agriculture

MDPI

Article

Factors Influencing the Accumulation of Free Asparagine in Wheat Grain and the Acrylamide Formation in Bread

Anna Szafrańska [1,*], Grażyna Podolska [2], Olga Świder [1], Danuta Kotyrba [1], Edyta Aleksandrowicz [2], Agnieszka Podolska-Charlery [3] and Marek Roszko [1]

[1] Institute of Agricultural and Food Biotechnology—State Research Institute, 36 Rakowiecka St., 02-532 Warsaw, Poland; olga.swider@ibprs.pl (O.Ś.); marek.roszko@ibprs.pl (M.R.)
[2] Institute of Soil Science and Plant Cultivation—State Research Institute, Czartoryskich 8, 24-100 Puławy, Poland; grazyna.podolska@iung.pulawy.pl (G.P.)
[3] National Food Institute, Technical University of Denmark, Kemitorvet Building 201, 2800 Kongens Lyngby, Denmark
* Correspondence: anna.szafranska@ibprs.pl

Abstract: Asparagine is one of the precursors of acrylamide that can form during bread production. The aim of this work was to determine the effect of genotype, environment, sulfur fertilization, and the interaction of those factors on the asparagine content, technological value of wheat, and acrylamide level in bread. The research material consisted of five wheat cultivars grown in two locations in Poland with nitrogen fertilization of 110 kg ha^{-1} and sulfur fertilization of 30 kg ha^{-1}. The standard ISO method for analyzing the milling and baking properties of wheat was used. The UHPLC-MS/MS method for analyzing the amino acids and the GC/MS method for acrylamide in bread were implemented. The analysis of variance results indicated that the location influenced the total variance in the measured asparagine content and quality of wheat the most, followed by the cultivar and then by the interaction between the environment and cultivar. Sulfur fertilization had no significant effect on the asparagine content, but slightly lowered the gluten quality and loaf volume of bread. However, sulfur fertilization in connection with the cultivar characterized by low starch damage had a positive effect on lowering the acrylamide in bread. Asparagine content in wheat and acrylamide in bread varies mostly depending on cultivar and environment.

Keywords: asparagine; acrylamide; baking value; starch damage; sulfur fertilization; wheat cultivars

Citation: Szafrańska, A.; Podolska, G.; Świder, O.; Kotyrba, D.; Aleksandrowicz, E.; Podolska-Charlery, A.; Roszko, M. Factors Influencing the Accumulation of Free Asparagine in Wheat Grain and the Acrylamide Formation in Bread. *Agriculture* **2024**, *14*, 207. https://doi.org/10.3390/agriculture14020207

Academic Editor: Carlos Guzmán

Received: 17 December 2023
Revised: 22 January 2024
Accepted: 26 January 2024
Published: 28 January 2024

1. Introduction

Wheat is known for being one of the most important crops grown in the world. In 2021, the total global production equaled 770 million tons, with an average yield of 3.6 t ha^{-1} and a harvested area of about 220 million ha [1]. Bread is a staple food for a large proportion of the world's population. Wheat bread, without a doubt, is an integral part of the daily diet and delivers not only carbohydrates and protein but also crucial health-promoting ingredients, especially when consumed as whole grain [2]. However, in the process of baking bread, acrylamide is formed, which may be a health risk, because acrylamide has been classified as being probably cancerogenic to humans (Group 2A). It has also been shown to have neurological and reproductive effects at high doses in rodent toxicology studies. Acrylamide forms mainly from free asparagine and carbonyl sources as part of the Maillard reaction [3]. Numerous food processing parameters, including temperature, formulation, and cooking time, affect acrylamide formation from asparagine. Acrylamide can be found in most cereal products, including bread and breakfast cereals, however, in particularly high concentrations in crispbread, cookies, and gingerbread [4].

A number of investigations focused on strategies to minimize the levels and the formation of acrylamide in wheat products. Based on the results, the reduction in heat and the addition of specific ingredients—for example, cysteine or asparaginase—alongside prolonged fermentation time is proposed [5]. However, all technological efforts to reduce acrylamide might be in vain if unsuitable raw material is used. Amrein et al. [6] proved that in French fries and potato chips, acrylamide content is dependent on the potato cultivar. Additionally, several studies showed that cultivars play a very important role in acrylamide content in baking products. Claus et al. [7] described that acrylamide content in wheat cultivars varied in the range of 8.7 to 24.9 mg/100 g. Also, Taeymans et al. [8] reported a range of 7.4 to 66 mg/100 g. Claus et al. [7] stated that, generally, wheat varieties belonging to the good quality category of wheat—E and A—show higher acrylamide levels than cultivars belonging to the low category—B and Ck. However, these authors also note that, among high-quality category A cultivars, it is possible to find cultivars with a high crude protein content and relatively low acrylamide concentration. The differences result from different amounts of asparagine in the grain of wheat [7,9]. Rapp et al. [10] showed that the mean asparagine content ranged from 143.25 to 392.75 mg/kg for the different wheat cultivars. Malunga et al. [11] found that the asparagine content of Canadian wheat varied from 302 to 965 and 116 to 336 mg kg^{-1} for whole meal and white flour, respectively. Genotype was a factor that had a significant effect on the asparagine content, suggesting that breeding strategies are an interesting avenue to pursue in order to produce cultivars with lower levels of this amino acid.

For this reason, focusing studies on the reduction of the precursors of acrylamide in wheat grains by lowering the asparagine content seems like the best way forward. Asparagine accumulates under stress conditions as a biological response to the restriction of protein synthesis. Stressful conditions can be caused by drought, salt in the soil, pathogen attack, toxicity, solar radiation, or nutrient deficiencies [9,12,13]. Asparagine often plays a pivotal role in the active site of enzymes [14,15]. Interestingly, the biosynthesis of all essential amino acids can be achieved from asparagine as a donor of fixed nitrogen. High free asparagine concentrations in grain indicate a poor efficiency of nitrogen utilization, defined as the conversion of assimilated nitrogen to grain protein, resulting in lower-quality grain. Asparagine content in flour and dough significantly affected acrylamide content [7]. Furthermore, it was found that asparagine concentration in grain was affected by both genotype and production environment [7,9,11,13]. In the Claus et al. [7] study, both asparagine and acrylamide content were shown to be dependent on weather conditions during the growing season, especially temperature.

Nitrogen is a vital component of amino acids; therefore, nitrogen fertilization has a strong impact on crude protein and free asparagine contents in flour [16,17]. Higher doses of nitrogen influenced the increase of asparagine in wheat grains [7,18]. In addition, sulfur was shown to have an important role in acrylamide formation and its precursors [16,17]. Curties and Halford [16] demonstrated a significant effect of sulfur fertilization on asparagine content in wheat grain. In conditions of sulfur deficiency in the soil, the amount of asparagine in grain was 26 mmol/kg (3435 mg/kg), while under sufficient sulfur conditions, the amount was 1.6 mmol/kg (211 mg/kg). Halford et al. [17] showed that even flours from wheat grown in the field with an intermediate level of sulfur application (10 kg sulfur per hectare) contained more acrylamide after processing than flours from wheat grown with the application of 40 kg sulfur per hectare.

