

The effects of sowing date and seeding rate on spelt and common wheat protein composition and characteristics

M. Mikos-Szymańska^{1*} and G. Podolska²

¹Fertilizer Department, New Chemical Syntheses Institute, Al. Tysiąclecia Państwa Polskiego 13a, 24-110 Puławy, Poland;

²Department of Cereal Crop Production, Institute of Soil Science and Plant Cultivation - State Research Institute, Czartoryskich 8, 24-100 Puławy, Poland; marzena.mikos-szymanska@ins.pulawy.pl

Received: 16 December 2014 / Accepted: 14 August 2015

© 2015 Wageningen Academic Publishers

RESEARCH ARTICLE

Abstract

We investigated the influence of sowing date and seeding rate on protein composition and quality parameters in two winter spelt cultivars (*Triticum aestivum* ssp. *spelta* L.) and one common winter wheat cultivar (*Triticum aestivum* ssp. *vulgare* L.). Wheat was grown during a 3 year experiment (2007-2010) at the Microplots Experimental Station in Puławy, Poland. The first experimental factor was the seeding rate: 300, 450 and 600 grains/m². The second factor was the sowing date: optimal and delayed (2 weeks delayed versus optimal). The third factor was cultivar: two spelt cultivars (Rokosz and STH 8) and one common wheat cultivar (Sukces). Detailed analysis by reversed-phase high-performance liquid chromatography of protein subunits revealed many significant differences between grain samples in relation to the tested sources of variation (sowing date, seeding rate and genotype). Delayed sowing and the lowest seeding rate slightly reduced the relative ratio of gliadins to glutenins, which points to a shift in the elastic properties of gluten proteins. However, neither sowing date nor seeding rate affected wet gluten, gluten index or sedimentation value (based on analysis of variance). These gluten quality parameters were affected only by the genotypes used and the year of cropping.

Keywords: cereal quality, spelt, wheat

1. Introduction

Wheat is an important and extensively used cereal in human and animal diets worldwide. Increasing grain yields and food quality is crucial for improving wheat crop competitiveness. Such increases may be accessible based on the selection of adapted cultivars and adequate management practices (Silva *et al.*, 2014).

Grain yield and seed quality in wheat are dependent on environment conditions, genetic factors, crop management practices and the interaction between these factors (Bansod *et al.*, 2013; Coventry *et al.*, 2011; Kumar *et al.*, 2012; Lindhauer, 2012; Podolska *et al.*, 2005; Yan and Holland, 2010). Temperature and light exposure during the maturity phase are the main factors determining the protein and gluten content of wheat (Podolska and Filippek, 2010; Tashiro and Wardlaw, 1999). When the seeding date is delayed,

the periods from tillering to shooting and from tillering to earing are shortened compared with wheat planted on the optimal seeding date (Podolska and Wyzinska, 2011). The genotypic response of wheat to different planting dates varies in terms of yield-contributing characters due to different genetic potentials. The decline in these characters is more prominent in cultivars requiring more days to heading under normal planting conditions, since increasing temperatures shorten the heading period (Tashiro and Wardlaw, 1999). When optimum conditions are provided, the grain filling period is longer than that under late sowing conditions, when there is high temperature stress at maturity. Many high yielding wheat cultivars have been recommended for general cultivation in the past. Late planting results in poor tillering; when the tillering period is reduced (Joshi *et al.*, 1992), plants produce fewer tillers per plant, with lower grain weight and fewer grains per plant (El-Gizawy, 2009; Razzaq *et al.*, 1986). An adequate

sowing date positively affects grain yield (Akhtar *et al.*, 2006; Coventry *et al.*, 2011; Kumar *et al.*, 2000; Ouda *et al.*, 2005; Podolska and Wyzinska, 2011; Silva *et al.*, 2014), causing the plants to better adjust their physiology and phenology to environmental conditions (De *et al.*, 1983; Ribeiro *et al.*, 2009; Wheeler *et al.*, 1996).

Therefore, temperature and the duration of anthesis and grain filling are important factors in determining the baking quality classification of wheat (Jiang *et al.*, 2009). The sowing date determines the baking quality pattern of wheat (Motzo *et al.*, 2007; Triboui and Triboui-Blondel, 2002). Singh *et al.* (2010) investigated the effects of varying sowing times on the protein quality of two wheat genotypes differing in their water requirements, finding that delayed sowing (associated with high temperatures during grain filling) resulted in an overall increase in total grain protein content. Exposure to low temperatures under early sowing conditions increased insoluble glutenin (IG) content but reduced total protein accumulation in both genotypes. IG and residue protein levels increased, while soluble glutenin contents decreased, under rain-fed conditions. The authors concluded that sowing time affects the flour properties of wheat cultivars.

Labuschagne *et al.* (2009) also demonstrated that sowing time affects the protein content of wheat grain. Late-sown wheat usually blooms late, and the grain filling period coincides with the occurrence of high temperatures. Periods of extreme temperatures and drought during grain filling are the main sources of variability of the quality parameters of wheat flour. For example, reduced grain filling duration due to drought or high temperatures reduces the duration of glutenin biosynthesis (Labuschagne *et al.*, 2009). Thus, the impact of high temperatures on the accumulation of proteins can be modified by altering the sowing date (Wardlaw, 1994).

Seeding density is directly correlated with and affects the amount of light reaching the leaf surface. Consequently, sowing rate influences the intensity of photosynthesis, which subsequently affects protein content and protein quality. Optimum seeding rate is considered to be an important management factor for improving wheat yields. This factor is particularly important in wheat production because it is under the farmer's control in most cropping systems (Laghari *et al.*, 2011; Slafer and Satorre, 1999). Optimum plant densities vary greatly between areas, climatic conditions, soil, sowing times and varieties (Darwinkel *et al.*, 1977). If optimal seeding rates are exceeded, yield reductions often occur (Beuerlein and Lafever, 1989; Harrison and Beuerlein, 1989). Seeding rates significantly affect biological yield (Ayaz *et al.*, 1997), wheat stand (Stoppler *et al.*, 1990), spike number and spike weight (Ozturk *et al.*, 2006). Higher seeding rates compensate for reduced tiller development by promoting the formation of

more main stem spikes, which can be favourable, especially for cultivars that tend to produce fewer tillers (Coventry *et al.*, 1993; Staggenborg *et al.*, 2003). Furthermore, a close relationship exists between wheat stands and yield components (Zhen-Wen *et al.*, 1988).

Seeding density is important for wheat yields, since it directly affects the number of ears per unit area. As a consequence, other yield components are affected, such as the number of grains per ear and individual grain weight (Lloveras *et al.*, 2004; Ozturk *et al.*, 2006). Currently, the optimal seeding density used for wheat is based on the cultivar cycle, as well as its dual ability as a forage and grain crop (Valerio *et al.*, 2013). Nevertheless, the differential performance of cultivars (including tiller formation and survival), as well as the crop environment, should be taken into account when determining proper seed densities. In favourable environments, yields are uniform due to regular tiller formation and uniform distribution of photosynthetic products, which contribute to grain yield (Rickman *et al.*, 1983).

Spelt wheat is an ancient subspecies of modern wheat that can be grown at higher elevations and under harsh climatic conditions (Bonafaccia and Fabjan, 2003; Pospíšil *et al.*, 2011). Very few differences between spelt and modern wheat have been confirmed experimentally. Compared with common wheat, spelt has a higher mineral and protein content (Capouchová, 2001), although some studies have shown that spelt has a lower protein content than common wheat (Grela, 1996; Ranhotra *et al.*, 1995). The protein content of spelt grain ranges from 13.0 to 16.5% (Abdel-Aal and Hucl, 2002; Capouchová, 2001). Spelt gluten tends to be more extensible and less elastic than gluten from modern wheat, resulting in the production of typical, weaker spelt dough (Abdel-Aal *et al.*, 1997; Mikos and Podolska, 2012; Ranhotra *et al.*, 1995).

