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The Quality of Winter Wheat Grain by Different Sowing Strategies and Nitrogen Fertilizer Rates: A Case Study in Northeastern Poland

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Abstract: The present study was undertaken to determine the effect of different sowing strategies and spring nitrogen (N) fertilizer rates on the technological quality of winter wheat (*Triticum aestivum* L.) grain in terms of its milling quality, protein complex quality, and enzyme activity (falling number). Winter wheat grain for laboratory analyses was produced in a small-area field experiment conducted between 2018 and 2021 in the AES in Bałcyny (53°35′46.4″ N, 19°51′19.5″ E, NE Poland). The experimental variables were (i) sowing date (early: 6 September 2018, 5 September 2019, and 3 September 2020; delayed by 14 days: 17–20 September; and delayed by 28 days: 1–4 October), (ii) sowing density (200, 300, and 400 live grains m^{-2}), and (iii) split application of N fertilizer in spring (40 + 100, 70 + 70, and 100 + 40 kg ha^{-1}) at BBCH stages 22–25 and 30–31, respectively. A sowing delay of 14 and 28 days increased the bulk density (by 1 and 1.5 percent points (%p), respectively), vitreousness (by 3 and 6%p, respectively), and total protein content of grain (by 1% an 2%, respectively). A sowing delay of 14 days increased grain hardness (by 5%), the flour extraction rate (by 1.4%p), and the falling number (by 3%) while also decreasing grain uniformity (by 1.9%p). In turn, a sowing delay of 28 days increased the wet gluten content of grain (+0.5–0.6%p) and improved the quality of the protein complex in the Zeleny sedimentation test (+1.5%). An increase in sowing density from 200 to 300 live grains m^{-2} led to a decrease in grain uniformity (by 2.6%p), the total protein content (by 1.5%), and the wet gluten content of grain (by 0.7%p). A further increase in sowing density decreased grain vitreousness (by 1.4%p). The grain of winter wheat fertilized with 40 and 100 kg N ha^{-1} in BBCH stages 22–25 and 30–31, respectively, was characterized by the highest hardness (64.7), vitreousness (93%), flour extraction rate (73.9%), total protein content (134 g kg^{-1} DM), wet gluten content (36%), and Zeleny sedimentation index (69 mL).

Keywords: *Triticum aestivum* L.; grain; milling quality; protein; gluten; sedimentation index; falling number

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1. Introduction

Wheat grain accounts for 21% of total dietary calories, 20% of protein, and 55% of carbohydrates consumed by 4.5 billion people around the world. Wheat a staple food for 36% of the global population [1–4] and one of the most popular cereal crops around the world [5–8]. Wheat, rice, and maize are the three main pillars of the human diet [5]. In 2021, the global harvests of maize, rice, and wheat reached 1210, 778, and 771 Tg, respectively, and accounted for 90% of the total cereal grain harvests worldwide [7]. However, wheat has a considerable advantage over other strategic cereals in terms of global food security because it easily adapts to varied environmental conditions due to its high plasticity [2,6,9,10]. Wheat is cultivated from 67° N in Scandinavia and Russia to 45° S in Argentina, including in elevated areas of tropical and sub-tropical regions [9]. The global area covered by wheat is estimated to be 216–221 million ha (2019–2021), which accounts for around 16% of gross cropped area [7]. In 2019–2021, the world's leading wheat producers were China (135 million Mg y^{-1}), India (107 million Mg y^{-1}), Russia (79 million Mg y^{-1}), the United States (49 million Mg y^{-1}), France (35 million Mg y^{-1}), Canada (30 million Mg y^{-1}), and

Ukraine (29 million Mg y^{-1}). Wheat is also a principal cereal in the European Union (135 million Mg y^{-1}, 2019–2021), including Poland (12 million Mg y^{-1}, 2019–2021). In 2019–2021, wheat accounted for 46% and 36% of total cereal production in the EU and Poland, respectively [7]. Due to the observed increase in the global human population, wheat will continue to play an important role as a strategic cereal crop for sustaining food security [10].

At present, hexaploid common wheat (*Triticum aestivum* L.) accounts for 95% of the wheat grown worldwide, whereas the remaining 5% is mostly tetraploid hard wheat (*T. durum* Dest.) [9]. Common wheat grown for consumption should be characterized by high yields and high technological quality [11,12]. The processing suitability of wheat is determined by grain quality attributes, including (i) the physical properties of grain that influence its milling quality [8,13,14] and (ii) the protein complex, the starch complex, and the activity of amylolytic enzymes [15]. Milling quality is defined as the processing suitability of grain for the milling industry by determining the properties of kernels responsible for high milling yields [16–18]. The milling quality of grain can be evaluated directly in laboratory milling tests [19], and it can be assessed indirectly by analyzing the physical properties of grain (hardness, vitreousness, bulk density, 1000-kernel weight, and uniformity) [14,20]. Kernels with hard (vitreous) and soft (floury) endosperm can be identified in grain hardness tests [21–23]. Hard endosperm strongly adheres to protein particles, which increases its compressive strength. Grain with floury endosperm is characterized by a lower milling yield (milled grain tends to clog the sieves), which increases with a rise in endosperm hardness [16,22,24]. Endosperm color is linked with vitreousness [17,25]. White endosperm is typically found in starchy kernels with low protein content. In turn, gray endosperm is characteristic of vitreous (hard) kernels that are high in protein but contain less starch [26,27]. Vitreousness and hardness are good predictors of grain filling [27], and seed coat thickness affects grain hardness. These parameters determine the bulk density of grain, namely the ratio of kernel weight to kernel volume [20,28–32]. To maximize flour extraction rates in common wheat, bulk density should be a minimum of 72 kg hL^{-1} and optimally exceed 76 kg hL^{-1} [10,33,34]. Wheat kernels should also be characterized by uniform thickness (minimum 2.5 mm–thicker kernels are more desirable) because this parameter facilitates processing and increases milling yields [8,10,35]. In the milling and baking industry, the milling quality of wheat grain has to be monitored and controlled to support the production of high-quality flour and cereal products. The milling quality of grain is also an important consideration for farmers and agricultural producers, as it may affect grain purchase prices [10,19].

The protein content of grain also significantly affects flour quality [36,37]. Storage proteins (gliadin and glutenin) play an important role during dough formation and are chiefly responsible for the baking value of wheat flour [38–41]. Gliadins contribute to the extensibility and viscosity of dough, whereas glutenins enhance its elasticity and strength [42,43]. During mechanical kneading, the two proteins are combined in the presence of water to form a viscous and elastic mass (gluten). Gluten determines the water absorption capacity of flour and, consequently, the elasticity and springiness of the obtained dough [44]. The quality of the protein complex is evaluated based on the value of the sedimentation index in the sodium dodecyl sulfate (SDS) test or the Zeleny sedimentation test [45–47]. These tests rely on differences in the size of glutenin particles (high-molecular-weight glutenins, or HMW-GSs, and low-molecular-weight glutenins, or LMW-GSs) [48,49]. Grain with a higher content of HMW-GSs is characterized by higher values of the sedimentation index [10]. HMW-GSs are regarded as the critical determinants of the processing suitability of wheat grain [50–53] despite the fact that they account for only 5–10% of storage proteins in wheat kernels [4,54]. The endosperm of wheat kernels contains 20–30% LMW-GSs [4,54]. Wheat flours with a high HMW/LMW-GSs ratio are characterized by higher baking value [55,56]. The milling quality of wheat grain is also influenced by enzyme activity, which is measured in the falling number test [15,57]. Wheat grain for bread production should have a falling number of 250–350 s. The activity of amylolytic enzymes is very high in grain with a low falling number (<150 s) and low in grain with a high falling number (>400 s). In both cases, the produced bread is of low quality [58,59]. Protein complex quality and enzyme activity in wheat grain are key parameters in the food processing sector. They are also important for farmers because they determine the quality, technological properties, and nutritional value of flour, being taken into account when selecting cultivars and agronomic management strategies [10,13,15,33,58].

The technological quality of grain is determined mostly by the wheat cultivar and its genetic profile [10,60–63], as well as weather conditions in the generative growth phase of wheat plants [10,50,64–69]. However, mistakes in the wheat production technology can decrease grain quality [13,21,70–76]. Nitrogen (N) fertilization is an agricultural management practice that exerts the greatest impact on the technological quality of wheat grain [10,77]. Grain quality is significantly affected not only by the N rate, but also by the method and date of N application [23,78–80]. Under the agroecological conditions of Poland, the optimal rate of N fertilizer in wheat grown for human consumption is 160 to 180 kg ha^{-2}, and 40–50% of the rate should be applied at the beginning of spring growth [10]. Above all, the N rate affects the biomechanical properties of grain (1000-kernel weight, density, uniformity, and vitreousness) [31,81]. Higher spring N rates increase the bulk density [13] and vitreousness of wheat grain [31,82–84]. According to Kindred et al. [85] and Dargie et al. [86], N fertilization also improves grain filling (by reducing empty spaces and decreasing the content of α-amylase), delays ripening, and prolongs grain dormancy. However, increasing N rates result in smaller and less uniform grain, which can compromise milling yields [70,71]. Higher N rates also increase the content of protein [13,31,81,84,87,88] and gluten in grain [83,88–90]. In turn, lower N rates increase the concentration of gliadins and decrease the content of HMW-GSs and LMW-GSs in grain [90–92]. However, it should be noted that the impact of N fertilization on the content and quality of gluten is strongly correlated with genetic factors (cultivar) and weather conditions during grain filling and ripening [10,93,94]. In some cultivars of common wheat, high N rates are required to obtain grain that is abundant in protein and gluten and can be processed into flour with a high baking value. In other cultivars, the optimal values of these grain parameters can be achieved already at low N rates, and an increase in the N rate can decrease the content and quality of gluten. The above is observed when high N rates are accompanied by high total precipitation, which increases enzyme activity in kernels and triggers kernel germination in spikes [10,95–98]. High enzyme activity decreases the falling number, which often renders grain unsuitable for processing in the milling industry [58,99]. The technological quality of grain is influenced not only by the N rate, but also by N application timing [100,101]. Nitrogen applied in the early stages of wheat growth and development in spring (BBCH stages 22–31, Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie–Meier [102]) affects mainly grain yields, whereas N applied in later stages (BBCH stages 37–51) influences grain quality parameters, mostly total protein content, gluten content, and the sedimentation index [71,80,103–107]. Due to climate change, which affects N use efficiency, fertilization strategies should be thoroughly analyzed to produce winter wheat grain of high technological quality [10,76,93,94].