The use of wheat for consumption requires appropriate quality characteristics that are important from a bakery's point of view. Wheat baking quality parameters depend on cultivars, weather conditions, and fertility. Sulfur and nitrogen fertilizer application on winter wheat can have many benefits. Nitrogen is an essential nutrient important for wheat growth and protein biosynthesis. Low nitrogen availability can limit crop growth, yield, and quality [19]. Adequate sulfur is also required for the proper growth and development of wheat, sulfur being essential for the biosynthesis of amino acids, proteins, and chlorophyll, in particular [20]. Sulfur is a key element of enzymes involved in nitrogen metabolism [21–23]. Moreover, sulfur deficiency can decrease nitrogen assimilation [24]. Without an adequate supply of sulfur, wheat is unable to reach its full yield potential and cannot efficiently utilize nitrogen for protein biosynthesis [25]. Therefore, nitrogen use efficiency and crop yields are increased when nitrogen and sulfur are applied together [24,26,27]. Two critically important amino acids, methionine and cysteine, contain sulfur. Methionine is the initiating amino acid in the synthesis of all proteins, and cysteine plays a pivotal function in protein folding [28]. Nitrogen and sulfur deficiency are detrimental to baking quality due to their influence on the formation of disulfide bonds formed from the sulfhydryl groups of cysteine. Such bonds are important because they influence the viscoelasticity of dough [29].

Sulfur can be deposited in the soil in a variety of ways. According to Ref. [30], the US Federal Clean Air Act revisions introduced in 1990 have strongly improved air quality by regulating power plant air emissions. As a result, sulfur emissions have decreased by 80%, resulting in the reduction of sulfur deposition along with lower sulfur deposition. Lower deposition combined with a lack of sulfur fertilization results in soil sulfur deficiency throughout the world [31]. Although the topic of asparagine and acrylamide has been discussed for several years, the number of publications on the interactions between genotype, growing location, and sulfur, as well as their impact on the asparagine content and baking quality of wheat, is still limited and requires supplementation. We hypothesize that the use of sulfur will reduce the amount of free asparagine and affect the baking quality of winter wheat, but the effect of sulfur on reducing asparagine will depend on the cultivar.

Another factor that we chose to consider in this research is the influence of starch damage in wheat flour on the formation of acrylamide in bread. Damaged starch formation occurs during the milling of grain due to the mechanical shear between the mill rolls [32]. Among the factors affecting the amount of damaged starch in the flour are wheat grain hardness, wheat tempering conditions, and the setting of the mill (i.e., roll diameter and speed ratio, pressure, and gap size between the reduction rolls, milling time, and roll temperature) [32,33]. Starch damage strongly affects the behavior of dough during processing (e.g., water absorption, gelatinization, and rheological properties), as well as the quality of the finished product (e.g., color, shelf life) [32]. However, levels of damaged starch in the work of Wang et al. [34] and Mulla et al. [35] for wheat flours showed a strong positive correlation with acrylamide formation in bread. An increase in starch damage was also correlated with reduced sugar content in flour [34,35]. Another hypothesis of our work was to prove that starch damage might influence the acrylamide content in bread.

Therefore, the goal of this study was to evaluate the influence of wheat cultivars, sulfur fertilization, and growing location impact on the asparagine and acrylamide content and baking quality of winter wheat.

2. Materials and Methods

Plant material. This research work concerned five winter wheat cultivars representing two technological groups approved by the Polish National List of Agricultural Plant Varieties issued yearly by COBORU (The Research Center for Variety Testing). Group A comprises quality bread cultivars, namely 'Hondia' and 'Pokusa'. Group B comprises bread cultivars 'Belissa', 'Hybery F_1', and 'Bonanza'.

Field trials. Field trials were conducted during the 2020–2021 and 2021–2022 growing seasons at the Experimental Stations in Wielichowo (52°07′ N 16°21′ E) and Werbkowice (50°45′ N 23°45′ E), Institute of Soil Science and Plant Cultivation—State Research Institute (IUNG-PIB) Puławy, Poland. The soil in Werbkowice was typical chernozem, class I soil categorized as a very good wheat soil complex that is characteristic of the region. In Wielichowo, the soil was pseudopodsolic, class Iva, categorized as a very good rye complex. Winter rapeseed was the fore crop. The experiment was established using the long strip method. The first factor was the cultivar ('Hondia', 'Pokusa', 'Belissa', 'Hybery F_1', and 'Bonanza'), and the second factor was sulfur fertilization. Plots with no sulfur fertilization were used as control objects. Before the start of the trial, the soil was analyzed down to a depth of 30 cm. In Werbkowice, the soil was characterized by 7.9 pH_{KCl}, and the soil fertility indicators were 19.5 mg of K_2O, 10.2 mg of P_2O_5, and 0.16 mg of S per 100 g of soil, and 12.5 mg of Mg per 100 g of soil. In Wielichowo, the soil was characterized by 6.2 pH_{KCl}, and the soil fertility indicators were 19.2 mg of K_2O, 25.3 mg of P_2O_5, and 0.78 mg of S per 100 g of soil, and 6.4 mg of Mg per 100 g of soil. In both experimental stations, the nitrogen in the planned total dose of 110 kg ha^{-1} was applied in divided doses. The first dose of 60 kg ha^{-1} was applied after resumption of spring vegetation and the second dose of 50 kg ha^{-1} was applied at the stem elongation stage. One dose of sulfur was 30 kg ha^{-1} and was applied after the resumption of spring vegetation together with nitrogen fertilization. Winter wheat was sown on 5 October (Werbkowice) and 28 September (Wielichowo) in an optimal sowing term at a seed rate of 450 germinable kernels per m^2. The area of the harvested plot was 0.7 ha. Pre-sowing practices, pre-sowing plowing, and mineral fertilization (NPK) were accomplished before sowing. A 350 kg ha^{-1} dose of Polifoska 6-NPK (6 20 30) fertilizer was applied, which was at a concentration equivalent to 21 kg of N, 70 kg of P_2O_5, and 105 kg of K_2O per ha. All winter wheat cultivars were chemically protected against pests. Weed control consisted of 1.0 L ha^{-1} of Kantor Forte 195SE and 0.5 L ha^{-1} Puma Universal 0.69 EW. Disease control consisted of 1.0 L ha^{-1} of Tilt Turbo at the flag leaf stage and 2.0 L ha^{-1} of Adaxar Plus after blossoming. Pest control consisted of 0.3 L ha^{-1} of Fury 100 EW at the flag leaf stage. The crop was harvested at full maturity on 2 August 2021 and on 28 July 2022.

Weather conditions during the growing season of winter wheat were assessed on the basis of the Hydrothermal Coefficient of Selyaninov (HTC). During the growing seasons from February to August, the HTC was 1.04 (2021) and 1.14 (2022) in Werbkowice and 1.32 (2021) and 1.28 (2022) in Wielichowo. This indicates that in Werbkowice, the year was dry, while in Wielichowo, it was optimal. Particularly large differences occurred in July during the grain ripening period. In Werbkowice, the HTC was 0.54 (2021) and 0.62 (2022), while the HTC in Wielichowo was 1.43 (2021) and 1.32 (2022). Therefore, during the grain ripening period, there was a drought in Werbkowice, while in Wielichowo, there were optimal weather conditions.

Because of a comparable impact of factors (cultivar and sulfur fertilization) in both years and taking into account the large amount of work needed to determine milling tests and the assessment of rheological characteristics of dough and baking tests, the cultivar samples from both years were mixed appropriately for further research.