Wieser (2000) compared the qualitative and quantitative composition of gluten proteins of spelt, durum, emmer and einkorn wheat with that of common wheat. The analysis revealed that gluten protein groups and types identified in common wheat (ω -, α -, and γ -gliadins; high molecular weight glutenin subunit (HMW-GS) and low molecular weight glutenin subunit (LMW-GS)) are present in all species. According to reversed-phase high-performance liquid chromatography (RP-HPLC) analysis, α -gliadins are predominant in most cases, followed by γ -gliadins and LMW-GS. Minor components generally include ω -gliadins and HMW-GS. Common wheat cultivars contain the highest proportion of total glutenins and HMW subunits, which are important for bread-making quality. Schober *et al.* (2006) conducted a study aimed at understanding the chemical properties of spelt wheat gluten and classified the European spelt cultivars in terms of gluten quality. They also compared the protein composition of these cultivars

to that of new wheat cultivars, finding that gluten quality is widely variable and that there is an interaction between cultivars and growing locations, which affects gluten rheology, quality parameters and the contents of various protein classes. Most studies have focused on growth and yield rather than grain quality. Growth, productivity, quality parameters and protein composition can be affected by planting the wheat crop earlier or later than the optimum date and by different seeding rate practices.

Therefore, the aim of the present study was to compare the quality parameters of new spelt wheat cultivars with a high-quality winter wheat cultivar and to determine if and how two factors important for production technology, such as sowing time and seeding rate, influence the quality traits and protein composition of wheat. The results reveal differences in protein composition between new spelt cultivars and common wheat, which are useful for the baking and milling industry. Moreover, this study provides insights into improving the cultivation of spelt wheat to achieve a higher quality raw material.

2. Materials and methods

Field experiments

The new winter spelt wheat cultivars 'Rokosz' and 'STH 8' (HRS, Strzelce, Poland) and common winter wheat variety 'Sukces', a high-quality cultivar with a high yield potential, were grown in the experimental fields in Puławy, Poland in 2007-2010. Two sowing dates (optimal and delayed, i.e. 2 weeks after the optimal date) and three seeding rates (300, 450 and 600 grains/m²) were utilised. The optimal sowing dates were as follows: 1st October 2007, 16th September 2008 and 18th September 2009. The delayed sowing dates were as follows: 15th October 2007, 30th September 2008 and 2nd October 2009.

The experiment was performed using good wheat complex soil, class IVa. Grain seeds were dressed with mortar (Baytan Universal; Bayer Crop Science AG, Monheim, Germany) at a dose of 200 g/100 kg. 'Polifoska' mineral fertiliser was applied at 80 kg/ha. In the spring, nitrogen fertilisation (as ammonium nitrate in quantities of 90 kg N/ha) was applied in two doses: (1) 50 kg N/ha during the beginning of tillering (BBCH 21) (Lancashire *et al.*, 1991); and (2) 2.40 kg N/ha at the beginning of the stem elongation stage (BBCH 30) (Lancashire *et al.*, 1991).

Depending on the occurrence of diseases or pests, plant protective measures were applied. At BBCH 59 in 2008, Karate 025 EC (Syngenta Limited, Guildford, UK) was sprayed at a dose of 0.2 l/ha. In 2009, the following pesticides were sprayed: at BBCH 12, Pirimor 500 WG (Syngenta Crop Protection AG, Basel, Switzerland) at a dose of 0.25 kg/ha; at BBCH 56, Karate 025 EC at a dose of 0.2 l/

ha; and at BBCH 83, Artea 330 EC at a dose of 0.5 l/ha. In 2009, at BBCH 21, Owadofos 540 EC (Zakłady Chemiczne Organika-Azot S.A., Jaworzno, Poland) at a dose of 1 l/ha was sprayed to control *Oscinella frit*.

Removal of weeds in the microplots was carried out manually. Harvesting was performed during the full maturity period (BBCH 89).

Grain quality analysis

Milling was carried out using a Perten Laboratory Mill 3010 (with 0.5 mm sieve; Perten Instruments, Hägersten, Sweden) to produce wholemeal. Wet mineralisation of the samples was performed with concentrated sulphuric acid and hydrogen peroxide. Nitrogen content was determined using a spectrophotometric flow injection system, and grain protein content of wholemeal was calculated using a nitrogen-to-protein factor of 5.7 ($N \times 5.7$). The quantity and quality of wet gluten were determined using a Glutomatic system (Perten, Sweden; ICC, 1994). The sodium dodecyl sulphate (SDS) sedimentation test was performed in duplicate on wholemeal according to Axford *et al.* (1978).

Protein composition

To determine the content of a particular protein fraction, a 3 g grain sample was ground in an IKA A10 laboratory mill (LTF Labortechnik GmbH & Co. KG, Wasserburg, Germany) in such a manner that all particles could be sieved through a 400 µm mesh sieve. The samples were defatted with petroleum ether in Soxhlet extractors (16 h). After evaporation of the solvent, 100 mg portions of powder were weighed and placed in Eppendorf tubes, and the following three protein fractions were extracted according to Wieser *et al.* (1998):

1. albumins + globulins: double extraction of 1 cm³ of the mixture (0.4 mol/l NaCl + 0.067 mol/l HKNaPO₄ with a pH of 7.6);
2. prolamins: triple extraction of 1 cm³ of the mixture (60% ethanol);
3. glutelins: double extraction of 1 cm³ of the mixture (50% propanol 1 + 2 mol/l urea + 0.05 mol/l Tris-HCl (pH=7.5) + 1% dithioerythritol under nitrogen).

The first two protein fractions were extracted at room temperature using an Eppendorf thermomixer (10 min extraction). Glutelins were extracted at 60 °C in the thermomixer. After each extraction, the mixture was centrifuged at 11,000×g. The collected fractions were lyophilised and dissolved in 2 cm³ of the respective phase (1-3), cleaned through a Spartan-3NY filter (GE Healthcare, Pittsburgh, PA, USA) with a 0.45 µm mesh and transferred to glass vials. The determinations were performed using a Hewlett Packard Series 1050 HPLC system with the following parameters (Agilent Technologies/Hewlett

Packard, Walldbronn, Germany): column RP-18 Vydac 218TPP54, 5 µm, 250×4.6 mm; pre-column Zorbax 3000SB-C18 4.6×12.5 mm; column temperature 45°C; mobile phase flow rate 1 ml per min; and injection size of 20 µl. The separation was performed using a two-component gradient. The proportion of component A was as follows: 0 min, 75%; 5 min, 65%; 10 min, 50%; 17 min, 25%; 18 min, 15%; and 19 min, 75%. The first gradient (A) was water containing 0.1% trifluoroacetic acid (TFA), while the second gradient (B) was acetonitrile containing 0.1% TFA. Subunits of gluten proteins (ω -, α/β - and $\gamma\gamma$ -gliadins; high molecular glutenin subunits; and low molecular glutenin subunits) were identified based on their second derivatives of UV spectra and retention times (Konopka *et al.*, 2007). Each group of protein was fractionated in at least two runs. After subtraction of the baseline signal (from solvent utilised for extraction), the corrected chromatograms showed no significant variation between runs. Commercially produced Bio-Rad bovine serum albumin (Bio-Rad Laboratories, Life Science Group, Hercules, CA, USA) and Sigma-Aldrich Gliadin G3375 (Saint Louis, MO, USA) were used to prepare standard solutions, which were used for quantification of the main groups of proteins (linear regression coefficients were above $R^2=0.98$ for both assays) via a Bio-Rad Bradford protein assay. Among the main groups of protein, the subunits (for example, ω -, α/β - and $\gamma\gamma$ -gliadins) were quantified based on the proportion of the peak area of the identified subunit to the areas of all peaks. The detection was carried out using a detector manufactured by the same company, and the reading was performed at a wavelength of 210 nm. The results were analysed using HPLC 3D ChemStation software (Hewlett Packard, Palo Alto, CA, USA). The protein contents were expressed in mAU × s. Assays of protein fractions were carried out at the Department of Processing and Chemistry of Plant Raw Materials, Faculty of Food Sciences, University of Warmia and Mazury in Olsztyn, Poland. Only wheat samples from 2009 were subjected to protein composition analysis.