Different sowing strategies (sowing date and sowing density) are evaluated mostly in terms of their effect on wheat yields, but they are rarely analyzed in the context of grain quality. In Poland, 250–350 live grains m^{-2} should be sown between 15 and 20 September [10]. According to Zende et al. [108], grain characterized by high milling quality can be obtained only when wheat is sown on the optimal date. Delayed sowing generally decreases bulk density [109–113], grain hardness, and milling yield [112,114]. In turn, the grain of late-sown wheat is more abundant in protein and gluten, which increases the sedimentation index [108,112,115–120]. The effect of sowing density on the quality of wheat grain has been rarely investigated in the literature, and the results are inconclusive [11,73,74,121,122]. Geleta et al. [121] and Zecevic et al. [74] found that high sowing density (650 vs. 500 live grains m^{-2}) had a beneficial influence on the milling quality of grain in several cultivars of winter wheat. In the cited studies, densely sown wheat produced a higher number of main shoots and larger and heavier kernels with a more desirable protein/starch ratio. In less dense stands, grain ripening was delayed, and the plants produced a higher number of secondary shoots (tillers) and smaller and lighter kernels with low bulk density and low quality [74,121]. In turn, Guerrini et al. [11] and Sun et al. [123] found that higher sowing density decreased 1000-kernel weight but did not affect the bulk density of wheat grain. In the work of Soofizada et al. [122], Caglar et al. [73], and Zecevic et al. [74], higher sowing density increased the protein content, gluten content, and the sedimentation index of wheat grain. In view of global climate change, optimal sowing strategies should be defined again to ensure stable production of grain characterized by high processing suitability [112].

Previous studies have mostly focused on evaluating the effect of single agronomic factors on the quality of winter wheat grain. However, the optimal sowing and N management strategies aimed at improving the technological quality of winter wheat grain have not been identified to date. The present study can contribute to optimizing winter wheat cultivation practices in terms of grain quality. The objective of this study was to determine the effect of split spring application of N fertilizer, sowing date, and sowing density on the milling quality (bulk density, vitreousness, uniformity, and flour extraction rate), protein complex quality, and enzyme activity (total protein content, gluten content, Zeleny sedimentation index, and falling number) of winter wheat grain grown in northeastern Poland. The findings from this study can be used to formulate recommendations for optimizing winter wheat cultivation practices, including sowing date, sowing density and N fertilization, to improve the technological quality of grain.

2. Materials and Methods

2.1. Field Experiment

Winter wheat (*Triticum aestivum* L.) grain was produced in 2018–2021 during a small-area field experiment conducted in the Agricultural Experiment Station in Bałcyny (53°35′46.4″ N, 19°51′19.5″ E, elevation 137 m, north-eastern Poland, *Dfb* according to Köppen's classification), owned by the University of Warmia and Mazury in Olsztyn. The experiment had a split-plot design with three replicates. The experimental variables were as follows: (i) sowing date: early (6 September 2018, 5 September 2019, and 3 September 2020), delayed by 14 days (17–20 September), and delayed by 28 days (1–4 October); (ii) sowing density: 200, 300, and 400 live grains m^{-2}; (iii) split application of N fertilizer in spring (BBCH stages 22–25 and 30–31; full tillering stage and first node stage, respectively): 40 + 100, 70 + 70, and 100 + 40 kg ha^{-1} (ammonium nitrate, 34% N). The third portion of N fertilizer (40 kg ha^{-1}; ammonium nitrate, 34%) was applied in flag leaf, just visible, still rolled (BBCH stage 37) in all plots.

Plot size was 15 m^2 (10 m by 1.5 m). The forecrop was winter oilseed rape (*Brassica napus* L.). All field treatments were consistent with the agronomic requirements of winter wheat and good agricultural practices. The experimental conditions (soil type and chemical properties) and the production technology of winter wheat were described in detail by Lachutta and Jankowski [124].

2.2. Grain Analysis

Grain uniformity was assessed in a mechanical grain separator (ZBPP sp. z o. o., Bydgoszcz, Poland) according to Polish Standard PN R–74110:1998 [125]. Wheat kernels (100 g) were placed on the top screen (2.5 × 25 mm) and separated at 310 rpm. The grain remaining on the top screen after 3 min was weighed. Grain uniformity was expressed as the percentage of grain remaining on the top screen in the total weight of the sample. The analysis was conducted on three grain samples (100 g each) from each plot.

The bulk density of winter wheat grain was determined using a density analyzer with a volume of 1 dm^3 (ZBPP sp. z o. o., Bydgoszcz, Poland) according to Polish Standard PN-EN ISO 7971–3:2019–03 [126]. Bulk density was measured in three grain samples from each plot.

Grain hardness (86% dry matter, DM) was determined with the InfratecTM 1241 grain analyzer (FOSS, Hillerod, Denmark), which relies on the near-infrared transmittance technology in the wavelength range of 570–1050 nm. The analyses were conducted in three grain samples from each plot.

Vitreousness was determined by analyzing the horizontal cross-sections of 50 winter wheat kernels from each plot. The examined kernels were cut in half with a commercial grain cutter (Farinotom, ZBPP sp. z o. o., Bydgoszcz, Poland). Kernels were classified as vitreous if >75% of their cross-sectional area had a gray color. Grain vitreousness was expressed as the percentage of kernels with vitreous endosperm in the total number of the analyzed kernels, with vitreousness being determined according to Polish Standard PN R–74008:1970 [127].

The flour extraction rate was determined as the percentage of flour obtained from a grain sample (on a weight basis). Flour was obtained by grinding wheat grain in a laboratory mill (Brabender, Quadrumat Junior, Duisburg, Germany). The moisture content of the grain was brought to 14% before milling. The initial moisture content of wheat grain was 10.5–13.0%. The amount of water (with a temperature of 20 °C) required to adjust the moisture content of a 1500 g grain sample to 14% was determined. Grain was conditioned in closed glass containers and stored in a cooled incubator (ICP 500, Memmert, Eagle, WI, USA) for 48 h. The analysis was conducted on three grain samples (150 g each) from each plot.

Total protein content was determined with the AgriCheck instrument (Bruins Instruments, Puchheim, Bayern, Germany), which measures near-infrared transmittance in the wavelength range of 730–1100 nm. Wet gluten content was determined by the gravimetric method with a gluten washing device according to Polish Standard PN-EN ISO 21415–2:2015–12E [128]. Dough was prepared by combing 10 g of sifted flour (250 µm mesh size) and 4.8 cm^3 of 2% NaCl solution in a vortex mixer (ZBPP sp. z o. o., Bydgoszcz, Poland). Gluten was separated in a dual-chamber glutomatic system (ZBPP sp. z o. o., Bydgoszcz, Poland) with the addition of 250–280 mL of 2% NaCl solution. The separated gluten was dried in a gluten centrifuge (ZBPP sp. z o. o., Bydgoszcz, Poland) at 3000 rpm for 60 s. The total protein content and wet gluten content were determined in three grain samples from each plot.

The sedimentation index was determined in the Zeleny sedimentation test with the use of the SWD-89 measuring device with a laboratory shaker (ZBPP sp. z o. o., Bydgoszcz, Poland) according to Polish Standard PN-EN ISO 5529:2010 [129].

The falling number was determined in the Hagberg–Perten test [130,131] with the use of the SWD-SŻ falling number system (ZBPP sp. z o. o., Bydgoszcz, Poland) according to Polish Standard PN-EN ISO 3093:2010 [132].

The bulk density, vitreousness, uniformity of grain, total protein content and wet gluten content of grain, as well as the Zeleny sedimentation index and the falling number were determined in the laboratory of ZBPP sp. z o. o. in Bydgoszcz, Poland. Grain hardness and the flour extraction rate were determined in the laboratories of the University of Warmia and Mazury in Olsztyn.

2.3. Weather Conditions

Weather conditions (mean daily temperature and precipitation) in the growing seasons of winter wheat (2018/2019, 2019/2020, and 2020/2021) were described by Lachutta and Jankowski [124]. Weather conditions between the flowering and fully ripe (harvest) stages are described in detail in the present study. In these phenological growth stages, temperature and precipitation exert the greatest influence on the technological quality of winter wheat grain [10]. Meteorological data (mean daily temperature and total precipitation) were acquired with the use of the PM Ecology automatic weather station (PM Ecology Ltd., Gdynia, Poland) in the AES in Bałcyny. In each growing season, the number of growing degree days (GDD) (Equation (1)) and the Sielyaninov hydrothermal index [133] (Equation (2)) were determined between the beginning of flowering and the milk stage (BBCH stages

61–73), between the milk stage and the dough stage (BBCH stages 73–83), between the dough stage and the fully ripe stage (BBCH stages 83–89), and between the beginning of flowering and the fully ripe stage (BBCH stages 61–89). The Sielyaninov hydrothermal index measures effective precipitation in a given period (as the ratio of precipitation to evaporation, which is determined mainly by the mean daily temperature).

$$GDD = \Sigma\,(MDT - T_{base}) \quad (1)$$

where:

GDD—growing degree days (°C),

MDT—mean daily temperature (°C),

T_{base}—the base temperature for GDD calculations was 5 °C (period of active plant growth) [134].

$$K = \frac{\sum P}{\sum (T \times 0.1)} \quad (2)$$

where:

K—Sielyaninov index (K: 0–0.5-extreme dry spell, 0.6–1.0-dry spell, 1.1–2.0-humid spell, >2.1-wet spell),

ΣP—total precipitation in the analyzed period (mm),

ΣT—total mean daily temperature in the analyzed period (°C),

0.1—constant.