Technological quality analysis. Test weight (ISO 7971-3:2019) [36], protein content (ISO 20483:2013) [37], gluten quantity (ISO 21415-2:2015) [38], Zeleny sedimentation index (ISO 5529:2007) [39], and ash content (ISO 2171:2007) [40] were evaluated to determine the baking value of five wheat cultivars. Grain samples were ground to whole meal flours using hammer mill FN 3100 (Perten InstrumentsAB, Hägersten, Sweden) for protein and gluten content analyses. The Sedimat laboratory mill (Brabender GmbH & Co. KG, Duisburg, Germany) was used to prepare samples for the Zeleny sedimentation test.

Amino acid analysis. Sample preparation. Free amino acid content was analyzed based on the method described by Świder et al. [41] with slight modifications. In brief, 2 g of a sample was placed in a centrifuge tube and spiked with internal standard (1.7—diaminoheptane solution, Merck, Darmstadt, Germany). In order to perform extraction, 40 mL of trichloroacetic acid (5% solution, Avantor Performance Materials, Gliwice, Poland) was added and the sample was shaken thoroughly. After centrifugation (10,000$\times$ g for 10 min.), 100 μL of the supernatant was mixed in a 15 mL volume centrifuge tube with 2.5 mL of di-sodium tetraborate (3% water solution, J.T. Baker, Pol-Aura, Gliwice, Poland) and 2.5 mL of dansylchloride (20 mMol in acetonitrile, abcr GmbH, Karlsruhe, Germany) to derivatize the analyzed compounds. Derivatization was performed by shaking in a water bath at 40 °C for 1 h. Then, 10 μL of formic acid (98–100% purity, Avantor Performance Materials, Gliwice, Poland) was added to stop derivatization. The sample was filtered into a chromatographic vial using a syringe filter (pore size: 0.22 μm, Captiva Econofilters, Agilent, Santa Clara, CA, USA). Amino acid standards were supplied by Merck (Darmstadt, Germany) and their assays were as follows: asparagine, arginine, aspartic acid, threonine, valine, methionine, phenylalanine, leucine, isoleucine, lysine, tyrosine, histidine $\geq$98%, glutamine, proline, ornithine, glutamic acid, and serine $\geq$99%.

Chromatographic analysis. UHPLC-MS/MS (ultra-high performance liquid chromatography coupled with a high-resolution mass spectrometer) (Q Exactive Orbitrap Focus MS Thermo Fisher Scientific, Waltham, MA, USA) analysis was performed to determine free amino acid concentration. The compounds were separated on the Cortecs UPLC C18 2.1 × 100 mm, 1.6 µm column (Waters, Milford, MA, USA). Phase A consisted of water:ACN (90:10)/0,1% FA/5 mM ammonium formate, and phase B contained can:water (90:10)/0.1% FA/5 mM ammonium formate. LC-MS-grade water and LC-MS-grade acetonitrile were supplied by Witko (Łódź, Poland), and formic acid 98–100% and ammonium formate ≥97% for LC-MS were supplied by Chem-Lab (Zedelgem, Belgium). The following liquid phase gradient at the flow rate 0.3 mL/min was applied: A:B (%)0–2 min.—90:10—waste, 2–22 min.—0:100, 22–25 min.—0:100, 25–26 min.—90:10, 26–28 min.—90:10. MS was equipped with heated electrospray ionization (HESI) source. Analysis was performed in positive polarization mode with an injection volume of 2.5 µL. The detailed MS parameters were set as follows: spray voltage: 3 kV, capillary temperature: 256 °C, sheath gas flow rate: 48, auxiliary gas flow rate: 11, sweep gas flow rate: 2, probe heater temperature: 413 °C, S-lens RF level: 50, resolution: 70,000 in simultaneous scan and 35,000 in all ion fragmentation. Acquisition and analysis of the data were performed using Xcalibure 4.2.47 software.

Milling and baking quality analysis. Wheat grain samples weighing approx. 2.5 kg each were moistened to 16% with water. After resting for 16 ± 1 h, the wheat samples were milled using an MLU-202 laboratory flour mill (Bűhler AG, Uzwil, Switzerland) to obtain flour for the measurement of the rheological characteristics of dough by mixolab acc. to ISO 17718:2013 [42] (KPM Analytics, Chopin Technologies, Villeneuve-la-Garenne, France) and to perform laboratory baking test. Flour yield was in the range of 68.1 to 75.4%. Damage starch of flour was analyzed according to ISO 17715:2013 [43] by Chopin SDmatic (KPM Analytics, Chopin Technologies, Villeneuve-la-Garenne, France).

Baking test. The wheat flour samples were evaluated by a standard baking test for pan bread [44]. The dough was prepared by one-stage method at 28–30 °C by mixing flour (100%), water (acc. To water absorption specified by Mixolab + 3%), yeast (3%), and salt (1%) using a laboratory mixer (KitchenAid, Benton Harbor, MI, USA). The dough in bulk was fermented for 60 min in a proofing chamber at 30 °C/75% RH. Then, after 30 min, kneading by hand was performed. The dough was next divided into three dough pieces with a mass of 250 g each that were molded round and placed in baking pans in a proofing chamber for 38–47 min (the time needed for optimal dough development). The loaves were baked in an oven (230 °C, 30 min) (Piccolo Wachtel Winkler, Wachtel ABT GmbH, Pulsnitz, Germany). The bread samples were assessed after 20 ± 1 h of cooling for loaf volume, bread crumb hardness (Instron 1140, Instron, Norwood, MA, USA), and acrylamide content using GC/MS after bromination [45]. Anion exchange solid phase extraction combined with octadecyl silica was used to perform sample cleanup. Based on validation experiment results, it was confirmed that the method performs well at low acrylamide concentration levels with LOQ values at 10 µg kg^{-1}, and recovery relative standard deviation below 6%. The validation process was performed according to ISO/IEC 17025:2017 [46] requirements. Detailed statistical parameters of the described method have been reported by Roszko et al. [45].

Statistical analysis. The results were statistically analyzed using the three-way analysis of variance (ANOVA) with subsequent Tukey's HSD test with the significance level of $p < 0.05$. The three main factors were localization, sulfur fertilization applied, and the wheat cultivar used. A principal component analysis (PCA) was carried out on the average results of each cultivar. Data were analyzed using Statistica v. 13 software (Tibco Inc. Palo Alto, CA, USA).

3. Results and Discussion

3.1. Technological Value of Wheat Samples

The tested wheat samples were varied in terms of basic quality characteristics used in assessing suitability for processing grain for consumption purposes. The analysis of variance results (Table 1 and Table S1) indicated that the localization had the biggest influence on the total variance in the measured technological parameters of wheat grain, followed by cultivar, and then by the interaction between the environment and cultivar.

Table 1. F values calculated in the three-way ANOVA made for the qualitative parameters of grain of the examined wheat samples.