Statistical analysis

Statistical evaluation was carried out using Statgraphics Centurion v. XVI (Statpoint Technologies, Inc., Warrenton, VA, USA). Analysis of variance (multifactor ANOVA) was performed at a significance level of $\alpha=0.05$.

3. Results and discussion

Weather conditions

The average climatic conditions (1971 to 2007) of the location and the weather conditions during the investigated growing seasons are presented in Figures 1 and 2. In all investigated growing seasons, mean temperatures from March to July were higher than the long-term average values. Mean monthly precipitation differed among years

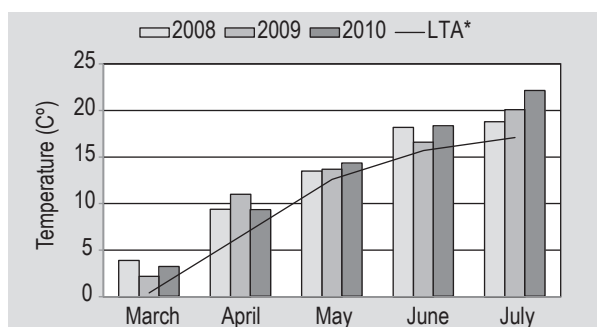


Figure 1. Mean monthly temperatures and long-term averages (LTA*; 1971–2007) during wheat vegetation (°C) in 2008–2010.

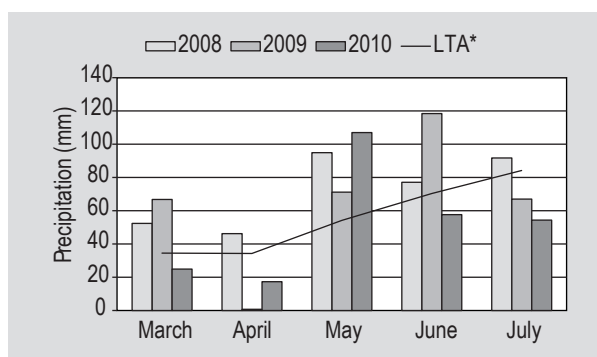


Figure 2. Total monthly precipitation and long-term averages (LTA*; 1971–2007) during wheat vegetation (mm) in 2008–2010.

of the study. In 2008, the monthly precipitation was above average from March to July. Growing season 2009 was characterised by considerably below average precipitation in April (0.7 mm) and above average precipitation in June (118.5 mm). In 2009, the monthly precipitation was below average in April and July. Growing season 2010 was characterised by below average precipitation in March, April, June and July and above average precipitation in May. In 2008, the average temperatures during wheat flowering and grain development were 18.2 and 18.8 °C in June and July, respectively, but in 2009, the average temperature in July was 20.1 °C. In 2010, the average temperature in June was similar to that in 2008 (18.3 °C), but 2010 was characterised by high temperatures in July (average of 22 °C).

Quality parameters

All wheat grain quality traits analysed in this study were significantly affected by genotype, year of cropping and year–genotype interactions (Table 1). Compared with cv. Sukces (common wheat), STH 8 and Rokosz (spelt wheat) were characterised by higher grain protein content (by 5.7 and 7.6%, respectively; Table 2). Differences between genotypes were expected to be large, since common wheat and spelt are wheat species with marked genetic and morphological differences. The protein contents

Table 1. Multifactor ANOVA for wheat quality traits.¹

Source of variation	Df	P (g/kg)		WG (%)		GI (%)		SV (ml)	
		Mean squares	F-ratio	Mean squares	F-ratio	Mean squares	F-ratio	Mean squares	F-ratio
Year (Y)	2	904.56	307.29*	109.21	109.35*	1,049.3	25.80*	79.68	5.65*
Genotype (G)	2	347.11	117.92*	80.38	80.48*	4,492.6	110.47*	1,482.74	105.08*
Sowing date (SD)	1	0.73	0.25	0.17	0.17	63.25	1.56	13.50	0.96
Seeding rate (SR)	2	12.75	4.33*	2.27	2.28	34.72	0.85	8.07	0.57
Y × G	4	34.37	11.68*	4.72	4.72*	444.85	10.94*	55.74	3.95*
Y × SR	4	2.50	0.85	0.30	0.30	33.45	0.82	2.91	0.21
Y × SD	2	10.84	3.68	0.61	0.61	57.81	1.42	26.17	1.85
G × SR	4	6.33	2.15	0.94	0.94	32.65	0.80	3.30	0.23
G × SD	2	7.59	2.58	2.88	2.89	73.95	1.82	0.89	0.06
SR × SD	2	3.29	1.12	1.30	1.30	45.48	1.12	13.55	0.96
Y × G × SR	8	3.43	1.17	0.62	0.63	19.47	0.48	7.30	0.52
Y × G × SD	4	10.28	3.49	2.71	2.72	50.57	1.24	12.22	0.87
Y × SR × SD	4	4.90	1.66	1.22	1.22	12.14	0.30	2.22	0.16
Y × SR × SD	4	2.70	0.92	0.71	0.71	43.08	1.06	14.78	1.05
Residual	8	2.94		1.00		40.67		14.11	
Total	53								

* $P \leq 0.05$.
¹ P = protein content; WG = wet gluten content; GI = gluten index; SV = sodium dodecyl sulphate sedimentation value; Df = degrees of freedom.

Table 2. Yearly and mean values of quality traits evaluated in this study.^{1,2}

Trait	Year	STH 8 (spelt wheat)	Rokosz (spelt wheat)	Sukces (common wheat)	Mean
P (g/kg)	2008	116.3b	116.3b	111.8b	114.8b
	2009	124.9a	128.7a	115.1a	122.9a
	2010	109.6c	112.0c	104.8c	108.8c
	Mean	116.9b	119.0a	110.6c	
WG (%)	2008	30.1b	28.4b	26.4b	28.3b
	2009	31.1a	32.4a	27.8a	30.4a
	2010	27.6c	26.2c	22.8c	25.5c
	Mean	29.6a	29.0a	25.7b	
GI (%)	2008	39a	59a	76b	57.9a
	2009	37b	47c	50c	57.5a
	2010	35c	56b	81a	44.5b
	Mean	37c	54b	69a	
SV (ml)	2008	40.7c	53.7c	63.5a	52.6b
	2009	49.5a	59.5a	61.0b	56.7a
	2010	42.7b	57.0b	61.3b	53.7b
	Mean	44.3c	56.7b	61.9a	

¹ P = protein content; WG = wet gluten content; GI = gluten index; SV = sodium dodecyl sulphate sedimentation value.
² For each variable, means in the same column and the last row followed by the same letter are not significantly different at $P \leq 0.05$.

of cv. STH 8, Rokosz and Sukces were as follows: 116.3, 116.3 and 111.8 g/kg in 2008; 124.9, 128.7 and 115.1 g/kg in 2009; and 109.6, 112.0 and 104.8 g/kg in 2010,

respectively (Table 2). Similar results were obtained in previous studies showing that the protein contents of spelt wheat range from 86.0-136.0 g/kg (Majewska *et al.*, 2007;

Rachoń and Szumiło, 2009). In a previous study (Mikos and Podolska, 2012), we determined that foreign spelt wheat cultivars grown in Poland contain 120–145 g/kg of protein. The differences in protein contents in wheat grains between years are related to the different weather conditions, particularly during the grain maturity period. According to Linina *et al.* (2014), the grain protein content of wheat significantly varies depending on the cultivar and meteorological conditions. The current results indicate that moderate temperatures during the anthesis and flowering periods, high temperatures during grain maturity (in 2009) and moderate precipitation are favourable for protein synthesis. These results are in agreement with the results of Labuschagne *et al.* (2009), who showed that after anthesis, temperature and rainfall during grain filling strongly affect grain protein content and composition. Sowing date did not have a significant effect on the wheat quality traits examined (Table 3), whereas the seeding rate had a statistically significant effect only on protein content; protein content decreased with increasing seeding rate (Table 4). These results disagree with the results of Ozturk *et al.* (2006), who reported that protein concentration was unaffected by seeding rate.