2.4. Statistical Analysis

The obtained data (bulk density, vitreousness, uniformity, flour extraction rate, total protein content, wet gluten content, Zeleny sedimentation index, and the falling number) were analyzed in ANOVA using Statistica software, version 13 [135]. Post hoc multiple comparisons were performed with the use of Tukey's test (HSD) in subsequent stages of statistical analyses. Data were regarded as statistically significant at $p \leq 0.05$. The results of the F-test for fixed effects in ANOVA are presented in Table S1. A linear regression method was used to evaluate the relationship between meteorological variables and the studied agronomic parameters. The values of Pearson's correlation coefficient (R) were regarded as significant at $p \leq 0.01$ and $p \leq 0.05$ (Table S2).

3. Results

3.1. Weather Conditions

Weather conditions during the phenological growth stages of winter wheat (growing seasons of 2018/2019, 2019/2020, and 2020/2021) were described by Lachutta and Jankowski [124]. This article focuses on weather conditions in growth stages that are critical for grain quality, i.e., from flowering to the fully ripe stage [10]. Mean daily temperature and precipitation between the flowering and harvest (BBCH stages 61–89) of winter wheat varied considerably across the experimental years (2018–2021) (Table 1). The mean thermal time between flowering and harvest was 696–796, 630–649, and 695–706 °C GDD in 2019, 2020, and 2021, respectively. In the first growing season, the mean thermal time between flowering and harvest increased by 22 and 100 °C GDD when sowing was delayed by 14 and 28 days, respectively. Delayed sowing induced a particularly high increase in GDD in BBCH stages 83–89 (93 vs. 118–187 °C). In the remaining years, delayed sowing did not lead to significant differences in GDD during flowering and ripening. In these phenological growth stages, precipitation was determined at 154–178 (1st growing season), 127–128 (2nd growing season), and 134–153 mm (3rd growing season). In the first growing season, late-sown plants were exposed to higher precipitation between flowering and ripening. In the second growing season, rainfall distribution in BBCH stages 61–89 was weakly differentiated by the sowing date. In the third growing season, more abundant precipitation was noted in the flowering stage and in the early stages of grain ripening (BBCH stages 61–73 and BBCH stages 73–83, respectively) in late-sown stands. The last stages of grain ripening

(BBCH 83–89) occurred during a dry spell (41 vs. 0–2 mm). In general, optimal values of the Sielyaninov hydrothermal index were noted during wheat flowering and grain ripening (humid spell). However, in the second growing season, a dry spell (K = 0.35–0.98) was observed between the milk stage and harvest (BBCH stages 73–89) (Table 1).

Table 1. Phenological development of winter wheat and weather conditions (2018/2019, 2019/2020, and 2020/2021).

Parameter	Growing Season	Sowing Date	Growth Stage			
			BBCH 61–73	BBCH 73–83	BBCH 83–89	BBCH 61–89
Growing Degree Days (°C)	2018/2019	6 September	445	158	93	696
		20 September	450	156	118	718
		4 October	447	166	187	796
	2019/2020	05 September	349	180	120	649
		19 September	359	153	107	630
		3 October	338	160	132	630
	2020/2021	3 September	351	202	152	705
		17 September	390	171	134	695
		1 October	381	172	152	706
Mean daily temperature (°C)	2018/2019	6 September	21.5	16.3	16.6	19.2
		20 September	21.5	16.1	18.0	19.4
		4 October	21.5	15.2	20.5	19.5
	2019/2020	5 September	19.0	17.0	18.3	18.2
		19 September	18.8	17.7	18.4	18.5
		3 October	19.1	17.3	18.2	18.4
	2020/2021	3 September	20.3	21.8	20.2	20.7
		17 September	21.3	22.1	19.9	21.2
		1 October	21.6	22.2	20.2	21.4
Total precipitation (mm)	2018/2019	6 September	92.0	49.9	12.4	154.3
		20 September	91.9	49.9	29.7	171.5
		4 October	101.4	52.8	23.5	177.7
	2019/2020	5 September	94.6	25.1	7.9	127.6
		19 September	107.4	13.4	5.7	126.5
		3 October	111.0	9.9	6.3	127.2
	2020/2021	3 September	55.1	56.9	41.0	153.0
		17 September	59.2	79.2	0.0	138.4
		1 October	65.2	73.2	2.3	140.7
Sielyaninov index (K)	2018/2019	6 September	1.59	2.19	0.93	1.64
		20 September	1.58	2.21	1.83	1.77
		4 October	1.74	2.18	0.95	1.66
	2019/2020	5 September	1.99	0.98	0.48	1.43
		19 September	2.20	0.63	0.39	1.49
		3 October	2.42	0.44	0.35	1.47
	2020/2021	3 September	1.18	2.17	2.03	1.65
		17 September	1.16	3.59	0.00	1.52
		1 October	1.31	3.29	0.11	1.53

K: 0–0.5-extreme dry spell, 0.6–1.0-dry spell, 1.1–2.0-humid spell, >2.1-wet spell. BBCH 61–73: beginning of flowering–milk stage; BBCH 73–83: milk stage to dough stage, BBCH 83–89: dough stage to fully ripe stage; BBCH 61–89: beginning of flowering to fully ripe stage.

3.2. Milling Quality

Grain uniformity was negatively correlated with the mean daily temperature between flowering and harvest (BBCH stages 61–89) (Figure 1). The most uniform grain (83.0%) was harvested in the second growing season (Table 2) when the mean daily temperature was low at BBCH stages 61–89 (18.2–18.5 °C) (Table 1). Grain uniformity was lowest (69.7%) in the third growing season (Table 2), which was characterized by the highest mean daily temperature between flowering and harvest (20.7–21.4 °C) (Table 1). Late-sown plants were exposed to higher mean daily temperatures between flowering and ripening (BBCH stages 61–89) (Table 1), which decreased grain uniformity by 1.9 percent points (%p) (Table 2). Sowing date exerted the greatest influence on grain uniformity in the first growing season when a sowing delay of 14 days decreased grain uniformity by 6.1%p (Figure 2a), mainly due to higher mean daily temperatures between flowering and ripening stages (20.7 vs. 21.2–21.4 °C) (Table 1). An increase in sowing density from 200 to 300 live grains m^{-2} induced a 2.6%p decrease in grain uniformity (Table 2). The split spring N rate had no significant effect on grain uniformity (Table S1).

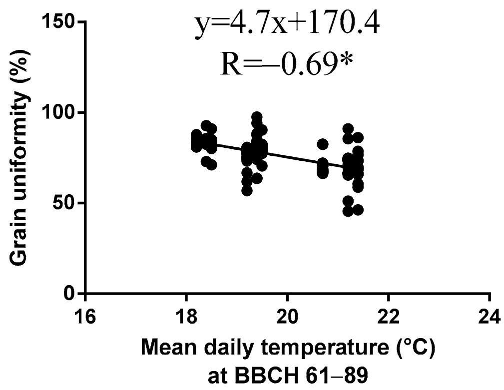

Figure 1. Linear regression between grain uniformity and mean daily temperature at BBCH 61–89. * significant at $p \leq 0.05$.

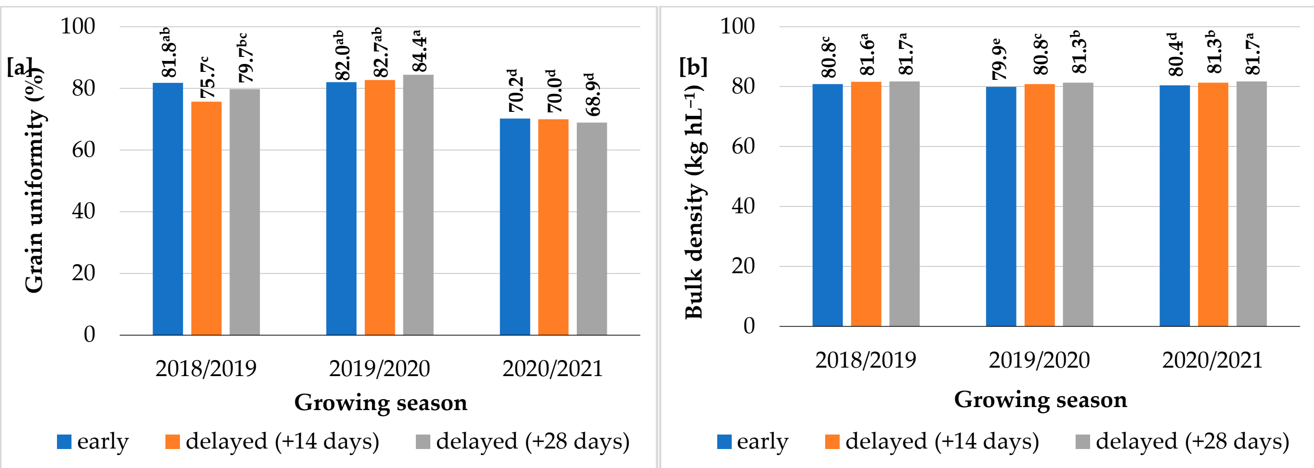

Figure 2. The effect of the sowing date on (**a**) grain uniformity and (**b**) the bulk density of winter wheat grain (2018/2019, 2019/2020, 2020/2021). Early sowing: 3–6 September; delayed sowing (+14 days): 17–20 September; delayed sowing (+28 days): 1–4 October. Means followed by the same letters are not significantly different at $p \leq 0.05$ in Tukey's test.

The bulk density of winter wheat grain ranged from 80.7 (2019/2020) to 81.1–81.3 kg hL^{-1} (2018/2019 and 2020/2021). Bulk density increased by 1.0%p and 1.5%p when winter wheat was sown, with a delay of 14 and 28 days, respectively (Table 2). In the first growing season, the grain of wheat sown in the middle of September had the highest bulk density

(81.6 kg hL^{-1}). In the second and third growing seasons, bulk density was highest (81.3 and 81.7 kg hL^{-1}, respectively) in winter wheat stands sown at the beginning of October (Figure 2b). Sowing density and the split spring N rate did not induce significant differences in the bulk density of grain, regardless of weather conditions (Table S1).

Table 2. Milling quality of winter wheat grain.