	Sources of Variation						
Parameter	**Localization (A)**	**S Fertilization (B)**	**Cultivar (C)**	**A × B**	**A × C**	**B × C**	**A × B × C**
Test weight	205.30 **	0.22	28.85 **	2.33	12.03 **	0.85	2.82
Protein content	2695.88 **	1.02	78.34 **	2.61	162.79**	3.09 *	6.92 **
Gluten content	2160.08 **	12.51 **	92.71 **	2.52	84.61 **	0.60	1.56
Zeleny index	84.70 **	0.42	9.97 **	0.58	14.52 **	0.91	0.74
Ash content	15.80 **	0.00	1.54	0.10	1.71	0.20	0.42
Asparagine	3418.61 **	1.50	43.33 **	27.35 **	17.04 **	2.82	4.88 **

Significant at: * $p < 0.05$, ** $p < 0.01$.

Wheat samples grown on location in Wielichowo were characterized by the highest average value of the test weight (74.0 kg hl^{-1}), which is an important predictor of the flour extraction rate for wheat (Table 2). However, these samples showed lower protein and gluten content (13.8% and 27.8%, respectively), with lower gluten quality (Zeleny index) and ash content than wheat grown in the Werbkowice location. Wheat grain samples from the Werbkowice location were also characterized by the highest value of asparagine content (525.1 mg kg^{-1}). Field location significantly influences the protein content and asparagine content. The analysis of variance results (Table 1) indicated that localization predominated (91.1%) the total variance in the measured free asparagine concentration in tested whole meal samples. The genotype, as well as the interaction localization and cultivar, contributed only 4.6 and 1.8%, respectively. For the wheat cultivars tested in this study, fertilization had the least impact on free asparagine content, contributing only 0.4% to the variance in free asparagine concentration. Similar to the result of Xie et al. [47], the interaction between genotype and fertilization and the interaction between localization and fertilization contributed less than 2% to the total variance.

The protein content of grain in the Werbkowice location was, on average, 2.5 p.% (percent points) higher than in Wielichowo. Additionally, we found interaction cultivar × localization in protein content (Table 1). In Wielichowo, sulfur fertilization did not cause significant changes in protein content, while in Werbkowice, sulfur fertilization caused an increase in protein content in the 'Hybery F_1' and 'Bonanza' cultivars (Table S2). For the cv. 'Pokusa' grown in Werbkowice, sulfur fertilization resulted in a reduction of the asparagine content in the grain. However, in Wielichowo, sulfur caused a decrease in the asparagine content in the seeds of the 'Belissa' and 'Bonanza' cultivars. Previous research also investigated environmental factors and agronomic differences that significantly influenced the free asparagine content in wheat grain [48]. They attempted to find answers to a question of how different environmental conditions and certain agronomic practices impact asparagine content in grain. Research from Malunga et al. [49] also showed that the asparagine concentration was significantly influenced by location and was responsible for 80% of the variation compared to 13% for the cultivar factor. Tafuri et al. [50] proved that several Italian cultivars seemed to be highly influenced by the environment, whereas others indicated relative stability in free asparagine content across locations and years. Xie et al. [47] stated that cultivating wheat in suitable environments, together with the selection of wheat genotypes with lower potential to form free asparagine content, is the most effective proposed action to control free asparagine content in Canadian wheat.

Table 2. Technological value of grain of wheat cultivars tested in the experiment.

Factor	Test Weight (kg hl^{-1})	Protein Content (N × 5.7) (%d.m.)	Gluten Content (%)	Zeleny Index (cm^3)	Ash Content (% d.m.)	Asparagine Content (mg kg^{-1})
Localization (A)						
Wielichowo	74.0 ^{a}	13.8 ^{b}	27.8 ^{b}	55 ^{b}	1.59 ^{b}	276.2 ^{b}
Werbkowice	71.5 ^{b}	16.3 ^{a}	34.2 ^{a}	63 ^{a}	1.68 ^{a}	525.1 ^{a}
Fertilization (B)						
N + 0S	72.7	15.0	30.7 ^{b}	59	1.63	392.4
N + 30S	72.8	15.1	31.2 ^{a}	59	1.63	408.9
Cultivar (C)						
Hondia	73.6 ^{ab}	14.6 ^{d}	29.9 ^{d}	56 ^{b}	1.60	378.3 ^{cd}
Pokusa	72.9 ^{bc}	15.8 ^{a}	33.4 ^{a}	63 ^{a}	1.65	397.1 ^{c}
Belissa	71.3 ^{d}	15.3 ^{b}	31.4 ^{b}	60 ^{ab}	1.59	426.8 ^{b}
Hybery F$_1$	73.8 ^{a}	14.6 ^{d}	29.7 ^{d}	56 ^{b}	1.66	363.5 ^{d}
Bonanza	72.2 ^{c}	15.0 ^{c}	30.7 ^{c}	58 ^{b}	1.64	437.4 ^{a}

a, b, c, d—values marked with the same letters do not differ significantly at $p < 0.05$ according to Tukey's multiple test.

In our study, the applied form of sulfur fertilization had no significant effect on the mentioned technological grain parameters, except gluten content (Table 1). Similar to the results of Klikocka et al. [51], the application of sulfur caused a slight positive effect on the increase in gluten content. Sulfur addition had no negative effect on the grain characteristics for processing for baking purposes. In our results, the asparagine content was higher in low-sulfur soil (Werbkowice) compared with high-sulfur soils (Wielichowo). Our results are consistent with the research of Wilson et al. [52] and Muttucumaru et al. [53]. In the research of Wilson et al. [52] and Muttucumaru et al. [53], free asparagine content in grain cultivated in low-sulfur soils was strikingly high—up to 30 times more than that cultivated in soil with normal sulfur levels. Wilson et al. [52] showed that low nitrogen use efficiency genotypes produced grain with greater asparagine content than high nitrogen use efficiency genotypes under sulfur-deficient conditions, compared to other research. Despite that, sulfur application reduces asparagine content to baseline levels. According to Xie et al. [47], fertilization had the least impact on asparagine content. Sulfur fertilization strategies for reducing free asparagine concentration in wheat were not always effective. In the research conducted by Stockmann et al. [15] in non-sulfate-deficient soils, sulfur fertilization within a conventional farming system did not influence the protein content and free asparagine amount significantly. Sufficient sulfur accumulation in the grains is presumably supplied from the available sulfur in the soil during the grain-filling period [15,54]. However, different accumulation of asparagine content in different cultivars, as a result of sulfur fertilization, was observed in both locations. The asparagine content in Werbkowice was found to be lower by 16.3 mg kg^{-1} for cv. 'Pokusa' and higher for cv. 'Hondia', 'Belissa', 'Hybery F$_1$', and 'Bonanza' (on average, 52 mg kg^{-1}) for grain with N + 30S treatment compared to N treatment. For the Wielichowo location, the differences were from -34 mg kg^{-1} for cv. 'Belissa' to +9 mg kg^{-1} for cv. 'Pokusa') (Table S2).

Tested wheat cultivars were characterized by high protein and gluten content with good potential for bread making. Among analyzed wheat cultivars, cv. 'Pokusa' was characterized by the highest value of wheat for baking purposes with the highest value of protein and gluten content and the best gluten quality determined by the Zeleny index (Table 1). The 'Hondia' and 'Hybery F$_1$' cultivars, despite the highest test weight, showed a slightly lower baking value.