The wet gluten content of cv. STH 8, Rokosz and Sukces was 30.1, 28.4 and 26.4% in 2008; 31.1, 32.4 and 27.8% in 2009; and 27.6, 26.2 and 22.8% in 2010, respectively (Table 2).

Table 3. Mean values of quality traits in relation to different sowing dates.^{1,2}

Sowing date	P (g/kg)	WG (%)	GI (%)	SV (ml)
Optimal	115.4ns	28.0ns	54ns	53.8ns
Delayed	115.6ns	28.1ns	52ns	54.8ns

¹ P = protein content; WG = wet gluten content; GI = gluten index; SV = sodium dodecyl sulphate sedimentation value.

² ns = not significant at $P \leq 0.05$.

Table 4. Mean values of quality traits in relation to different seeding rates.^{1,2}

Seeding rate (grains/m ²)	P (g/kg)	WG (%)	GI (%)	SV (ml)
300	116.3a	28.5ns	54ns	54.4ns
450	115.6ab	28.0ns	52ns	53.6ns
600	114.6b	27.8ns	54ns	54.9ns

¹ P = protein content; WG = wet gluten content; GI = gluten index; SV = sodium dodecyl sulphate sedimentation value.

² ns = not significant. For each variable, means followed by the same letter in the same column are not significantly different at $P \leq 0.05$.

Among 3 year averages, compared with common wheat, STH 8 and Rokosz were characterised by higher wet gluten content (by 15.2 and 12.8%, respectively; Table 2). These results are in agreement with previous studies showing that spelt wheat grain has higher wet gluten content than common wheat grain (Abdel-Aal *et al.*, 1996; Chrenková *et al.*, 2000; Lacko-Bartošova and Rádlová, 2007). Using previously reported values, we found that STH 8 and Rokosz have lower wet gluten contents than German spelt wheat cultivars (Schweizer Altgold Zeinnars Weisser, Bastard, Burgdorf, and Oberlander), whose gluten contents range from 31–37% (Makowska *et al.*, 2008). Majewska *et al.* (2007) showed that flours of spelt wheat cultivars such as Ceralio, Schwabekorn, Frankenkorn, Holstenkorn, Schwabenspelz, Ostro and Oberkulmer Rothkorn have significantly higher wet gluten contents (27.3–45.6%) than flour of common wheat cv. Korweta (22.5%). The highest wet gluten contents were found in Ostro and Oberkulmer Rotkorn flours (35.6 and 38.9%, respectively). Krochmal-Marczak and Sawicka (2011) showed that the wet gluten content in spelt wheat (Frankencorn, Ceralio and Schwabencorn) ranges from 23.5–27.5%. According to Capouchová (2001), spelt wheat gluten is of lower quality than common wheat gluten, which is in agreement with the current study.

Furthermore, we found that the gluten index of STH 8, Rokosz and Sukces was 39, 59 and 76% in 2008; 37, 47 and 50% in 2009; and 35, 56 and 81% in 2010, respectively (Table 2). Based on 3 year averages, common wheat had the highest gluten index (69%) compared with spelt wheat cultivars (37 and 54%, respectively) (Table 2). In a previous study (Mikos and Podolska, 2012), we detected high variability in the gluten index, ranging from 10.2 to 34.2%. For baking purposes, wheat with a gluten index of 60–90% is best. Wheat with a gluten index of less than 50 is more difficult to process; the dough is sticky and is mainly suitable for biscuits, with potential application for two-layer flat breads (Konopka *et al.*, 2007).

SDS sedimentation values characterise the viscoelastic features and quality of proteins and indicate the potential for the fermentation process to occur in dough. According to Slovak technical norms (STN 46 1100-2), wheat should have a minimum SDS sedimentation value of 45 ml to be designated as bread-making wheat, which is marked with the letter B. In this study, the SDS sedimentation values, depending on year and cultivation, ranged from 42.7 to 63.5 ml. Among mean values over the 3 year period, significantly higher values were found in the common wheat cv. Sukces (61.9 ml) compared with the spelt cultivars (44.3 and 56.7 ml, respectively) (Table 2). These results are in agreement with those of other studies (Bonafaccia *et al.*, 2000; Mikos and Podolska, 2012), namely, that the SDS sedimentation values of spelt wheat grain are lower than those of common wheat grain. As shown in Table 4, seeding rate had no

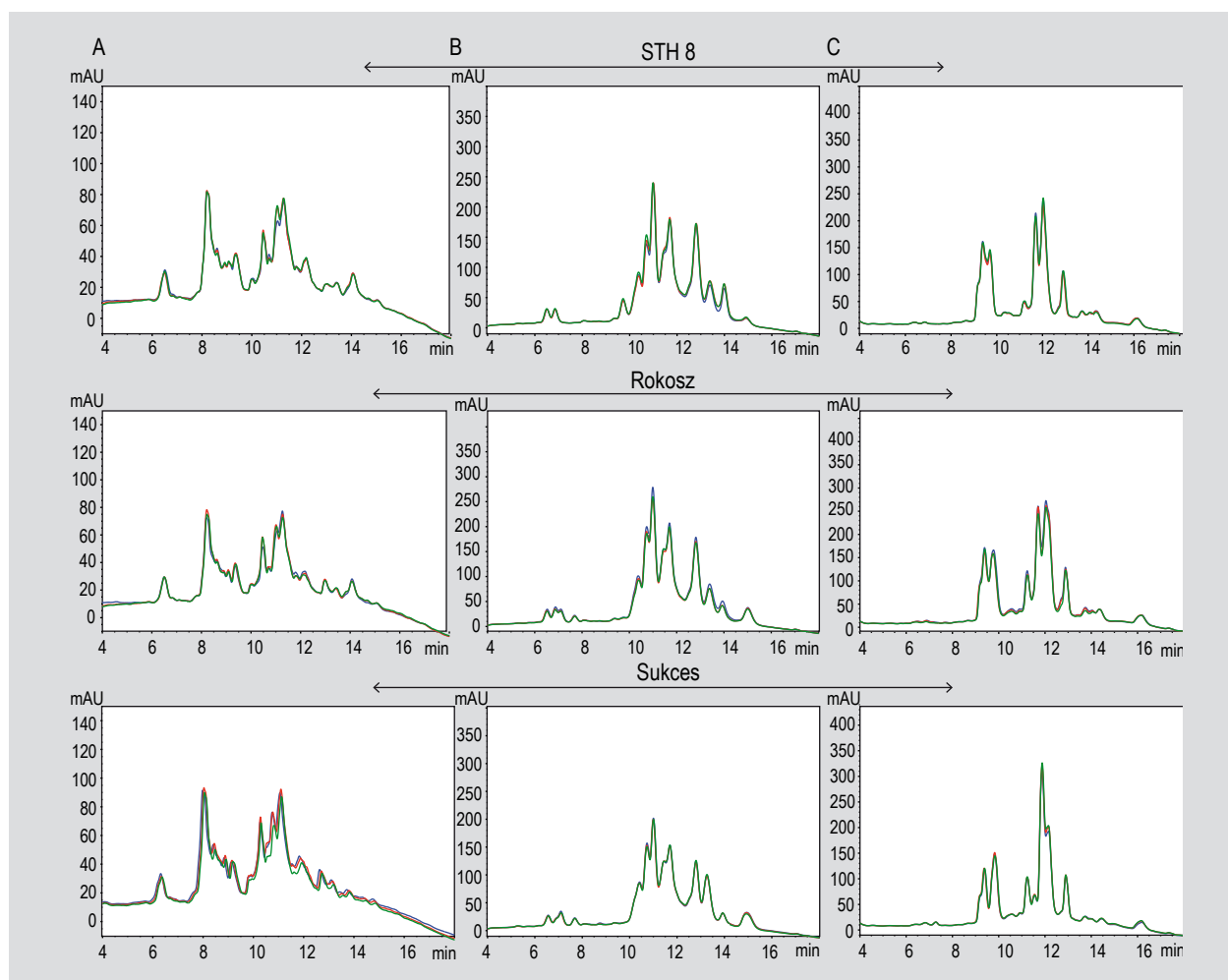


Figure 3. Reversed-phase high-performance liquid chromatography chromatograms of (A) albumins and globulins, (B) gliadins and (C) glutenins from cultivars STH 8, Rokosz and Sukces under the optimal sowing date at different seeding rates (data from 2009) (blue curve = 300 grains/m²; red curve = 450 grains/m²; green curve = 600 grains/m²).

significant effect on wet gluten content, gluten index or SDS sedimentation value.