Parameter	Uniformity (%)	Bulk Density (kg hL^{-1})	Hardness	Vitreousness (%)	Flour Extraction Rate (%)
Growing season					
2018/2019	79.1 ^{b}	81.3 ^{a}	62.7 ^{b}	90.9 ^{b}	73.2 ^{b}
2019/2020	83.0 ^{a}	80.7 ^{c}	58.8 ^{c}	88.9 ^{c}	71.2 ^{c}
2020/2021	69.7 ^{c}	81.1 ^{b}	68.0 ^{a}	97.1 ^{a}	74.7 ^{a}
Sowing date, mean for 2018–2021					
Early	78.0 ^{a}	80.4 ^{c}	61.4 ^{b}	89.2 ^{c}	71.8 ^{b}
Delayed (+14 days)	76.1 ^{b}	81.2 ^{b}	64.3 ^{a}	92.5 ^{b}	73.2 ^{a}
Delayed (+28 days)	77.7 ^{ab}	81.6 ^{a}	63.8 ^{a}	95.2 ^{a}	74.0 ^{a}
Sowing density (live grains m^{-2}), mean for 2018–2021					
200	78.8 ^{a}	81.0	63.4	93.1 ^{a}	73.2
300	76.2 ^{b}	81.1	63.2	92.0 ^{ab}	72.9
400	76.9 ^{b}	81.1	63.0	91.7 ^{b}	73.0
Split spring N rate (kg ha^{-1}), mean for 2018–2021 †					
40 + 100	76.7	81.1	64.7 ^{a}	93.2 ^{a}	73.9 ^{a}
70 + 70	77.8	81.1	62.0 ^{b}	91.9 ^{b}	73.0 ^{ab}
100 + 40	77.3	81.1	62.9 ^{b}	91.8 ^{b}	72.1 ^{b}

† BBCH stages 22–25 + BBCH stages 30–31. Early sowing: 3–6 September; delayed sowing (+14 days): 17–20 September; delayed sowing (+28 days): 1–4 October. Means followed by the same letters are not significantly different at $p \leq 0.05$ in Tukey's test. Means without letters indicate that the main effect is not significant.

Grain hardness was negatively correlated with grain uniformity (Table S2) and positively correlated with main daily temperature between flowering and harvest (Figure 3). Winter wheat plants produced the hardest grain (68.0) in the third growing season (2020/2021) (Table 1), which was characterized by the highest mean daily temperature between flowering and harvest (20.7–21.4 °C) (Table 1). Grain hardness was 8% and 14% lower in the first and second growing season, respectively (the mean daily temperature was 1.7 °C and 2.7 °C lower in BBCH stages 61–89, respectively). Grain hardness increased by 5% when wheat was sown with a 14-day delay (mid-September) relative to the early sowing date (Table 2). Late-sown plants were exposed to higher temperatures during flowering and ripening (Table 1), which explains the observed increase in grain hardness (Figure 3). Sowing density (200, 300, and 400 live grains m^{-2}) had no effect on grain hardness (Table S1). The split spring N rate of 40 + 100 kg ha^{-1} (BBCH stages 22–25 and 30–31, respectively) promoted an increase in grain hardness. An increase in the early spring N rate with a simultaneous decrease in the N rate applied in the stem elongation stage (70 + 70 or 100 + 40 kg ha^{-1}) decreased grain hardness by 3–4% (Table 2).

Grain vitreousness ranged from 88.9% to 97.1% (Table 3). The regression analysis revealed a positive correlation between vitreousness and mean daily temperature in the dough stage (BBCH stages 83–89) (Figure 4); therefore, grain vitreousness was highest (97.1%) in the third growing season (Table 2), which was characterized by the highest mean daily temperature in BBCH stages 83–89 (19.9–20.2 °C) (Table 1). Late-sown plants were exposed to higher mean daily temperatures in the dough stage (Table 1), which increased grain vitreousness by 3%p (+14 days) and 6%p (+28 days) (Table 2). There was a lack of interaction between grain vitreousness and the sowing date in the third growing season exclusively (Figure 5), which could be attributed to the absence of a relationship between the sowing date and the mean daily temperature in the dough stage (Table 1). Grain vitreousness decreased by 1.4%p when sowing density was increased from 200 to 400 live grains m^{-2}. An increase in the first spring N rate (BBCH stages 22–25) from 40 to 70 or 100 kg ha^{-1} with a simultaneous decrease in the N rate in BBCH stages 30–31 (from 100 to 70 and 40 kg ha^{-1}) increased grain vitreousness by 1.3–1.4%p (Table 2).

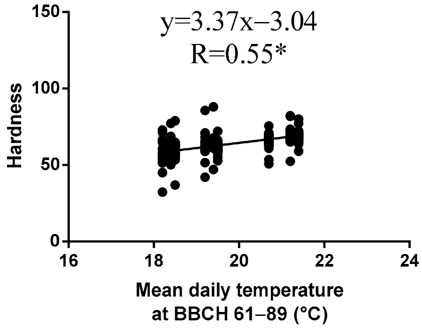

Figure 3. Linear regression between grain hardness and mean daily temperature at BBCH stages 61–89. * significant at $p \leq 0.05$.

Table 3. Protein complex quality and enzyme activity in winter wheat grain.

Parameter	Total Protein Content (g kg^{-1} DM)	Wet Gluten Content (%)	Zeleny Sedimentation Index (mL)	Falling Number (s)
Growing season				
2018/2019	137 [a]	34.0 [b]	68.1 [b]	364 [b]
2019/2020	129 [c]	34.3 [b]	68.2 [b]	328 [c]
2020/2021	132 [b]	38.3 [a]	69.0 [a]	381 [a]
Sowing date, mean for 2018–2021				
Early	131 [c]	35.4 [b]	68.0 [b]	352 [b]
Delayed (+14 days)	132 [b]	35.3 [b]	68.3 [ab]	364 [a]
Delayed (+28 days)	134 [a]	35.9 [a]	69.0 [a]	358 [ab]
Sowing density (live grains m^{-2}), mean for 2018–2021				
200	134 [a]	36.0 [a]	68.8	360
300	132 [b]	35.3 [b]	68.1	359
400	132 [b]	35.3 [b]	68.3	354
Split spring N rate (kg ha^{-1}), mean for 2018–2021 †				
40 + 100	134 [a]	36.0 [a]	68.9 [a]	356
70 + 70	133 [b]	35.5 [b]	68.4 [ab]	359
100 + 40	131 [c]	35.2 [b]	68.0 [b]	357

† BBCH stages 22–25 + BBCH stages 30–31. Early sowing: 3–6 September; delayed sowing (+14 days): 17–20 September; delayed sowing (+28 days): 1–4 October. Means followed by the same letters are not significantly different at $p \leq 0.05$ in Tukey's test. Means without letters indicate that the main effect is not significant.

The flour extraction rate ranged from 71.2% (2019/2020) to 74.7% (2020/2021) (Table 2), and it was significantly differentiated by the sowing date and the split spring N rate (Table S1). The flour extraction rate was lowest (71.8%) in early sown stands (beginning of September). The analyzed parameter increased from 1.4 to 2.2%p when sowing was delayed by 14 and 28 days. The flour extraction rate peaked (73.9%) in response to the spring N rate of 40 and 100 kg ha^{-1} applied in BBCH stages 22–25 and 30–31, respectively.

An increase in the early spring N rate with a simultaneous decrease in the N rate applied in BBCH stages 30–31 (100 + 40 kg ha^{-1}) decreased the flour extraction rate by 1.8%p (Table 2).

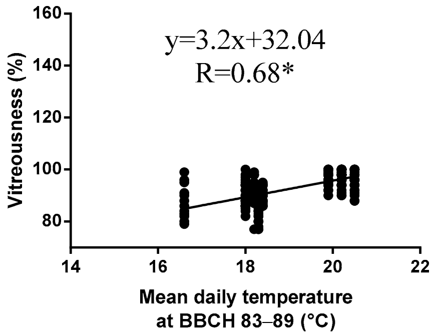

Figure 4. Linear regression between grain vitreousness and mean daily temperature at BBCH stages 83–89. * significant at $p \leq 0.05$.

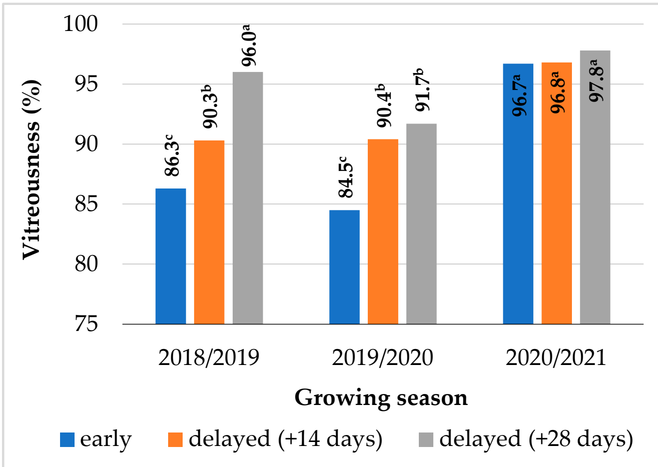

Figure 5. The effect of the sowing date on grain vitreousness (2018/2019, 2019/2020, 2020/2021). Early sowing: 3–6 September; delayed sowing (+14 days): 17–20 September; delayed sowing (+28 days): 1–4 October. Means followed by the same letters are not significantly different at $p \leq 0.05$ in Tukey's test.

3.3. Protein Complex Quality and Enzyme Activity in Grain

The total protein content of winter wheat grain was positively correlated with GDD, precipitation, and the Sielyaninov hydrothermal index between flowering and harvest (BBCH stages 61–89) (Figure 6). Weather conditions were least favorable in the second growing season (Table 1), which resulted in the lowest total protein content of grain (129 g kg^{-1} DM) (Table 3). In the second and third growing season, weather conditions were more favorable during wheat flowering and grain ripening (Table 1), which increased the total protein content of grain (132–137 g kg^{-1} DM) (Table 3). Delayed sowing increased total protein content by 1% (+14 days) and 2% (+28 days) (Table 3). A sowing delay of 14 and 28 days induced a particularly high increase in the total protein content of grain in the first growing season (4% and 8%, respectively) (Figure 7a). In this season, late-sown plants were exposed to higher GDD (696 vs. 718–796 °C), higher precipitation (154 vs. 172–178 mm), and higher values of the Sielyaninov index (1.64 vs. 1.66–1.77) (Table 1) between flowering and harvest, and these parameters are positively correlated with the total protein content of grain (Figure 6). The total protein content of winter wheat grain decreased by 1.5% when sowing density was increased from 200 to 300 live grains m^{-2} (Table 3). It should also be noted that the effect exerted by sowing density on the total protein content of grain was significantly modified by weather conditions during the growing season and the sowing date (Table S1). In the first growing season, a reduction in total protein content was observed already when sowing density was increased from 200 to 300 live grains m^{-2}.

