value is assessed based on a comparison of its amino acid composition against the composition of a reference protein that should theoretically fully meet a given organism's protein requirements. Based on these premises, values were calculated for indices that take into account the content of exogenous amino acids in the protein of Persian, Indian dwarf, and common wheat grain; these were the limiting amino acid index and the essential amino acids index.

The limiting amino acid index (CS) expresses the ratio of amino acid content in the tested protein to the content of that amino acid in chicken egg protein, which is considered to be the reference for optimal nutritional value. Analysis of CS indices showed that the first limiting amino acid in Persian wheat and Indian dwarf wheat grains was lysine (Table 8), as the average CS values for this amino acid were 32.4% and 32.0%, respectively, which were the lowest CS values of all the examined exogenous amino acids. In research by Konvalina et al. (2011), the quality of the protein of old bread

Table 8

The values of the limiting amino acid index (CS) for Met, Lys and the value of the exogenous amino acid index (EAAI) for the grain protein of *T. Triticum aestivum* cv. Torridon, *T. persicum* cv. Persa, and *T. sphaerococcum* cv. Trispa (%)

Tillage method	Wheat species	CS Met	CS Lys	EAAI
			(g kg^{-1} DM)	
Plowing	*T. aestivum*	29.7±1.1 *b*†	32.1±1.2 *a*	52.6±2.3 *b*
	T. persicum	35.2±5.2 *a*	31.4±2.2 *a*	56.1±2.1 *a*
	T. sphaerococcum	33.7±1.8 *a*	29.0±4.3 *a*	53.7±3.7 *ab*
Shallow tillage	*T. aestivum*	29.7±0.9 *b*	31.9±2.4 *a*	52.2±1.6 *b*
	T. persicum	43.9±16.8 *a*	33.5±9.0 *a*	52.1±2.6 *b*
	T. sphaerococcum	31.8±5.2 *b*	32.8±1.5 *a*	59.5±4.3 *a*
	T. aestivum	29.7±1.0 *B*	30.9±3.7 *A*	52.4±1.9 *B*
Mean	*T. persicum*	39.5±12.9 *A*	32.4±6.5 *A*	54.1±3.1*AB*
	T. sphaerococcum	32.8±3.9 *B*	32.0±1.8 *A*	56.3±5.0 *A*
Plowing		32.8±3.9 *A*	30.8±3.1 *A*	50.4±3.1 *A*
Shallow tillage		35.1 ±11.7 *A*	32.7 ±5.3 *A*	54.6 ±4.6 *A*

† Mean values ± standard deviation (SD) in a column followed by different letters indicate significant differences between treatments at $p \leq 0.05$.

wheat cultivars was also limited by lysine, and the CS index value was 37-39%. Research by Jiang et al. (2008) also indicates lysine as the main limiting amino acid in wheat. In the present study, the quality of common wheat protein was first limited by methionine (29.7%), which was the second limiting amino acid for ancient wheat, while the second limiting amino acid for common wheat was lysine (Table 8). According to Anjum et al. (2005) and Shewry (2007), for cereal grains, the first limiting amino acid is, as in our studies, lysine. Regardless of the tillage system, the CS limiting amino acid index value for methionine was the largest for the Persian wheat grain protein. The wheat genotype had no significant impact on the CS index value for Lys (Table 8).

The essential amino acids index (EAAI) is defined as the geometric mean of the set of ratios of exogenous amino acid contents in the tested protein to their contents in a reference protein and is expressed as a percentage (Table 8). It should be assumed that this index provides for a fuller interpretation of the protein nutritional value than does the CS indicator, as its value depends on the participation of all exogenous amino acids. According to some authors (Krejčířová et al. 2007), however, with the EAAI, the surpluses of certain amino acids can compensate for deficiencies of others in the biosynthesis of the protein, whereas in human and animal nutrition no exogenous amino acids can be lacking. For this reason, a biological verification of the results of the chemical determinations is needed. The average EAAI for grains of the analyzed wheat was 54.3 (Table 8), which was significantly lower than for winter wheat grains (Spychaj-Fabisiak et al. 2014) for the plowing tillage system (55.6). A significant interaction of experimental factors was found to shape the nutritional value of the proteins as expressed by the EAAI. Among the plowing tillage, the value of this index was significantly greater for Persian wheat than other wheat; meanwhile, among shallow tillage crops, the index value was highest for Indian dwarf wheat. The technological quality of wheat from organic system, in which soil fertility is built based on the long-term accumulation of organic matter, varies in many respects from the technological quality of wheat under conventional cultivation. The most significant differences occur in the content of raw protein and parameters characterizing the quality of the wheat protein complex. In organic farming, which has no fast-acting mineral fertilizers, there is often a nitrogen deficit and less accumulation of storage proteins in wheat (Krejčířová et al. 2007). Simplified tillage methods can be harder to implement in organic farming because organic farming principles do not allow synthetic fertilizers to be used to compensate for the delayed and reduced mineralization of nutrients from organic matter that results from reduced tillage (Peigne et al. 2015, Fernandez et al. 2019*a*,*b*).

CONCLUSIONS

Based on the qualitative analysis of wheat grain from organic farming, it was found that the mineral composition of the grain depended mainly on the wheat genotype. Primary wheats - Persian wheat (*T. persicum*) and Indian dwarf wheat (*T. sphaerococcum*) were characterized by significantly higher content of P, Cu, Fe and Zn in grain compared to common wheat (*T. aestivum*). In addition, Persian wheat grain cultivated both in the plow and shallow tillage system contained significantly more K and Zn compared to Indian dwarf wheat. The content of individual amino acids in the grain protein also differs significantly depending on the wheat species. Compared to common wheat, the protein of the ancient wheat species had higher contents of most amino acids, as well as higher measures of its biological value (the limiting amino acid index CS and the integrated index of exogenous amino acids EAAI). The higher exogenous amino acids contents were obtained from plowing tillage for *T. persicum* and shallow tillage for *T. sphaerococcum*. The preferable level of exogenous amino acids in the grain of Indian dwarf wheat and Persian wheat generally corresponded with the highest nutritional value of protein as measured by EAAI values. In conclusion, the cultivation of ancient Persian wheat and Indian dwarf wheat in organic farming is a chance to obtain grain with increased nutritional value.

Abbreviations: Ala, alanine; Arg, arginine; Asp, aspartic acid; CS, limiting amino acid index; DM, dry matter; EAAI, exogenous amino acid index; Glu, glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Tyr, tyrosine; Val, valine;

Author contributions

Conceptualization, W.K., and M.S.; methodology, W.K., and M.S; validation, W.K., M.S., and T.K.; formal analysis, M.S.; investigation, W.K., and M.S., data curation, W.K., and T.K.; writing-original draft, W.K., M.S., T.K and. E.T.; writing—review and editing, W.K., M.S., T.K and. A.N.; visualization, W.K., and M.S.; project administration, M.S.; funding acquisition, M.S.

Conflicts of interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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