The free asparagine content in wheat varied widely both within cropping systems and cultivars [15]. This was also confirmed in this study as the asparagine content in tested wheat samples ranged from 235.6 (cv. 'Hondia', N fertilization) to 587.9 mg kg^{-1} (cv. 'Bonanza', N + 30S fertilization) (Table S2). Therefore, wheat grown in Poland has similar asparagine levels to wheat cultivated in other parts of the world, i.e., in Canada where asparagine content ranges from 302.2 to 700.3 mg kg^{-1} for whole meal flour [11], and was lower than those of whole meal obtained from hard red spring cultivated in North Dakota and Nebraska, USA (357–1037 mg kg^{-1} [55] and 200–110 mg kg^{-1} [13], respectively). The asparagine content of wheat grown in Europe was in a wider range (320–1560 mg kg^{-1}) [56], while that of wheat cultivated in Australia was relatively lower (137–437 mg kg^{-1}) [57]. Free asparagine content in a total of 54 bread wheat cultivars for the Italian market ranged from 0.55 to 2.84 mmol kg^{-1} dry matter (73 to 375 mg kg^{-1}) [50]. Malunga et al. [49] suggested that breeding strategies should aim to identify cultivars that are low asparagine accumulating and are stable across different growing environments. These observations suggest that five tested wheat cultivars are not stable in diverse environments. However, since only two crop years and two growing locations were used in this research, multiple years, locations, and cultivars are required to understand the differences in polish conditions. In an ideal scenario, cultivars in which the influence of localization on the asparagine content is not significant would be found.

Asparagine content was negatively correlated with test weight (r = −0.831) and positively correlated with protein content (r = 0.855), Zeleny index (r = 0.706), and ash content (r = 0.614) (Table S3). Higher protein content may increase levels of free asparagine. Stockmann et al. [15] reported a poor relationship between protein and free asparagine for nitrogen trials, whereas the conventional sulfur trial showed a good correlation for both traits. Ohm et al. [55] found significant but low simple linear correlations between free asparagine content and test weight, as well as wheat protein content and bread volume, suggesting that high asparagine content might be associated with good bread quality.

The significant interaction between localization and fertilization was stated only for asparagine content (Table 1). The interaction between localization and cultivar had a significant impact on test weight, protein content, gluten content, Zeleny index, and asparagine, contributing 11.8, 17.5, 11.6, 27.6, and 1.8% of the total variance, respectively (Table S1). The interaction between fertilization and cultivar was found only for protein content, whereas interactions between all three tested factors—localization × fertilization × cultivar—were found only for protein content and asparagine content and contributed only 0.7 and 0.5% of the total variance, respectively.

3.2. *Quality Characteristics of Wheat Flour Samples Obtained from Tested Cultivars*

Flour samples from wheat cultivars obtained in laboratory milling were evaluated in terms of quality parameters describing the properties of both protein and starch complexes. Both indirect and direct methods were used for conducting laboratory baking tests.

The ANOVA results (Tables 3 and S4) indicated that both localization and cultivar, as well as the interaction between these two factors, had the greatest share in the total variance in the measured quality parameters that characterized the protein complex of flour. Localization had the greatest share in the total variance of such quality parameters as protein content (63.8% in the total variance) and protein weakening in points C2 (78.9%) and C_{18} (81.3%), respectively (Table S4). For the wheat samples tested in this study, the cultivar had the second strongest impact on such parameters as water absorption, development time T1, gluten index, gluten content, and stability, contributing to 58.5%, 47.0%, 43.3%, 40.6%, and 30.7% of the variance in their concentration, respectively.

Results of the quality characteristics of the protein and starch complex of flour are presented in Table 4. Flour samples obtained from grain from the Werbkowice localization were characterized by significantly higher protein and gluten content than samples obtained from the Wielichowo location. Even though the quality of gluten measured by the gluten index was slightly lower, it still met the requirements for good baking value of flour for bread production [58]. Protein quantity and quality influenced the water absorption of flour and the technological properties of dough measured by mixolab. Flours from grain samples from the Werbkowice location, which were also characterized by higher ash content, showed higher water absorption values (on average 57.3%) compared to

Wielichowo location (54.4%). However, the lowest quality of gluten samples from the Werbkowice location caused significantly lower values of dough stability and torque in points C2 and C_{18}.

Table 3. F values calculated in the three-way ANOVA made for the qualitative parameters of the flour obtained from the grain of the examined wheat samples.

	Sources of Variation					
Parameter	**Localization (A)**	**S Fertilization (B)**	**Cultivar (C)**	**A × B**	**A × C**	**B × C**
Protein content	530.45 **	1.25	23.05 **	1.25	50.07 **	0.62
Gluten content	93.06 **	0.45	34.22 **	2.82	24.06 **	0.95
Gluten index	135.16 **	0.02	63.21 **	6.45	44.00 **	2.43
Ash content	102.38 **	0.03	7.68 *	1.44	6.79 *	1.35
Water absorption	232.96 **	2.14	127.96 **	0.70	30.58 **	0.26
Development time, T1	2.78	28.44 **	214.14 **	1.00	224.03 **	8.44 *
Stability	13.06 *	7.60	5.45	1.98	4.23	1.42
C2	770.06 **	27.56 **	14.44 *	5.06	24.44 **	3.50
C_{18}	586.51 **	10.51 *	7.87 *	8.18 *	16.78 **	3.51
Falling number	868.97 **	0.86	21.85 **	10.85 *	20.81 **	1.62
Starch damage	2.24	0.31	17.47 **	0.00	14.40 *	0.76
C3	2653.80 **	21.76 **	108.00 **	13.49*	62.60 **	4.55
C4	1263.83 **	0.03	40.29 **	5.98	13.93 *	0.42
C5	2311.20 **	0.04	52.44 **	15.96 *	14.06 *	1.31
Final temp. of gelatinization D3	0.60	11.43 *	1.49	1.20	7.31 *	2.67

Significant at: * $p < 0.05$, ** $p < 0.01$.

Table 4. Quality characteristics of protein complex of flour obtained from the tested cultivars.

Factor	Protein Content (N × 5.7) (%d.m.)	Gluten Content (%)	Gluten Index (%)	Ash Content (% d.m.)	Water Absorption (%)	Development Time, T1 (min)	Stability (min)	C2 (Nm)	C_{18} (Nm)
Localization (A)									
Wielichowo	12.3 ^{b}	31.2 ^{b}	91 ^{a}	0.50 ^{b}	54.4 ^{b}	3.4	10.0 ^{a}	0.52 ^{a}	0.54 ^{a}
Werbkowice	14.3 ^{a}	35.4 ^{a}	82 ^{b}	0.56 ^{a}	57.3 ^{a}	3.5	9.4 ^{b}	0.40 ^{b}	0.41 ^{b}
Fertilization (B)									
N + 0S	13.3	33.4	86	0.53	55.7	3.6 ^{a}	9.9	0.47 ^{a}	0.48 ^{a}
N + 30S	13.4	33.1	86	0.53	56.0	3.3 ^{b}	9.5	0.45 ^{b}	0.46 ^{b}
Cultivar (C)									
Hondia	13.0 ^{cd}	31.8 ^{b}	90 ^{bc}	0.50 ^{b}	53.2 ^{d}	3.4 ^{b}	10.2	0.48 ^{a}	0.49 ^{a}
Pokusa	14.0 ^{b}	35.9 ^{a}	84 ^{c}	0.53 ^{ab}	57.6 ^{b}	4.5 ^{a}	9.5	0.46 ^{a}	0.48 ^{ab}
Belissa	13.5 ^{bc}	36.6 ^{a}	76 ^{d}	0.52 ^{ab}	59.0 ^{a}	4.2 ^{a}	9.1	0.44 ^{b}	0.45 ^{b}
Hybery F_1	12.7 ^{d}	29.8 ^{b}	88 ^{bc}	0.54 ^{ab}	54.0 ^{d}	3.1 ^{b}	9.7	0.48 ^{a}	0.48 ^{ab}
Bonanza	13.4 ^{bc}	32.4 ^{b}	94 ^{a}	0.56 ^{a}	55.4 ^{c}	2.0 ^{c}	10.0	0.45 ^{ab}	0.46 ^{ab}

a, b, c, d—values marked with the same letters do not differ significantly at $p < 0.05$ according to Tukey's multiple test.