In the second growing season, total protein content decreased only when sowing density was increased to 400 live grains m^{-2}. In turn, sowing density had no effect on the total protein content of grain in the third growing season (Figure 7b). An increase in sowing density decreased the total protein content of grain only in stands sown with a delay of 14 and 28 days (by 2% and 3%, respectively) (Figure 7c). Total protein content was highest when winter wheat was supplied with 40+100 kg N ha^{-1} in BBCH stages 22–25 and 30–31, respectively. An increase in the first spring N rate with a simultaneous decrease in the N rate applied in BBCH stages 30–31 (70 + 70 or 40 + 100 kg ha^{-1}) decreased the total protein content of grain by 1% and 2%, respectively (Table 3).

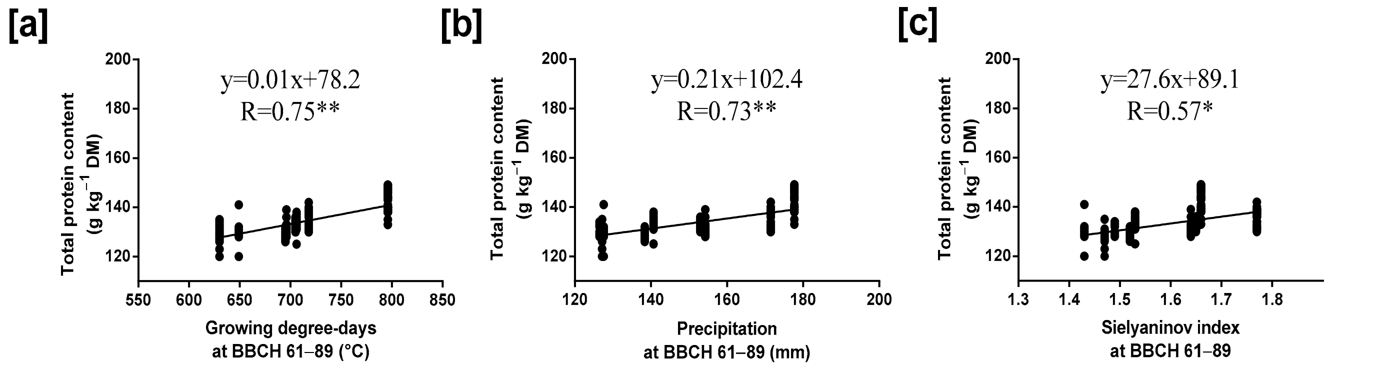

Figure 6. Linear regression between the total protein content of winter wheat grain and (**a**) growing degree days at BBCH stages 61–89; (**b**) precipitation at BBCH stages 61–89; (**c**) Sielyaninov index at BBCH stages 61–89. * significant at $p \leq 0.05$; ** significant $p \leq 0.01$.

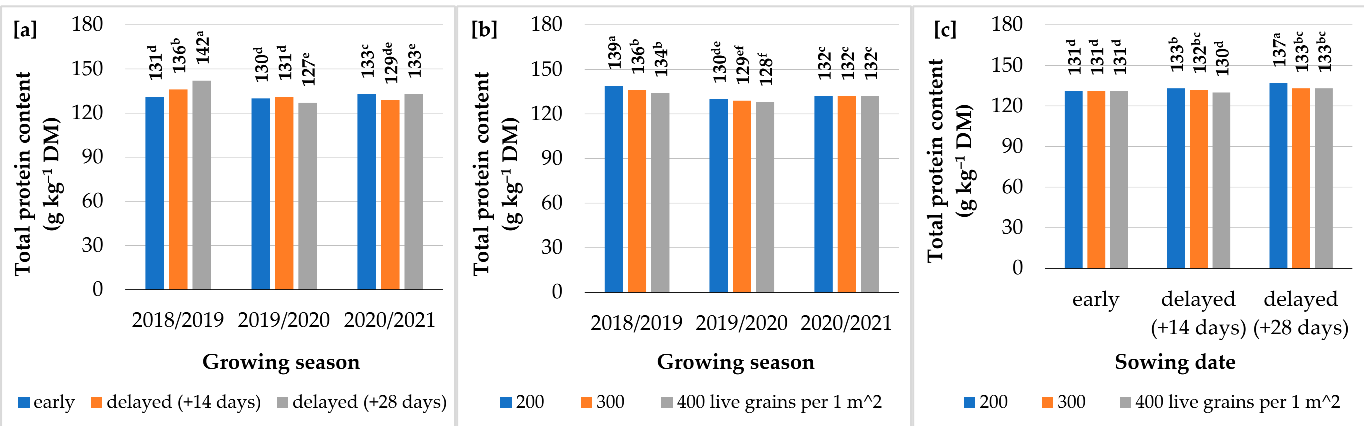

Figure 7. The effect of (**a**) the sowing date, (**b**) sowing density (2018/2019, 2019/2020, 2020/2021), and (**c**) the interaction between the sowing date and sowing density (mean for 2018–2021) on the total protein content of winter wheat grain. Early sowing: 3–6 September; delayed sowing (+14 days): 17–20 September; delayed sowing (+28 days): 1–4 October. Means followed by the same letters are not significantly different at $p \leq 0.05$ in Tukey's test.

The wet gluten content of winter wheat grain was negatively correlated with grain uniformity and positively correlated with grain vitreousness (Table S2). Wet gluten content was also influenced by the mean daily temperatures in BBCH stages 61–89, and by precipitation and the Sielyaninov index in BBCH stages 73–83. An increase in the values of these parameters promoted the accumulation of wet gluten in grain (Figure 8). Therefore, wet gluten content was highest in grain harvested in the third growing season (38.3%), and it was 4.3%p and 4.0%p lower in the first and second growing season, respectively. A sowing delay of 28 days increased wet gluten content by 0.5–0.6%p. In stands sown with a 28-day delay, a greater increase in wet gluten content (2.2%p) was observed in the first growing season (Figure 9a). In this season, late-sown plants were exposed to more favorable weather conditions during flowering and grain ripening. In turn, higher precipitation (by 6%) and higher values of the Sielyaninov index (by 52–65%) were observed in the milk stage (BBCH stages 73–83) (Table 1). An increase in sowing density from 200 to 300 live grains m^{-2} decreased wet gluten content by 0.7%p (Table 3). A greater decrease in the wet gluten content of grain (by 0.8%p and 1.5%p) was noted in late-sown stands (+14 and +28 days, respectively). In early sown stands (beginning of September), an increase in sowing density did not induce significant differences in wet gluten content (Figure 9b). The split application of different N rates in spring had no effect on the wet gluten content of winter wheat grain (Table S1).

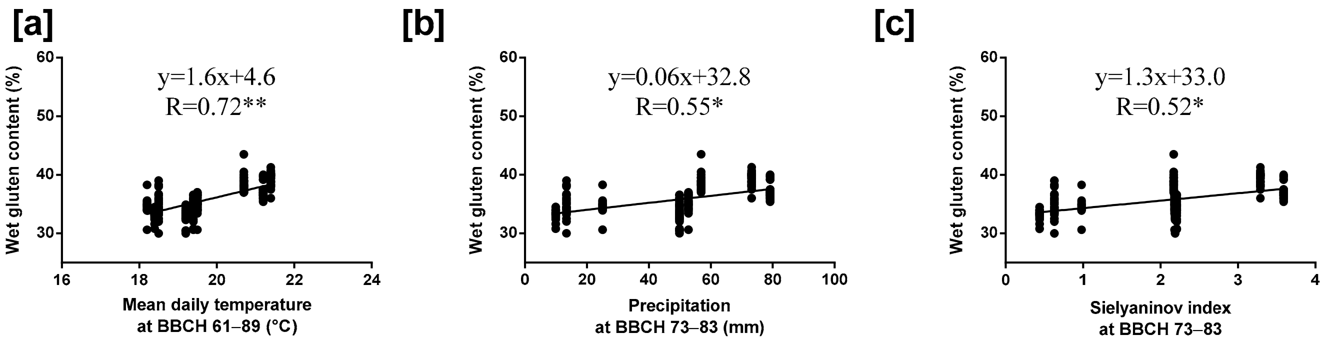

Figure 8. Linear regression between the wet gluten content of winter wheat grain and (**a**) mean daily temperature at BBCH stages 61–89; (**b**) precipitation at BBCH 73–83; (**c**) Sielyaninov index at BBCH stages 73–83. * significant at $p \leq 0.05$; ** significant at $p \leq 0.01$.