Protein composition

An analysis of chromatograms reveals the typical arrangements of protein fractions (Figures 3-4). In this study, we found that the spelt wheat protein fractions had different compositions from those of common wheat. We also confirmed the influence of agrotechnical factors on protein fractions. Our results indicate that albumins and globulins constituted 21.45 to 25.75% of total proteins in the grains of the cultivars examined (Table 6). Both sowing date and seeding rate influence albumin and globulin contents; the albumin and globulin contents in wheat grain were significantly higher in wheat with a delayed sowing date versus the optimal sowing date. Moreover, significantly lower amounts of albumins and globulins were detected at a seeding rate of 400 grains/m² compared with 300 and 600 grains/m², with reductions of 0.32 and 0.25%,

respectively (Table 5). By contrast, Olszewski *et al.* (2009) showed that for common wheat (cv. Tonacja and Sukces), delaying the sowing date had no influence on albumin or globulin contents. The results indicate that if differences in the levels of particular protein groups occur, their ratios are highly stable and are not altered by the sowing dates and seeding rates examined. Although albumin-globulins do not have a significant effect on dough quality, they are nutritionally important due to their high essential amino acid contents (Singh *et al.*, 2012).

As shown in Table 6, the gliadin contents of common and spelt wheat were higher than the albumin and globulin contents, ranging from 42.43% (cv. Sukces) to 46.70% (cv. STH 8) of total proteins. The results also confirm that the gliadin content is cultivar dependent, which is in agreement with the results of Konopka *et al.* (2007) and Singh *et al.* (2012). The smallest sub-fractions of gliadins were ω -gliadins, which ranged from 4.41% (STH 8) to 5.49% (Sukces) of total proteins. The largest sub-fractions were

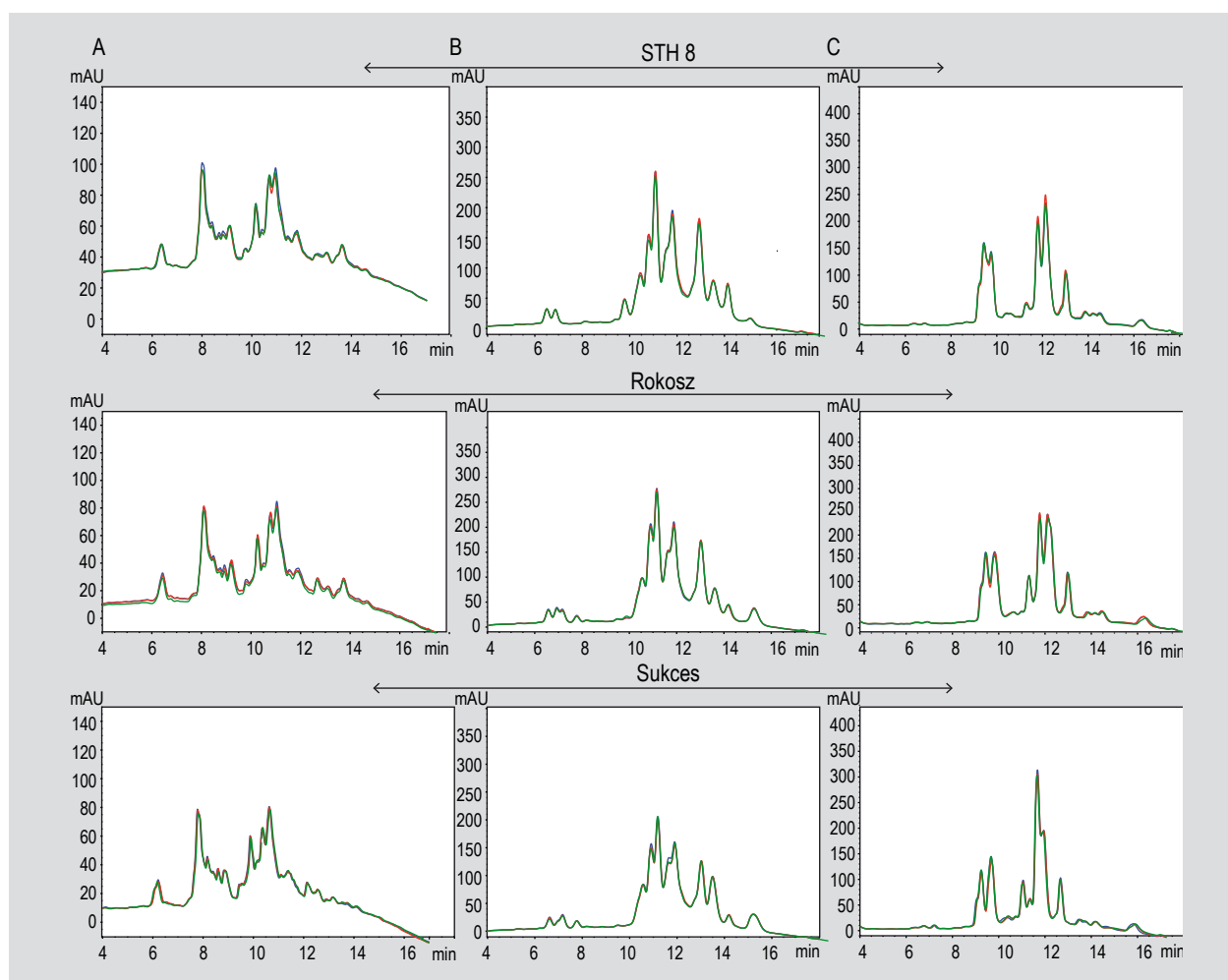


Figure 4. Reversed-phase high-performance liquid chromatograms of (A) albumins and globulins, (B) gliadins and (C) glutenins from cultivars STH 8, Rokosz and Sukces under a delayed sowing date at different seeding rates (data from 2009) (blue curve = 300 grains/m²; red curve = 450 grains/m²; green curve = 600 grains/m²).

α/β -gliadins, comprising 24.54% (Sukces) to 27.46% (STH 8) of total proteins. The γ -gliadin contents ranged from 12.4% (Sukces) to 14.8% (STH 8) of total proteins (Table 6). Slightly different gliadin contents in common wheat were reported by Konopka *et al.* (2007), who detected 2% fewer ω -gliadins, 7% fewer α/β -gliadins and 2% fewer γ -gliadins compared with our results. However, similar to our study, ω -gliadins comprised the smallest fractions and α/β -gliadins comprised the largest (Table 5). Delayed sowing date caused a significant increase in α/β -gliadin and γ -gliadin contents and a decrease in ω -gliadin contents. We found that the levels of ω -gliadins, α/β -gliadins and γ -gliadins increased with increases in seeding rate (Table 5).

According to the data in Table 6, glutenins comprised from 29.8% (STH 8) to 33.9% (Rokosz) of total proteins. The differences in glutenin contents between cultivars suggest that wheat cultivars have different optimal temperatures for the biosynthesis of glutenins, which is in agreement with studies (Labuschagne *et al.*, 2009; Singh *et al.*, 2012)

demonstrating the different temperature requirements for polymeric glutenin accumulation in different wheat cultivars. Delayed sowing date caused a significant increase in glutenin contents. Moreover, the glutenin contents decreased with increasing seeding rate (Table 5). The baking value of flour is favourably influenced by an increase in the contents of high molecular weight glutenin subunit (HMW-GS) and its ratio to low molecular weight glutenin subunit (LMW-GS) (Daniel and Triboi, 2002; MacRitchie, 1999). The HMW-GS to LMW-GS ratio ranged from 0.38 (Sukces) to 0.45 (STH 8; Table 5). The decrease in HMW-GS levels is, therefore, unfavourable.