The addition of sulfur fertilization had no effect on flour protein and gluten content, and neither had any effect on gluten quality, ash content, and water absorption of flours (Table 4). However, we observed lower values of development time and torque in points C2 and C_{18}, which indicates that dough is at risk of becoming less stable and weaker during processing [59]. The differences were statistically significant, but not from a technological point of view. Sulfur application in the research of Wilson et al. [52] caused increased average water absorption of flour, development time, and dough stability determined by farinograph.

Flour obtained from tested wheat cultivars was differentiated in terms of protein properties. Cultivar 'Pokusa' was characterized by the highest baking value—with the highest protein and gluten content (14.0% and 35.9%, respectively) (Table 4). The lowest baking value was found for cv. 'Hondia' and 'Hybery F_1', which were also characterized by the lowest water absorption values (53.2 and 54.0%, respectively). Cultivar 'Belissa', with one of the greatest protein and gluten contents and the lowest gluten index, was characterized to be the most favorable in terms of water absorption of flour (59.0%) compared to the rest of the cultivars. All tested cultivars showed protein weakening—a dough torque in point C2 below 0.5 Nm, which indicates the appropriate quality to produce bread [60]. High values of dough torque measured 18 min after the mixolab test started (C_{18}) indicate a better tolerance to the weakening of the gluten structure during mixing and gradually increasing the temperature of the dough [60].

The interaction between localization and fertilization was stated only for the dough torque measured 18 min after the test started (C_{18}) (Table 3). The interaction between localization and cultivar was found for protein content, gluten content, gluten index, ash content, water absorption, dough development time, C2, and C_{18}, whereas the interaction between fertilization and cultivar was found only for development time.

Asparagine concentration was positively correlated with factors affecting bread-making quality: gluten content (r = 0.597) and water absorption of flour (r = 0.616) (Table S3). With the increase of asparagine content in flour, the gluten index decreased (r = −0.514), as well as the dough torque in point C_{18} (r = −0.952, respectively). No significant correlation between the asparagine content of wheat and protein or gluten content was found in the research of Malunga et al. [11]. However, weak correlations were found between asparagine content and wheat protein [13], gluten content, gluten index, the Zeleny sedimentation index, and water absorption [56].

The importance of the level of alpha-amylase activity is crucial for the dough fermentation process and for obtaining high-quality bread. Alpha-amylase activity and starch properties of tested flours were characterized by several parameters. The ANOVA results (Tables 3 and S4) indicated that localization had the greatest share in the total variance in the measured quality parameters characterizing the starch complex of flour, such as starch retrogradation C5 (88.8% in the total variance), stability of hot starch paste C4 (84.7%), starch gelatinization C3 (78.2%), and falling number (81.8%) (Table S4). For the wheat samples tested in this study, the cultivar was the second most impactful factor on parameters such as starch damage, contributing to 51.0% of the variance in their concentration.

Tested flours were characterized by average and low alpha-amylase activity, measured as falling number (in the range of 187 to 372 s). Flours obtained from grain cultivated in the Wielichowo localization were characterized by significantly higher falling numbers compared to the Werbkowice localization (Table 5). This lower alpha-amylase activity of grain samples from the Wielichowo localization directly affected the higher values of dough torque measured in points C3, C4, and C5 (2.15, 2.17, and 3.46 Nm, respectively) than from Werbkowice (Table 5).

Additional sulfur fertilization had no negative effect on the starch properties of tested flours. It influenced only the lower value of dough torque in point C3 measures of starch gelatinization and the lower final temperature of gelatinization (1.87 Nm and 74.8 °C, respectively) (Table 5). According to Klikocka et al. [51], sulfur addition had no effect on the starch content of tested wheat cultivars.

Alpha-amylase activity determined by falling numbers varied between the tested cultivars. Cultivar 'Belissa' was characterized by the highest value of falling number (on average, 317 s) compared to other cultivars (Table 5). The lowest falling number was stated for cv. 'Bonanza' (266 s). Despite the highest falling number value, cv. 'Belissa', along with cv. 'Pokusa' and cv. 'Bonanza', was characterized by the significantly lowest dough torque in points C3 (starch gelatinization), C4 (stability of hot starch paste), and C5 (starch retrogradation) of the mixolab curves. The highest values were stated for cv. 'Hondia' and 'Hybery F_1'.

Table 5. Quality characteristics of starch complex of flour obtained from the tested cultivars.

Factor	Falling Number (s)	Starch Damage (UCD)	C3 (Nm)	C4 (Nm)	C5 (Nm)	Final Temperature of Gelatinization, D3 (°C)
Localization (A)						
Wielichowo	342 [a]	18.2	2.15 [a]	2.17 [a]	3.46 [a]	75.7
Werbkowice	237 [b]	18.8	1.63 [b]	1.16 [b]	1.69 [b]	75.4
Fertilization (B)						
N + 0S	291	18.6	1.92 [a]	1.67	2.58	76.3 [a]
N + 30S	288	18.4	1.87 [b]	1.66	2.57	74.8 [b]
Cultivar (C)						
Hondia	288 [bc]	16.3 [c]	2.00 [a]	1.85 [a]	2.84 [a]	75.2
Pokusa	282 [bc]	20.3 [a]	1.79 [b]	1.51 [b]	2.36 [b]	74.8
Belissa	317 [a]	19.7 [ab]	1.81 [b]	1.62 [b]	2.47 [b]	76.3
Hybery F_1	296 [ab]	17.2 [bc]	2.04 [a]	1.90 [a]	2.95 [a]	75.6
Bonanza	266 [c]	18.9 [ab]	1.82 [b]	1.46 [b]	2.28 [b]	75.8

a, b, c—values marked with the same letters do not differ significantly at $p < 0.05$ according to Tukey's multiple test.

Starch damage is one of the most important criteria for assessing the quality of baking flour. It affects the water absorption of flour, the rheological properties of dough, the fermentation of the dough, and the structure of the bread crumb [61]. The starch damage of tested flours varied from 14.0 to 21.3 UCD and differed depending on the cultivar (Table 5). The highest average starch damage was found for cv. 'Pokusa', whereas the lowest value was found for cv. 'Hondia'. A wide range of starch damage indicates the possibility of using these flours in the production of many assortments of baking and pastry products. Wheat flours intended to produce bread and confectionery products are characterized by starch damage, usually in the wide range from 14 to 24 UCD [62]. According to Ma et al. [63], an increase in the starch damage of flour significantly decreased the falling number and increased the alpha-amylase activity. One of its benefits is greater susceptibility to amylolytic enzymes, which results in increased maltose and dextrin production during dough fermentation [32,34]. With the increase in the amount of fermentable carbohydrates in the dough, the activity of yeast and bacteria is stimulated [64]. In addition, the increase in the level of starch damage may also favor the increased reduction in carbohydrates, which are also involved in acrylamide formation. The conversion ratio of asparagine to acrylamide could be enhanced when starch damage is increased [34].