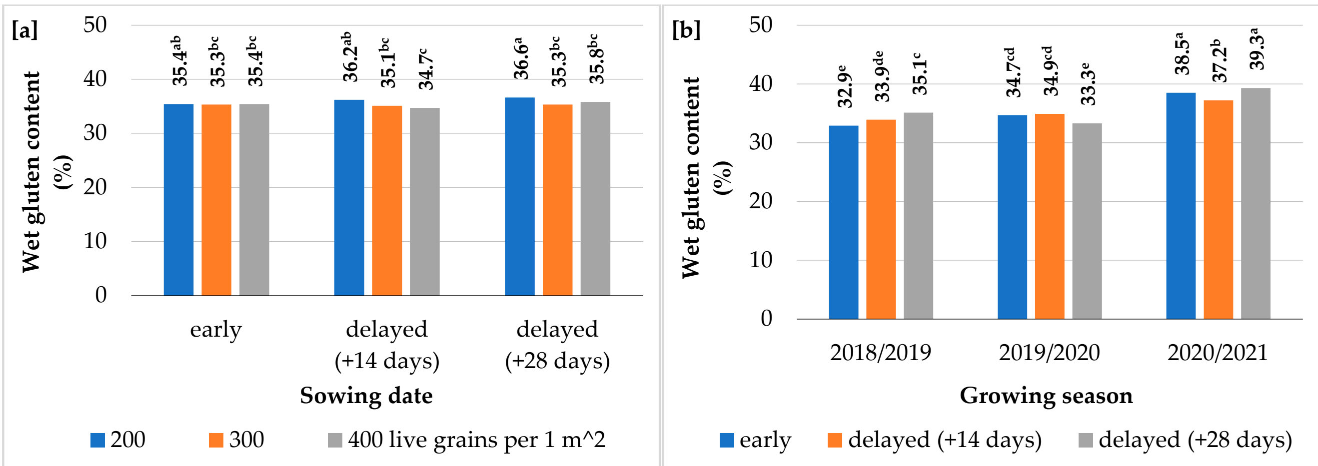

Figure 9. The effect of (**a**) the sowing date and (**b**) the interaction between the sowing date and sowing density on the wet gluten content of winter wheat grain (2018/2019, 2019/2020, 2020/2021). Early sowing: 3–6 September; delayed sowing (+14 days): 17–20 September; delayed sowing (+28 days): 1–4 October. Means followed by the same letters are not significantly different at $p \leq 0.05$ in Tukey's test.

The quality of the protein complex (evaluated in the Zeleny sedimentation test) was somewhat higher in the grain of winter wheat grain harvested in the third growing season than in the first and second growing season (69.0 vs. 68.1–68.2 mL). A 28-day delay in sowing improved the sedimentation index of grain (Table 3) regardless of year, sowing density, or the split spring N rate (Table S1). These results could be indirectly indicative of a higher content of HMW-GSs and higher baking quality of flour. The sedimentation index was not differentiated by sowing density (200, 300, and 400 live grains m^{-2}) (Table S1). The spring N rate of 40 + 100 kg ha^{-1} applied in BBCH stages 22–25 and 30–31, respectively, exerted a more favorable influence on protein complex quality. An increase in the first spring N rate (BBCH stages 22–25) with a simultaneous decrease in the N rate applied in

BBCH stages 30–31 (40 + 100 vs. 70 + 70 or 100 + 40 kg ha^{-1}) decreased the sedimentation index by 0.7% and 1.3%, respectively (Table 3).

The falling number was positively correlated with the wet gluten content of winter wheat grain (Table S2). The falling number was also positively correlated with a mean daily temperature between flowering and harvest, as well as with precipitation and the Sielyaninov index in the milk stage (Figure 10). Enzyme activity in grain (falling number) was highest in the growing seasons of 2018/2019 and 2020/2021 (364 and 381 s, respectively), and it was 10% and 14% lower, respectively, in the second growing season (Table 1). This season was characterized by the lowest mean daily temperature (18.2–18.5 °C) between flowering and harvest and a dry spell in the milk stage (9.9–25.1 mm, K = 0.44–0.98) (Table 1). Alpha-amylase activity was very high, but it remained within (250–350 s) or somewhat above the optimal range of values for the falling number, not exceeding the level at which flour is unsuitable for breadmaking (<400 s). The falling number increased by 3% when sowing was delayed by 14 days (Table 3), which could be attributed to the fact that late-sown plants were exposed to higher mean daily temperatures during flowering and ripening, and more abundant precipitation and higher values of the Sielyaninov index in the milk stage (Table 1). These parameters were positively correlated with the falling number (Figure 10). Sowing density and the split spring N rate caused no significant differences in the falling number (Table S1).

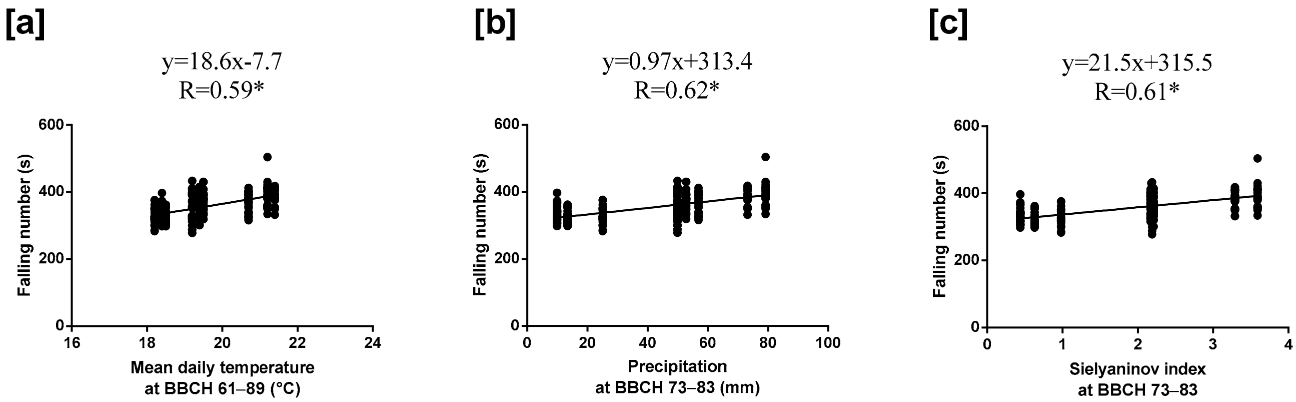

Figure 10. Linear regression between the falling number and (**a**) mean daily temperature at BBCH stages 61–89; (**b**) precipitation at BBCH stages 73–83; (**c**) Sielyaninov index at BBCH stages 73–83. * significant at $p \leq 0.05$.

4. Discussion

4.1. Milling Quality

Grain uniformity is an important indicator of grain quality and milling performance [136,137], and this trait is strongly influenced by the sowing date [112,138–142]. In the work of Meena et al. [112], early sown wheat produced the largest kernels (with a diameter of 2.87 and 2.90 mm). Kernel diameters decreased by 3–4% when sowing was delayed by 6 weeks. According to Meena et al. [112], the decrease in kernel size in late-sown stands could be attributed to higher temperature and lower precipitation during grain formation. The presence of a relationship between the sowing date and grain uniformity/kernel size was also noted by Panazzo and Eagles [138], Waraich et al. [140], Coventry et al. [141], and in this study [Table 2, Figure 1]. In the present study, grain uniformity was negatively correlated with mean daily temperatures between flowering and harvest (BBCH stages 61–89). The most uniform grain (83.0%) was harvested in a growing season with a low mean daily temperature at BBCH stages 61–89. In turn, wheat plants exposed to the highest mean daily temperature in BBCH stages 61–89 produced the least uniform grain (69.7%). Late-sown stands were exposed to higher mean daily temperatures during flowering and ripening, which decreased grain uniformity by 1.9%p. In a study by McKenzie [139], an increase in the sowing density of barley (*Hordeum vulgare* L.) from 150 to 350 grains m^{-2}

decreased kernel size by 5%. In the work of Forster et al. [142], the size of durum wheat kernels was reduced by 4% when sowing density was increased from 222 to 371 grains m^{-2}. In the current study, an increase in sowing density from 200 to 300 live grains m^{-2} also decreased grain uniformity by 2.6%p. In the experiment conducted by Sadowska et al. [143], an increase in the N rate from 50 to 150 kg ha^{-1} had no effect on the size of common wheat kernels. Varga [144] found no correlation between agricultural inputs in the production technology of wheat and kernel size (dimensions). Similar observations were made in the present study, where the split spring N rate had a minor influence on the uniformity of winter wheat grain.

Bulk density is an important indicator of grain development, grain structure, and the thickness of the seed coat [13]. Higher bulk density enhances the processing suitability of wheat grain [13,145]. In a study by Meena et al. [112], wheat grown on sandy-loamy soil in a semi-arid region of India was characterized by higher bulk density (80.2 kg hL^{-1}) in early sown stands (beginning of November) and significantly lower bulk density (78.3 kg hL^{-1}) in stands sown with a 6-week delay. According to the cited authors, delayed sowing led to premature ripening and drying of unripe grain in the filling stage due to high temperature. In a study conducted by Kaur et al. [111] in India, delayed sowing decreased the bulk density of wheat grain by 3%. Delayed sowing also considerably reduced the bulk density of grain grown in Australia, Brazil, India, and Iraq [109,110]. In contrast, in the present experiment, bulk density was highest (81.6 kg hL^{-1}) when sowing was delayed by 28 days, which could be attributed to the absence of a relationship between the bulk density of grain and weather conditions (GDD, mean daily temperature, precipitation, and the Sielyaninov index) between flowering and harvest. Densely sown wheat plants generally develop smaller and fewer spikes, which can contribute to an increase in kernel size [146]. In the current study, sowing density was not correlated with the bulk density of winter wheat grain, probably because the phenological development of grain occurred in periods when precipitation and nutrient levels were sufficient to counteract the decrease in the number and size of spikes. Similar observations were made by Otteson et al. [147] and Guerrini et al. [11], who found no correlation between sowing density and the bulk density of wheat grain. The bulk density of winter wheat grain can also be modified by adjusting the N rate [147–149]. Jankowski et al. [13] demonstrated that an increase in agricultural inputs in the production technology of winter wheat increased the bulk density of grain by 1%. In a study by Harasim and Wesołowski [148], the bulk density of wheat grain increased by 1% when the N rate was increased from 100 to 150 kg ha^{-1}. In turn, Jańczak-Pieniążek et al. [149] found that bulk density was associated with the cultivar of winter wheat. In open-pollinated cultivars, the bulk density of grain increased significantly (by 5%) when the N rate was increased from 110 to 150 kg ha^{-1}. In hybrid cultivars, the N rate had no influence on bulk density. No correlations between N fertilization and the bulk density of wheat grain were reported by either Otteson et al. [147], Guerrini et al. [11], or in this study [Tables S1 and 2].