4. Conclusions

The spelt cultivars (Rokosz and STH 8) were characterised by higher protein and wet gluten contents compared with common wheat cultivar Sukces. However, the gluten quality, which was determined by SDS sedimentation value and gluten index, was lower in spelt grain than in common

Table 5. Relative amounts (mAU) of protein types in spelt and common wheat grains (data from 2009).^{1,2}

Factor		Protein in kernel (mAU)	A+G	Gliadins (GLI)			
				ω	α/β	Γ	Total
Sowing date	optimal	64,600±3,083.1b	15,354±855.6b	3,334±264.8a	16,960±1,440.1b	8,715±848.0b	29,009±2,151.9b
	delayed	66,755±3,319.2a	15,505±595.5a	3,240±566.4b	17,471±1,381.2a	8,881±833.0a	29,592±1,978.5a
	LSD _{$\alpha=0.05$}	195.049	79.895	35.260	19.444	22.954	44.767
Seeding rate (grains/m ²)	300	65,776±3,922.2a	15,539±632.3a	3,196±518.7c	17,175±1,529.5b	8,733±889.2c	29,105±2,099.9b
	450	65,407±3,752.3b	15,243±675.1b	3,241±513.9b	17,132±1,421.1b	8,790±808.0b	29,163±2,044.9b
	600	65,844±2,433.5a	15,505±886.0a	3,425±214.9a	17,338±1,407.6a	8,870±867.5a	29,633±2,170.2a
	LSD _{$\alpha=0.05$}	407.78	193.393	37.601	60.135	48.035	98.568
Cultivar	STH 8 (spelt wheat)	64,502±1,164.9b	15,162±827.7b	2,847±117.9c	17,714±251.4b	9,568±137.8a	30,129±319.7b
	Rokosz (spelt wheat)	69,783±1,481.0a	14,967±153.2c	3,569±435.0a	18,532±716.9a	9,046±473.6b	31,147±1,007.8a
	Sukces (common wheat)	62,742±635.9c	16,159±311.6a	3,445±288.0b	15,400±193.8c	7,779±340.2c	26,625±293.8c
	LSD _{$\alpha=0.05$}	434.414	168.19	62.933	65.19	48.035	97.814

Factor		Glutenins (GLU)				GLI/GLU
		HMW-GS	LMW-GS	Total	HMW-GS/LMW-GS	
Sowing date	optimal	5,989±560.1b	14,248±1,339.7b	20,238±1,788.3b	0.42	1.43
	delayed	6,300±614.1a	15,358±1,858.6a	21,657±2,344.9a	0.41	1.36
	LSD _{$\alpha=0.05$}	22.183	15.313	32.832	-	-
Seeding rate (grains/m ²)	300	6,243±706.9a	14,890±2,007.1a	21,132±2,606.9a	0.41	1.37
	450	6,106±614.6b	14,895±1,741.5a	21,001±2,238.2b	0.40	1.38
	600	6,083±502.0b	14,623±1,416.6b	20,708±1,783.0c	0.41	1.43
	LSD _{$\alpha=0.05$}	37.683	30.933	50.101	-	-
Cultivar	STH 8 (spelt wheat)	6,047±152.1b	13,164±391.7c	19,213±527.4c	0.45	1.56
	Rokosz (spelt wheat)	6,864±252.0a	16,805±540.4a	23,669±780.9a	0.40	1.31
	Sukces (common wheat)	5,521±246.0c	14,438±1,108.3b	19,959±1,332.2b	0.38	1.33
	LSD _{$\alpha=0.05$}	23.800	66.266	82.721	-	-

¹ mAU = absorbance units determined by reversed-phase HPLC.

² A+G = albumins and globulins; HMW-GS = high molecular weight glutenin subunit; LMW-GS = low molecular weight glutenin subunit; ± = standard deviation; ns = not significant. For each variable, means followed by the same letter in the same column are not significantly different at $P \leq 0.05$.

wheat grain. Common wheat was characterised by the highest amounts of albumins and globulins and the lowest amounts of gliadins compared with the spelt cultivars. The delayed sowing date caused an increase in total protein levels in the kernel as well as increases in the contents of albumins and globulins, α/β -gliadins, γ -gliadins, total gliadins, HMW-GS, LMW-GS and total glutenins, but it did not change the HMW-GS to LMW-GS ratio. The reaction of Polish spelt cultivars to delayed sowing date was similar to that of the common wheat cultivar. The seeding rate had no effect on the wet gluten content in the wheat cultivars examined. As the seeding rate increased, the total amount

of gliadins decreased but the total amount of glutenins increased. Therefore, both the amount of available light (determined by sowing rate) and the sowing time influence the baking quality of wheat.

Based on the current results, favourable quality parameters of common and spelt wheat grains are obtained by delaying the sowing time (2 week delay) combined with a low or medium sowing rate (300 or 450 grains/m²).

Table 6. Proportion (%) of protein types in spelt and common wheat grains (protein = 100%) (2009).^{1,2}

Factor		A+G (% of total protein)	Gliadins (% of total protein)				Glutenins (% of total protein)		
			ω	α/β	γ	sum	HMW-GS	LMW-GS	sum
Sowing date	optimal	23.77a	5.16a	26.25ns	13.49a	44.90a	9.27ns	22.06b	31.33b
	delayed	23.23b	4.85b	26.17ns	13.30b	44.32b	9.44ns	23.01a	32.45a
Seeding rate (grains/m ²)	300	23.62a	4.86b	26.11ns	13.28ns	44.25c	9.49ns	22.64a	32.13a
	450	23.30b	4.96b	26.19ns	13.44ns	44.59b	9.34ns	22.77a	32.11a
	600	23.55a	5.20a	26.33ns	13.47ns	45.00a	9.24ns	22.21b	31.45b
Cultivar	STH 8 (spelt wheat)	23.51b	4.41b	27.46a	14.83a	46.70a	9.37ab	20.41c	29.78c
	Rokosz (spelt wheat)	21.45c	5.11a	26.56b	12.96b	44.63b	9.84a	24.08a	33.92a
	Sukces (common wheat)	25.75a	5.49a	24.54c	12.40b	42.43c	8.80c	23.01b	31.81b

¹ A+G = albumins and globulins; HMW-GS = high molecular weight glutenin subunits; LMW-GS = low molecular weight glutenin subunits.

² For each variable, means followed by the same letter in the same column are not significantly different at $P \leq 0.05$.

Acknowledgments

We thank our collaborators from the Chair of Food Plant Chemistry and Processing, University of Warmia and Mazury in Olsztyn for analyses of protein fractions and from the Main Laboratory of the Institute of Soil Science and Plant Cultivation – State Research Institute in Puławy for protein content analysis. This work, which is part of a PhD thesis, was funded by the National Science Centre in Poland (grant no. N N310 168139).