Mulla et al. [35] found a correlation between damaged starch content in the flour and acrylamide content in bread. The extent of milling is known to affect the contents by both reducing sugars [65] and amino acids [66]. This observation indicated that the mitigation of acrylamide in bread can be obtained by reducing starch damage in wheat flour [34]. To clearly unravel genotypic differences and their interaction with environmental factors and, especially, nitrogen and sulfur fertilization, further research is needed.

Similar to the research of Malunga et al. [11], no significant correlation was found with asparagine content. Asparagine content was negatively correlated with the falling number (r = −0.905) (Table S3). Doughs from flours with higher asparagine content were also characterized by lower consistency in points C3, C4, and C5 (r = −0.932, r = −0.964, and r = −0.977, respectively).

The interaction between localization and fertilization was found for the following quality parameters: falling number and dough torque in points C3 and C5 (Table 3). The interaction between localization and cultivar was found for falling number; starch damage; dough torque in points C3, C4, C5; and final temperature of gelatinization, whereas no interaction between fertilization and cultivar was detected.

3.3. Bread Quality Properties

The ANOVA results (Tables 6 and S5) indicated that the localization had the greatest share in the total variance of the measured acrylamide content in bread (55.8% in the total variance) and the color of the bread crumb (85.5%—parameter *a**). The wheat cultivar had the second most significant impact on parameters such as loaf volume (62.5% of the total variance), acrylamide content (13.5%), and bread crumb color (*L* 38.7% and *b** 55.2%).

Table 6. F values calculated in the three-way ANOVA made for the qualitative parameters for the bread obtained in the baking trial from the grain of the examined wheat samples.

	Sources of Variation						
Parameter	**Localization (A)**	**S Fertilization (B)**	**Cultivar (C)**	**A × B**	**A × C**	**B × C**	**A × B × C**
Loaf volume	12.22 **	18.03 **	68.75 **	1.47	20.02 **	1.74	1.51
Bread crumb hardness	119.35 **	25.87 **	4.33 **	3.08	6.25 **	29.19 **	37.75 **
Acrylamide content	1518.09 **	359.17 **	92.03 **	9.21 **	8.15 *	65.03 *	37.92 *
*L*crumb	404.92 **	0.61	85.12 **	1.59	11.63 **	0.84	0.81
*a**crumb	3059.32 **	12.69 **	62.99 **	0.61	25.49 **	4.68 **	12.79 **
*b**crumb	248.16 **	4.36 *	187.01 **	0.00	51.10 **	4.91 **	12.92 **
*L*crust	36.33 **	24.89 **	2.08	0.41	13.96 **	2.03	1.17
*a**crust	0.60	2.85	4.03 *	6.67 *	9.71 **	1.43	0.40
*b**crust	39.13 **	33.20 **	3.48 *	0.03	7.25 **	1.96	2.14

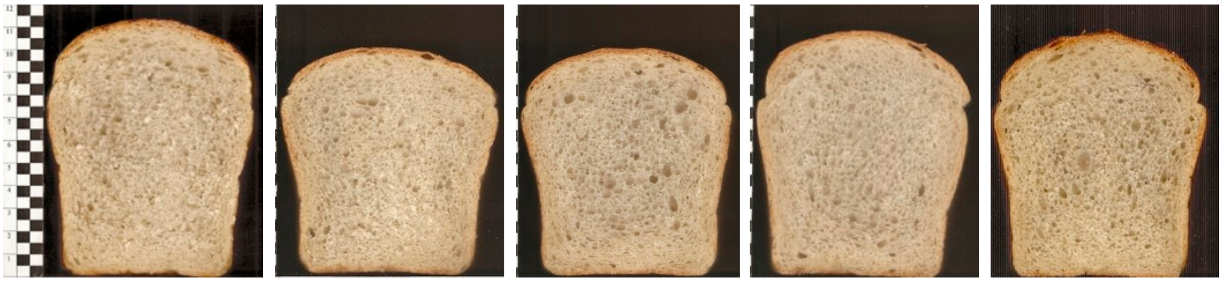
Significant at: * $p < 0.05$, ** $p < 0.01$.

The data on bread's physical characteristics are shown in Table 7. Bread obtained in laboratory baking was characterized by a proper appearance with the shape of a well-risen loaf and the right color of the crust, as well as a relatively uniform porosity of the crumb (Figures 1–4). The most favorable in terms of baking value and the possibility of using it for baking purposes was bread obtained from grain cultivated in Wielichowo with the highest bread volume (322 cm^3) and the lowest acrylamide content (11.9 μg 100^{-1} g) compared to samples from Werbkowice (Table 7).

Table 7. Quality characteristics of bread obtained in laboratory baking from tested cultivars.

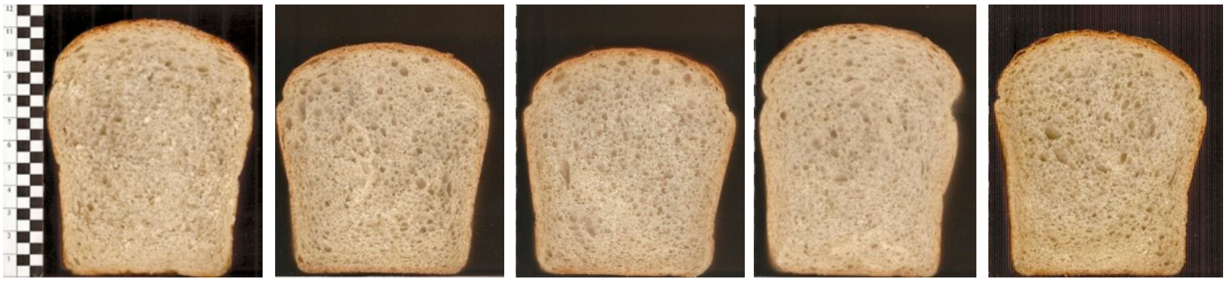
Factor	**Loaf Volume (cm^3/100 g)**	**Bread Crumb Hardness (N)**	**Acrylamide Content (μg 100^{-1} g)**	**Breadcrumb Color**			**Bread Crust Color**		
				L	***a****	***b****	***L***	***a****	***b****
Localization (A)									
Wielichowo	322 ^{a}	14.9 ^{a}	11.9 ^{b}	74.2 ^{a}	−1.07 ^{b}	18.44 ^{a}	53.36 ^{a}	13.97	30.45 ^{a}
Werbkowice	315 ^{b}	11.9 ^{b}	17.9 ^{a}	70.1 ^{b}	−0.23 ^{a}	17.60 ^{b}	49.73 ^{b}	13.83	27.88 ^{b}
Fertilization (B)									
N + 0S	323 ^{a}	12.7 ^{b}	16.4 ^{a}	72.2	−0.68 ^{b}	17.97 ^{b}	50.04 ^{b}	14.06	27.98 ^{a}
N + 30S	315 ^{b}	14.1 ^{a}	13.3 ^{b}	72.1	−0.62 ^{a}	18.08 ^{a}	53.04 ^{a}	13.70	30.35 ^{a}
Cultivar (C)									
Hondia	334 ^{a}	12.7 ^{b}	14.1 ^{b}	75.1 ^{a}	−0.58 ^{b}	17.25 ^{c}	52.34	14.23 ^{a}	30.02 ^{ab}
Pokusa	303 ^{c}	13.8 ^{ab}	14.2 ^{b}	69.2 ^{d}	−0.45 ^{a}	18.26 ^{b}	50.35	14.28 ^{a}	28.47 ^{ab}
Belissa	297 ^{c}	14.3 ^{a}	17.8 ^{a}	72.7 ^{b}	−0.72 ^{c}	18.81 ^{a}	51.64	13.95 ^{ab}	28.97 ^{ab}
Hybery F_1	337 ^{a}	12.9 ^{b}	13.9 ^{b}	72.3 ^{bc}	−0.75 ^{c}	17.08 ^{c}	52.60	13.24 ^{c}	30.10 ^{a}
Bonanza	323 ^{b}	13.5 ^{ab}	14.4 ^{b}	71.5 ^{c}	−0.75 ^{c}	18.72 ^{a}	50.78	13.81 ^{ab}	28.27 ^{b}