Grain hardness determines milling quality by influencing milling yield and the baking value of flour [150]. In the present study, grain hardness was negatively correlated with grain uniformity (an increase in uniformity led to a decrease in hardness). In the work of Meena et al. [112], early sown stands produced the hardest grain, while delayed sowing decreased grain hardness by 7–11%. Similar results were reported by Coventry et al. [141,151]. In the current experiment, grain hardness was positively correlated with mean daily temperatures between flowering and harvest. Late-sown stands were exposed to higher temperature during flowering and ripening, which increased grain hardness by 5%. Grain hardness was not affected by sowing density (200, 300, and 400 live grains m^{-2}), which corroborates the findings of McKenzie et al. [152] and Twizerimana et al. [153]. According to Souza et al. [154], grain hardness is influenced by cultivar, environmental conditions, location, and N availability. In the present study, the hardest grain was produced in winter wheat stands supplied with 40 + 100 kg N ha^{-1} in BBCH stages 22–25 and 30–31, respectively. An increase in the early spring N rate, accompanied by a decrease in the N rate at the beginning of stem elongation (70 + 70 or 100 + 40 kg ha^{-1}), decreased grain hardness by 3–4%. In the work of Zhong et al. [155], grain hardness peaked in response to 60 kg N ha^{-1} applied in BBCH stage 17 or 31, and it was 8% lower when N fertilizer was applied in BBCH stage 37. Hao et al. [156] found that grain hardness was reduced by around 10% when the total N rate of 210 kg ha^{-1} was split into two portions (applied at the beginning of stem elongation and during flowering) than when the same N rate was split into four portions (beginning of stem elongation, heading, flowering, and grain filling). In turn, Blandino et al. [157] reported that the hardness of wheat grain was not affected when the total N rate of 130 kg ha^{-1} was applied in one split (BBCH stage 23) or in two splits (BBCH stages 23 and 32). In a study by Valdés-Valdés et al. [158], N rates of 0 to 300 kg ha^{-1} did not induce differences in the hardness of wheat grain. Split application of N fertilizer had no effect on grain hardness in the work of Mor et al. [159] and Zhang et al. [44].

Vitreousness is an important property of endosperm [13]. Vitreous kernels contain more endosperm and protein and are harder than non-vitreous kernels [160]. In the present study, harder grain was also characterized by higher vitreousness and higher milling yield. Vitreousness was positively correlated with mean daily temperatures in the dough stage. Late-sown plants were exposed to higher temperatures in the dough stage, which increased grain vitreousness by 3%p (+14 days) and 6%p (+28 days). A relationship between the sowing date and grain vitreousness was not observed only in the growing season when delayed sowing was not associated with different temperatures in the dough stage. In turn, Forster et al. [142] reported that neither sowing date nor sowing density affected the vitreousness of durum wheat grain. In the work of Bożek et al. [161] and Karabínová et al. [162], the vitreousness of durum wheat and common wheat grain, respectively, was not significantly influenced by sowing density, either. In the current study, an increase in sowing density from 200 to 400 live grains m^{-2} decreased grain vitreousness by 1.4%p. The results of studies investigating the effect of agricultural inputs on grain vitreousness are inconclusive [13]. In the work of Jańczak-Pieniążek et al. [149], an increase in the N rate from 110 to 150 kg ha^{-1} in the production of open-pollinated wheat cultivars had no influence on grain vitreousness. Higher N rates increased grain vitreousness by 23–25%p in only two out of the seven examined hybrid cultivars of winter wheat. Jankowski et al. [13] reported that the vitreousness of winter wheat grain increased by 11%p in a high-input production technology. Higher N rates also increased the percentage of kernels with vitreous endosperm in the work of Budzyński et al. [70] and Narkiewicz-Jodko et al. [82]. In the present experiment, grain vitreousness was highest (93.2%) in winter wheat stands supplied with 40 + 100 kg N ha^{-1} (BBCH stages 22–25 and 30–31, respectively). An increase in the N rate in BBCH stages 22–25, with a simultaneous decrease in the N rate in BBCH stages 30–31 (70 + 70 or 100 + 40 kg ha^{-1}), decreased grain vitreousness by 1.3–1.4%p.

The flour extraction rate is a key parameter in analyses of the milling quality of grain, and it largely determines profits in flour production [13]. In a study by Meena et al. [112], the grain of early sown wheat was characterized by the highest flour recovery (63–68%), and delayed sowing decreased flour recovery by 3%p. A similar relationship between the sowing date and flour yield was reported by Gaire et al. [163] and Zheng et al. [164]. It should also be noted that milling yield is strongly correlated with kernel size, and the value of this parameter is highest in large and well-filled grain [13,165]. In the present study, late-sown wheat produced larger, harder, and more vitreous kernels, which increased flour extraction rates (by 1.4–2.2%p). Otteson et al. [147] found no correlation between sowing density and flour yield, and similar observations were made in the present study [Table 3]. In turn, in the work of Caglar et al. [73], flour yield was reduced by 3% when the seeding rate was increased from 325 to 625 grains m^{-2}. Milling yield is determined by the physical properties of kernels (size, hardness, vitreousness, and bulk density), which are strongly influenced by N fertilization. In the present study, the milling yield peaked (73.9%) in wheat stands supplied with 40 and 100 kg N ha^{-1} in BBCH stages 22–25 and 30–31, respectively. An increase in the early spring N rate with a simultaneous decrease in the N rate at the beginning of stem elongation (100 + 40 kg ha^{-1}) decreased the milling yield by 1.8%p. In turn, in the work of Zheng et al. [164], the flour yield was 1%p higher when N fertilizer was applied at a 6:4 ratio (BBCH stages 00 and 31) than a 7:3 ratio. In a study by Wu et al. [166], the application of the total N rate of 180 kg ha^{-1} in three splits (before sowing and during tillering and stem elongation at a ratio of 5:1:4, 7:1:2, and 5:4:1, respectively) had no effect on flour yield. Budzyński et al. [70], Otteson et al. [147], and Jankowski et al. [13] also found that the intensity of agricultural inputs in wheat production had no significant influence on flour yield.

4.2. Protein Complex Quality and Enzyme Activity in Grain

Wheat grain is a rich source of essential nutrients, including protein, in the human diet [167,168]. Protein content is the main indicator of wheat grain quality in commerce and processing [90]. The protein content of wheat grain ranges from 100 to 150 g kg^{-1} DM [9,11,90]. Genetic factors are responsible for approximately a third of the variation in the protein content of wheat grain [9]. The remaining two thirds of the variation are determined by environmental factors (soil quality, weather conditions) and agronomic practices, including sowing and fertilization [169]. The sowing date significantly affects the total protein content of grain mainly due to different temperatures during grain filling. When wheat is sown late, flowering is delayed and plants are exposed to high temperatures in the grain filling stage [170]. Thermal stress decreases kernel size, inhibits endosperm development, and increases protein concentration [171–173]. Moderate environmental stress (high temperature, water deficit during grain filling) can stimulate protein remobilization from vegetative organs to grain [64,174,175]. Meena et al. [112] found that delayed sowing significantly increased the protein content of grain (by 6–8%). Delayed sowing and exposure to higher temperatures also increased the protein content of grain in the work of Gooding et al. [115], Zende et al. [108], Motzo et al. [116], Sattar et al. [176], Farooq et al. [117], Singh et al. [118], and Shah et al. [177]. In the present study, the total protein content of grain was positively correlated with GDD, precipitation, and the Sielyaninov index between flowering and harvest (BBCH stages 61–89). Late-sown plants were exposed to higher GDD and higher precipitation in these phenological growth stages, which increased the total protein content of grain by 1–2%. The relationship between sowing density and the protein content of wheat grain is not unidirectional [11,147,178–184]. Otteson et al. [147], Nakano and Morita [180], Dragoş and Pîrşan [181], Jemal et al. [182], and Guerrini et al. [11] did not report any associations between sowing density and the protein content of grain. In the current study, an increase in sowing density from 200 to 300 live grains m^{-2} reduced the total protein content of grain by 1.5% on average. However, the decrease in protein content induced by higher sowing density was exacerbated when wheat was sown with a delay of 14 and 28 days (protein content decreased by 2–3%). In the work of Gooding et al. [178] and Han and Yang [179], the protein content of grain also decreased by around 3% when sowing density was increased from 180–200 to 270–400 live grains m^{-2}. A reverse relationship was reported by Hao et al. [184], who found that an increase in sowing density from 200 to 250 live grains m^{-2} induced a 6% increase in the protein content of grain. In turn, Zhang et al. [183] observed that the protein content of wheat grain was highest at a sowing density of 260 live grains m^{-2}, whereas lower or higher sowing densities decreased protein content by 3% [183]. Nitrogen fertilization is one of the key factors that affect the content and composition of protein in wheat grain [169]. Nitrogen is an essential component of amino acids, the building blocks of proteins, which is why N supply is critical in all stages of plant development [11,13,90,185]. However, the use of N fertilizers in crop production has to be reduced for environmental reasons, and alternative strategies are needed to maximize protein yields while decreasing the release of unused N to soil, water, and the atmosphere. For this reason, N fertilization should be optimized by selecting the appropriate total rate or split rate, application timing, and type of fertilizer [186,187]. The application of N fertilizer in the late stages of growth promotes protein accumulation in grain [186]. In a study by Landolfi et al. [185], an N rate of 80 or 160 kg ha^{-1} applied in two equal portions (50:50) in tillering and stem elongation stages increased the protein content of grain by 15–18%. In the work of Wieser et al. [90], the protein content of grain was 4–9% higher when N fertilizer was applied only before sowing than when N was applied both before sowing and in the stem elongation stage. In a study conducted by Landolfi et al. [188] in northern Italy, a total N rate of 160 kg ha^{-1} applied in three splits (tillering, stem elongation, heading) increased the total protein content by around 1% relative to two splits. In the experiment by Xue et al. [189], the total protein content of grain was 6% higher when N fertilizer was split into three portions (BBCH stages 00, 30, and 47) than two portions (BBCH stages 00 and 30). A meta-analysis conducted by Hu et al. [190] revealed that the protein content of wheat grain was 2–5% higher on average when the total N rate was split into three to four portions. In the work of Mor et al. [159], an N rate of 120 or 160 kg ha^{-1} increased the protein content of grain by 12% when split in two equal portions (BBCH stages 00 and 21), but by only 4% when split into five equal portions (BBCH stages 00, 21, 31, 39, and 65). In the present study, protein content peaked when winter wheat was supplied with 40 + 100 kg N ha^{-1} in BBCH stages 22–25 and 30–31, respectively. An increase in the first N rate with a simultaneous decrease in the second N rate (70 + 70 and 100 + 40 kg ha^{-1}) decreased the total protein content of grain by 1–2%. In turn, Schulz et al. [191] found no correlation between split N application and the protein content of grain produced in Germany. The influence of N fertilization on the protein content of wheat grain can be mediated by differences in the availability of soil mineral nitrogen (N_{min}) during the growing season [154].