References

- Abdel-Aal, E.S.M. and Hucl, P., 2002. Amino acid composition and *in vitro* protein digestibility of selected ancient wheats and their end products. *Journal of Food Composition and Analysis* 15: 737-747.
- Abdel-Aal, E.S.M., Hucl, P., Sosulski, F.W., Bhurud, P.R., 1997. Kernel, milling and baking properties of spring-type spelt and einkorn wheats. *Journal of Cereal Science* 26: 363-370.
- Abdel-Aal, E.S.M., Salama, D.A., Hucl, P., Sosulski F.W. and Cao W., 1996. Electrophoretic characterization of spring spelt wheat gliadins. *Journal of Agricultural and Food Chemistry* 44: 2117-2123.
- Akhtar, M., Cheema, M.S., Moazzam, J. and Ali, L., 2006. Effect of time of sowing on some important characters of wheat, *Triticum aestivum* genotypes. *Journal of Agricultural Research* 44: 255-261.
- Axford, D.W.E., McDermott, E.E. and Redman, D.G., 1978. Small-scale test of bread-making quality. *Milling Feed and Fertilizer* 161: 18-20.
- Ayaz, S., Shah, P. and Ali, M., 1997. Influence of seeding density and geometry of planting on emergence, tillering and biological yield of wheat. *Sarhad Journal of Agriculture* 13: 219-222.
- Bansod, B.S., Pandey, O.P., Rajesh, N.L. and Dhatteval, S., 2013. Optimisation of agricultural input application to enhance the crop quality and yield quantity in paddy under precision farming. *Quality Assurance and Safety of Crops & Foods* 5: 179-185.
- Beuerlein, J.E. and Lafever, H.N., 1989. Yield of soft red winter wheat as affected by row spacing and seeding rate. *Applied Agricultural Research* 4: 47-50.
- Bonafaccia, G. and Fabjan, N. 2003. Nutritional comparison of tartary buckwheat with common buckwheat and minor cereals. *Zbornik Biotehniške fakultete Univerze v Ljubljani, Kmetijstvo*, 8, 2, Oktober 2003, pp. 349-355.
- Bonafaccia, G., Galli, V., Francisci, R., Mair, V., Skrabanja, V. and Kreft, I., 2000. Characteristics of spelt wheat products and nutritional value of spelt wheat-based bread. *Food Chemistry* 68: 437-441.
- Capouchová, I., 2001. Technological quality of spelt (*Triticum spelta* L.) from ecological growing system. *Scientia Agriculturae Bohemica* 32: 307-322.
- Chrenková, M., Čerešňáková, Z., Sommer, A., Gálová, Z. and Král'ová V., 2000. Assessment of nutritional value in spelt (*Triticum spelta* L.) and winter wheat (*Triticum aestivum* L.) by chemical and biological methods. *Czech Journal of Animal Science* 45: 133-137.
- Coventry, D.R., Gupta, R.K., Poswal, R.S., Chhokar, R.S., Sharma, R.K., Yadav, V.K., Gill, S C., Mehta, A., Kleemann, S.G.L., Bonamano, A. and Cummins, J.A., 2011. Wheat quality and productivity as affected by varieties and sowing time in Haryana, India. *Field Crops Research* 123: 214-225.
- Coventry, D.R., Reeves, T.G., Brooke, H.D. and Cann, D.K., 1993. Influence of genotype, sowing date, and seeding rate on wheat development and yield. *Australian Journal of Experimental Agriculture* 33: 751-757.
- Daniel, C. and Triboi, E., 2002. Changes in wheat protein aggregation during grain development: effects of temperatures and water stress. *European Journal of Agronomy* 16: 1-12.

- Darwinkel, A., Hag, B.A. and Kuizenga, J., 1977. Effect of sowing date and seed rate on crop development and grain production of winter wheat. *Netherlands Journal of Agricultural Science* 24: 83-94.
- De, R., Satan, G., Turkhede, B.B., Lal, R.B., Singh, R.K. and Giri, G., 1983. Response of wheat cultivars to date of sowing under dry land conditions. *Journal of Agricultural Science* 10: 727-733.
- El-Gizawy, N., 2009. Effect of planting date and fertilizer application on yield of wheat under no till system. *World Journal of Agricultural Sciences* 5: 777-783.
- Grela, E.R., 1996. Nutrient composition and content of antinutritional factors in spelt (*Triticum spelta* L.) cultivars. *Journal of the Science of Food and Agriculture* 71: 399-404.
- Harrison, K.S. and Beuerlein, J.E., 1989. Effect of herbicide mixtures and seeding rate on soft red winter wheat (*Triticum aestivum*) yield. *Weed Technol.* 3: 505-508.
- International Association for Cereal Science and Technology (ICC), 1994. ICC standard method no. 155. Determination of wet gluten quantity and quality of whole wheat meal and wheat flour. ICC, Vienna, Austria.
- Jiang, D., Yue, H., Wollenweber, B., Tan, W., Mu, W., Bo, Y., Dai, T. and Cao, W., 2009. Effects of postanthesis drought and waterlogging on accumulation of high-molecular-weight glutenin subunits and glutenin macropolymers content in wheat grain. *Journal of Agronomy and Crop Science* 195: 89-97.
- Joshi, A.K., Rai, B. and Sing, M.P. 1992. Technology for late sowing wheat in eastern Uttar Pradesh. *Indian Farming* 42: 15.
- Konopka, I., Tańska, M., Pszczółkowska, A., Fordoński, G., Koziro, W. and Olszewski, J., 2007. The effect of water stress on wheat kernel size, color and protein composition. *Polish Journal of Natural Science* 22: 157-171.
- Krochmal-Marczak, B. and Sawicka, B., 2011. Wartość wypiekowa wybranych odmian ozimej pszenicy orkiszowej (*Triticum aestivum* ssp. *spelta*) uprawianych w warunkach Polski południowo-wschodniej. In: Hołubowicz-Kliza, G. (ed.) *Proceedings of Hodowla uprawa i wykorzystanie pszenicy orkisz w warunkach zmian klimatu*, Puławy, Poland, 28-29 June 2011. Institute of Soil Science and Plant Cultivation – State Research Institute, Puławy, Poland, pp. 16-17.
- Kumar, P., Dhillon, S., Kumar, P. and Kumar, S., 2012. Estimation of genetic parameters for morpho-physiological traits under terminal heat tolerance in bread wheat (*Triticum aestivum* L.) *Quality Assurance and Safety of Crops & Foods* 4: 136-158.
- Kumar, S., Bangarwa, A.S. and Kadian, V.S., 2000. Response of wheat varieties to sowing dates and nitrogen levels. *Annales Agriculture and Biological Research* 5: 99-103.
- Labuschagne, M.T., Elago, O. and Koen, E., 2009. Influence of extreme temperatures during grain filling on protein fractions, and its relationship to some quality characteristics in bread, biscuit, and durum wheat. *Cereal Chemistry* 86: 61-66.
- Lacko-Bartošova, M. and Rėdlová, M., 2007. The significance of spelt wheat cultivated in ecological farming in the Slovak Republic. In: *Proceedings of Organic Farming*, Prague, Czech Republic, 6-7 February 2007, pp. 79-81. Available at: http://organicfarming.agrobiology.eu/sbornik_proceedings.pdf.
- Laghari, G.M., Oad, F.C., Tunio, S., Chachar, Q., Gandahi, A.W., Siddiqui, M.H., Hassan, S.W.U. and Ali A., 2011. Growth and yield attributes of wheat at different seed rates. *Sarhad Journal of Agriculture* 27: 177-183.
- Lancashire, P.D., Bleiholder, H., Langelüddecke, P., Stauss, R., Van den Boom, T., Weber, E. and Witzemberger, A., 1991. A uniform decimal code for growth stages of crops and weeds. *Annals of Applied Biology* 119: 561-601.
- Lindhauer, M.G., 2012. Do classical wheat quality assessments deliver relevant results for modern wheat cultivars? *Quality Assurance and Safety of Crops & Foods* 4: 152.
- Linina, A., Ruza, A., Kunkulberga, D. and Rakcejewa, T., 2014. The influence of environmental conditions on winter wheat wholemeal protein content and rheological properties. In: *FoodBalt Conference Proceedings*, Jelgava, Latvia, 8-14 May 2014, pp. 66-71. Available at: http://ilufb.llu.lv/conference/foodbalt/2014/FoodBalt_Proceedings_2014.pdf.
- Lloveras, J., Manent, J., Viudas, J., López, A. and Santiveri, P. 2004. Seeding rate influence on yield and yield components of irrigated winter wheat in a mediterranean climate. *Agronomy Journal* 96: 1258-1265.
- MacRitchie, F., 1999. Wheat proteins: characterization and role in flour functionality. *Cereal Foods World* 44: 188-193.
- Majewska, K., Dąbkowska, E., Żuk-Golaszewskam K. and Tyburski, J., 2007. Wartość wypiekowa mąki otrzymanej z ziarna wybranych odmian orkisz (*Triticum spelta* L.). *Żywność. Nauka. Technologia. Jakość* 2: 60-71.
- Makowska, A., Obuchowski, W., Adler, A. and Sulewska, H., 2008. Charakterystyka wartości przemiałowej i wypiekowej wybranych odmian orkisz. *Fragmenta Agronomica* 1: 228-239.
- Mikos, M. and Podolska, G., 2012. Bread-making quality of old common bread (*Triticum aestivum* ssp. *vulgare* L.) and spelt (*Triticum aestivum* ssp. *spelta* L.) wheat cultivars. *Journal of Food, Agriculture and Environment* 10: 221-224.
- Motzo, R., Fois, S., Giunta, F. 2007. Protein content and gluten quality of durum wheat (*Triticum turgidum* subsp. *Durum*) as affected by sowing date. *Journal of the Science of Food and Agriculture* 87: 1480-1488.
- Olszewski, J., Podolska, G. and Pszczółkowska, A., 2009. Stres biotyczny i abiotyczny oraz czynniki agrotechniczne a zawartość w nasionach ważnych roślin uprawnych białek powodujących nietolerancje pokarmowe. In: *Biologicznie aktywne peptydy i białka żywności*, Ed by Dziuba J and Fornal L, Warsaw, pp. 388-408. WNT Wydawnictwa Naukowo-Techniczne, Warsaw, Poland.
- Ouda, S.A., El-Marsafawy, S.M., El-Kholy, M.A., Gaballah, M.S., 2005. Simulating the effect of water stress and different sowing dates on wheat production in South Delta. *Journal of Applied Sciences Research* 1: 268-276.
- Ozturk, A., Caglar, O. and Bulut, S., 2006. Growth and yield response of facultative wheat to winter sowing, freezing sowing and spring sowing at different seeding rates. *Journal of Agronomic Crop Science* 192: 10-16.
- Podolska, G., 2014. Czynniki siedliskowe i agrotechniczne wpływające na wartość technologiczną pszenicy ozimej. *Studia i Raporty IUNG-PIB*, Zeszyt 41: 99-115.