The concentration of gluten proteins in wheat grain is a very important consideration in the baking industry [13,90,192]. In this study, wet gluten content was positively correlated with grain vitreousness. Winter wheat grain produced in northeastern Poland contained 34.0–38.3% of wet gluten. Similar values were reported by Šip et al. [193] in Czechia, whereas much lower values were noted by Jaskulska et al. [66] and Jańczak-Pieniążek et al. [149] in Poland. In the work of Meena et al. [112], delayed sowing increased the wet gluten content of wheat by 9%p. In the present study, wet gluten content was also highest (35.9%) in late-sown wheat (early October). The analyzed parameter was determined by mean daily temperatures between flowering and harvest, as well as by precipitation and the Sielyaninov index in the milk stage. Late-sown wheat was exposed to more favorable temperatures during flowering and ripening, as well as to higher precipitation and higher values of the Sielyaninov index in the milk stage, which contributed to the accumulation of wet gluten in the flowering and milk stages. An increase in sowing density from 200 to 300 live grains m^{-2} decreased wet gluten content by 0.7%p. The negative impact of higher sowing density on wet gluten content was exacerbated by delayed sowing (decrease of 0.8–1.5%p). When winter wheat was sown early, increasing sowing density did not significantly affect the wet gluten content of grain. Zecevic et al. [74] also reported a decrease in the wet gluten content of grain (by 6%p) when sowing density was increased from 500 to 650 live grains m^{-2}. An increase in sowing density (180 vs. 270 grains m^{-2}) also reduced the wet gluten content of wheat grain (by 3–5%p) in the work of Han and Yang [179], Dragoş and Pîrşan [181] (400 vs. 500 live grains m^{-2}), and Twizerimana et al. [153] (112 vs. 225 kg ha^{-1}). A reverse correlation was observed by Caglar et al. [73] and Guerrini et al. [11], with the former finding that wet gluten content decreased by 11% when sowing density was reduced from 525 and 625 grains m^{-2} to 325 and 425 grains m^{-2}. Regardless of cultivar (open-pollinated or hybrid cultivars), winter wheat is highly sensitive to N fertilization, which affects both grain yield and grain quality, including the accumulation of gluten proteins [66]. In a study by Jańczak-Pieniążek et al. [149], an increase in the N rate from 110 to 150 kg ha^{-1} increased the wet gluten content of winter wheat grain by 7–11%p (open-pollinated cultivars) and 10–16%p (hybrid cultivars). Nitrogen fertilization also increased gluten concentration in the grain of winter and spring wheat in the work of Podolska et al. [79], Sułek and Podolska [89], and Dubis [88]. In contrast, Jankowski et al. [13] and Wojtkowiak et al. [194] did not observe any associations between N fertilization and wet gluten content, regardless of cultivar. In the current study, splitting the N fertilizer rate did not induce differences in the wet gluten content of grain. In the work of Mor et al. [159], wet gluten content was lowest when the total N rate of 120 or 160 kg ha^{-1} was applied in two equal portions (BBCH stages 00 and 21), and it was 4% higher when the N rate was split into five equal portions (BBCH stages 00, 21, 31, 39, and 65).

The baking quality of wheat is determined by both the quantity and quality of gluten proteins in grain. The sedimentation index is the main predictor of gluten quality, and its value denotes the size of protein aggregates [13,145,195]. Meena et al. [112] found that delayed sowing increased the sedimentation index by 4–5%. In the work of Knapowski and Ralcewicz [78], the sedimentation index was also higher (by 12%) when winter wheat was sown with a delay. In the current experiment, the sedimentation index increased by 1% when winter wheat was sown with a 28-day delay, regardless of weather conditions. In turn, in a study conducted in India, the sedimentation index was 7% lower when wheat was sown with a delay of 40 days [196]. In the present study, sowing density did not affect the sedimentation index, which is consistent with the findings of Piekarczyk [197] and Mikos-Szymańska and Podolska [198]. In the work of Twizerimana et al. [153], sowing density influenced the sedimentation index in only one year of the experiment, when an increase in the seeding rate from 112 to 225 kg ha^{-1} decreased the sedimentation index by 10%. In turn, Han and Yang [179] and Hao et al. [184] found that the sedimentation index decreased by 2–7% when the seeding rate was increased by 50–90 grains m^{-2}. The quality of the protein complex in wheat grain is also determined by agricultural inputs, including N fertilization [13]. Litke et al. [199] demonstrated that an increase in the N rate to 210 kg ha^{-1} led to a significant increase in the sedimentation index. Nitrogen also improved protein quality in the grain of winter and spring wheat in the work of Budzyński et al. [70], Podolska et al. [79], Piekarczyk [197], Ellmann [83], Dubis [88], Rossini et al. [200], Kizilgeci et al. [201], and Zhang et al. [44]. In this study, the split spring N rate of 40 + 100 kg ha^{-1} applied in BBCH stages 22–25 and 30–31, respectively, also enhanced the quality of grain protein. An increase in the first spring N rate (BBCH stages 22–25) with a simultaneous decrease in the second N rate applied in BBCH stages 30–31 (70 + 70 or 100 + 40 kg ha^{-1}) decreased the sedimentation index by 1% on average. Mor et al. [159] reported that the sedimentation index was 3% higher when the N rate was split into five rather than two portions. In a study by Hao et al. [156], the sedimentation index was 5% higher when the total N rate of 210 kg ha^{-1} was applied in four rather than two splits.

The falling number denotes the activity of α-amylase in grain [13,85,202]. In the present study, the falling number was positively correlated with the wet gluten content of grain. Flours with a falling number in the range of 250–320 s are most suitable for baking purposes, and this parameter can be decreased through the addition of α-amylase preparations [203]. In the current experiment, the falling number was very high (328–381 s), but it remained within or somewhat above the optimal range of values, not exceeding the level at which grain is unsuitable for breadmaking (<400 s). According to Grausgruber et al. [204], α-amylase activity in grain may vary depending on environmental and genetic factors, particularly weather conditions, during grain ripening. The falling number was also strongly correlated with weather conditions in this study. A sowing delay of 14 days increased the falling number by 3%, which could be attributed to the fact that late-sown plants were exposed to higher mean daily temperatures during flowering and ripening, as well as higher precipitation and higher values of the Sielyaninov index in the milk stage (these parameters were also positively correlated with the falling number). In contrast, the sowing date had no influence on the falling number in the works of Knapowski and Ralcewicz [78] or Forster et al. [142]. Sowing density is bound by a weak and multidirectional relationship with the falling number [142,205,206]. Forster et al. [142] found no correlation between the sowing density of durum wheat and the falling number. Sowing density was not associated with α-amylase activity (falling number) in common wheat grain in the work of Korres and Froud-Williams [205], Piekarczyk [197], and Forster et al. [142], or in this study [Table 3]. In contrast, Hao et al. [184] found that the falling number increased by 5% when sowing density was increased from 200 to 250 grains m^{-2}. In wheat grain, α-amylase activity is also determined by agricultural inputs, including N fertilization [206] and genotype [85,149]. In a study by Jańczak-Pieniążek et al. [149], an N rate of 150 kg ha^{-1} increased the falling number by 0.3–1.6% (open-pollinated cultivar) to 3.5% (hybrid cultivar). Linina and Ruza [206] reported a significant increase in the falling number up to the N rate of 180 kg ha^{-1}. Szentpétery et al. [207] found that split N application had a positive impact on the falling number. In the cited study, the application of 40 or 80 kg N ha^{-1} in the tillering stage and 40 kg N^{-1} ha in the flowering stage increased the falling number by 6–11% relative to the treatment where a single N rate of 80 or 120 kg ha^{-1} was applied in the tillering stage. According to Budzyński et al. [70], the absence of positive correlations between agricultural inputs and the falling number in many published studies can be attributed to unfavorable weather conditions [88,185,208–210]. In the present study, split N application did not induce significant differences in the falling number.

5. Conclusions

The present study demonstrated that the milling quality of winter wheat grain produced in northeastern Poland on Haplic Luvisol originating from boulder clay was significantly influenced by weather conditions, sowing strategies, and N fertilization. The sowing date affected the beginning and duration of phenological growth stages, which were characterized by different mean daily temperatures and precipitation. Grain uniformity and grain hardness were positively correlated with the mean daily temperature from the beginning of flowering until harvest, while grain vitreousness was positively correlated with the mean daily temperature in the dough stage. The total protein content of grain was positively correlated with GDD, precipitation, and the Sielyaninov index between flowering and harvest. Wet gluten content and the falling number were positively correlated with the mean daily temperature between flowering and harvest, as well as with precipitation and the Sielyaninov index in the milk stage. In general, delayed sowing exposed wheat plants to more favorable weather conditions during flowering and grain ripening, which increased the bulk density, vitreousness and hardness of grain, the flour extraction rate, the total protein content and wet gluten content of grain, the sedimentation index, and the falling number. The sowing density of 200 grains m^{-2} enhanced grain uniformity, grain vitreousness, total protein content, and wet gluten content. Higher N supply in the stem elongation stage (BBCH stages 30–31) had a positive influence on grain vitreousness and hardness, the flour extraction rate, the total protein content and wet gluten content of grain, and the sedimentation index. These results indicate that the milling quality of winter wheat grain produced in northeastern Poland can be improved by sowing in late September or early October at 200 grains m^{-2} and by applying N fertilizer at 40 and 100 kg ha^{-1} in BBCH stages 22–25 and 30–31, respectively.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14040552/s1, Table S1. F-test statistics in ANOVA; Table S2. Pearson's correlation coefficients denoting the relationship between wheat grain parameters.

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