- Podolska, G. and Filipiak, K., 2010. Plonowanie i wartość technologiczna ziarna pszenicy ozimej w zależności od rejonu uprawy. Zeszyty Problemowe Postępów Nauk Rolniczych 556: 203-210.
- Podolska, G., Krasowicz, S. and Sulek, A., 2005. Ocena ekonomiczna i jakościowa uprawy pszenicy ozimej przy różnym poziomie nawożenia azotem [Economic and quality evaluation of winter wheat cultivation in relation to different nitrogen fertilization levels]. Pamiętnik Puławski, 139: 175-188.
- Podolska, G. and Wyzińska, M., 2011. Reakcja nowych odmian pszenicy ozimej na gęstość i termin siewu. Polish Journal of Agronomy 6: 45-53.
- Pospišil, A., Pospišil, M., Svečnjak, Z. and Matotán, S., 2011. Influence of crop management upon the agronomic traits of spelt (*Triticum spelta* L.). Plant Soil Environment 57: 435-440.
- Rachoń, L. and Szumilo, G., 2009. Comparison of chemical composition of selected Winter wheat species. Journal of Elementology 14: 135-146.
- Ranhotra, G.S., Gelroth, J.A., Glaser, B.K. and Lorenz, K.J., 1995. Baking and nutritional qualities of a spelt wheat sample. LWT-Food Science and Technology 28: 118-122.
- Razzaq, A., Zada, K. and Shah, P. 1986. Effect of sowing dates and varieties on yield and yield components of wheat in Peshawar valley. Sarhad Journal of Agriculture 2: 29-40.
- Ribeiro, T. L. P., Cunha, G. R., Pires, J. L. F. and Pasinato, A. 2009. Phenological responses of Brazilian wheat cultivars to vernalization and photoperiod. Pesquisa Agropecuária Brasileira 44: 1383-1390.
- Rickman, R.W., Klepper, B.L. and Peterson, C.M., 1983. Time distributions for describing appearance of specific culms of winter wheat. Agronomy Journal 75: 551-556.
- Schober, T.J., Bean, S.R. and Kuhn, M., 2006. Gluten proteins from spelt (*Triticum aestivum* ssp. *spelta*) cultivars: a rheological and size-exclusion high performance liquid chromatography study. Journal of Cereal Science 44: 161-173.
- Silva, R.R., Benin, G., Almeida, J.L., Batista Fonseca, I.C. and Zucareli, C., 2014. Grain yield and baking quality of wheat under different sowing dates. Acta Scientiarum. Agronomy 36: 201-210.
- Singh, S., Gupta, A.K., Guptam, S.K. and Kaur, N., 2010. Effect of sowing time on protein quality and starch pasting characteristics in wheat (*Triticum aestivum* L.) genotypes grown under irrigated and rain-fed conditions. Food Chemistry 122: 559-565.
- Singh, S., Gupta, A.K., Guptam, S.K. and Kaur N., 2012. Influence of drought and sowing time on protein composition, antinutrients, and mineral contents of wheat. The Scientific World Journal: 485751.
- Slafer, G.A. and Satorre, E.H., 1999. An introduction to the physiological-ecological analysis of wheat yield. In: Satorre, E.H. and Slafer, G.A. (eds.). Wheat: ecology and physiology of yield determination. The Haworth Press, New York, NY, USA, pp. 3-12.
- Staggenborg, S.A., Whitney, D.A., Fjell, D.L. and Shroyer, J.P., 2003. Seeding and nitrogen rates required to optimize winter wheat yields following grain sorghum and soybean. Agronomy Journal 95: 253-259.
- Stoppler, H., Kolsch, E. and Vogtmann, H., 1990. The influence of sowing date, seed rate and variety on agronomic characteristics of winter wheat. Journal of Agronomy and Crop Science 90: 28-38.
- Tashiro, T. and Wardlaw, I.F., 1999. The response to high temperature shock and humidity changes prior to and during the early stages of grain development in wheat. Australian Journal of Plant Physiology 17: 551-561.
- Triboi, E. and Triboi-Blondel, A.M., 2002. Productivity and grain or seed composition: a new approach to an old problem. European Journal of Agronomy 16: 163-186.
- Valério, I.P., Carvalho, F.I.F., Benin, G., Silveira, G., Gonzalez da Silva, J.A., Nornberg, R., Hagemann, T., Souza Luche, H. and Costa de Oliveira, A., 2013. Seeding density in wheat: the more, the merrier? Scientia Agricola 70: 176-184.
- Wardlaw, I.F., 1994. The effect of high temperature on kernel development in wheat: variability related to pre-heading and post heading conditions. Australian Journal of Plant Physiology 21: 731-739.
- Wheeler, T.R., Hong, T.D., Ellis, R.H., Batts, G.R., Morrison, J.I.L. and Hadley, P., 1996. The duration and rate of grain growth, and harvest index, of wheat (*Triticum aestivum* L.) in response to temperature and CO₂. Journal of Experimental Botany 47: 623-630.
- Wieser, H., 2000. Comparative investigations of gluten proteins from different wheat species. I. Qualitative and quantitative composition of gluten protein types. European Food Research and Technology 211: 262-268.
- Wieser, H., Antes, S. and Seilmeier, W., 1998. Quantitative determination of gluten protein types in wheat flour by reversed phase high-performance liquid chromatography. Cereal Chemistry 75: 644-650.
- Yan, W., Holland, J.B., 2010. A Heritability-adjusted GGE biplot for test environment evaluation. Euphytica 171: 355-369.
- Zhen-Wen, Y., Van Sanford, D.A. and Egli, D.B., 1988. Effect of population density on floret initiation, development and abortion in winter wheat. Annals of Botany 62: 295-302.