L, lightness, (*L* = 0 is black; *L* = 100 is white); *a**, green-red opponent colors (−*a* = green; +*a* = red); *b**, blue-yellow opponent colors (−*b* = blue; +*b* = yellow). a, b, c, d—values marked with the same letters do not differ significantly at $p < 0.05$ according to Tukey's multiple test.



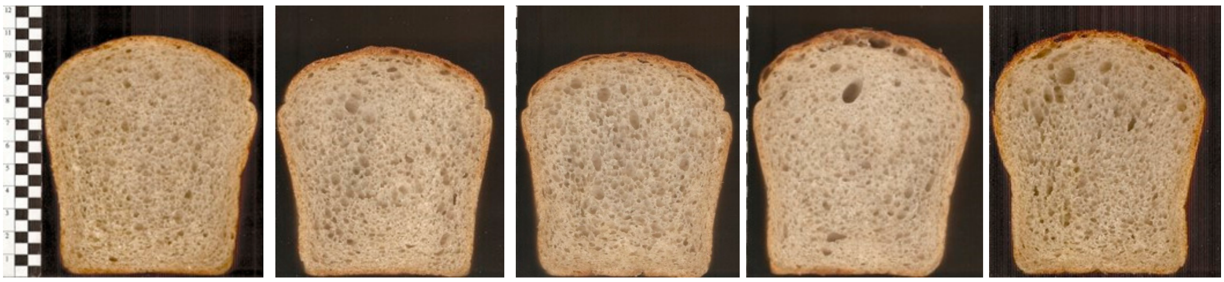
(**a**) (**b**) (**c**) (**d**) (**e**)

Figure 1. Cross-sectional view of bread from the tested wheat cultivars from Wielichowo localization with N + 0S fertilization: (**a**) Hondia; (**b**) Pokusa; (**c**) Belissa; (**d**) Hybery F_1; (**e**) Bonanza.



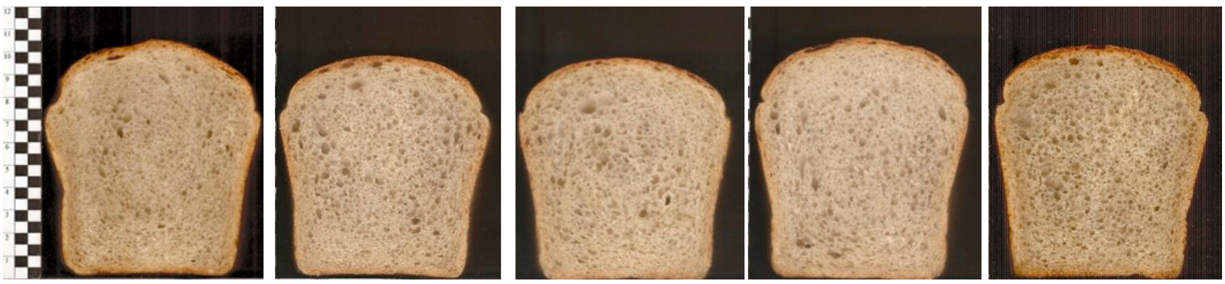
(**a**) (**b**) (**c**) (**d**) (**e**)

Figure 2. Cross-sectional view of bread from the tested wheat cultivars from Wielichowo localization with N + 30S fertilization: (**a**) Hondia; (**b**) Pokusa; (**c**) Belissa; (**d**) Hybery F_1; (**e**) Bonanza.



(**a**) (**b**) (**c**) (**d**) (**e**)

Figure 3. Cross-sectional view of bread from the tested wheat cultivars from Werbkowice localization with N + 0S fertilization: (**a**) Hondia; (**b**) Pokusa; (**c**) Belissa; (**d**) Hybery F_1; (**e**) Bonanza.



(**a**) (**b**) (**c**) (**d**) (**e**)

Figure 4. Cross-sectional view of bread from the tested wheat cultivars from Werbkowice localization with N + 30S fertilization: (**a**) Hondia; (**b**) Pokusa; (**c**) Belissa; (**d**) Hybery F_1; (**e**) Bonanza.

The addition of sulfur fertilization had a negative effect on the bread volume by lowering it from 323 cm^3 to 315 cm^3. Additionally, the hardness of the bread crumb was increased to 14.1 N (Table 7). Shahsavani and Gholami [67] and Li et al. [68] found that wheat grown at an intermediate sulfur fertilization rate yielded the highest loaf volume. Asparagine reductions achieved by increasing sulfur fertilization partially coincided with a loss in the functionality of bread.

An undoubtedly positive health effect of sulfur fertilization was lowering the acrylamide content in bread, on average, from 16.4 to 13.3 $\mu g\ 100^{-1}$ g (Table 7). Considering the results of individual samples in a given location, the highest reduction in acrylamide content was found for grain samples cultivated in Wielichowo (on average, 3.6 $\mu g\ 100^{-1}$ g lower) than in Werbkowice (on average, 2.5 $\mu g\ 100^{-1}$ g lower) (Table S2). Bread obtained from cv. 'Hondia' and 'Belissa' cultivated with sulfur fertilization was characterized by the greatest reduction in acrylamide content in both localizations (on average, 3.7 and 6.9 $\mu g\ 100^{-1}$ g lower).

Unlike the results of Claus et al. [7], bread obtained from wheat cultivars belongs to Class A—good category wheat (cv. 'Hondia', 'Pokusa')—and shows a lower value of acrylamide than from other bread category cultivars ('Belissa', Hybery F_1', 'Bonanza'). It is possible to indicate cultivars with good baking value, the bread obtained from which will be characterized by a low acrylamide content.

Among tested cultivars, cv. 'Hondia' and cv. 'Hybery F_1' with the lowest starch damage of flour were characterized by the highest bread volume, the lowest bread crumb hardness, and one of the lowest acrylamide contents. Cultivar 'Belissa', with the second highest degree of starch damage, was characterized significantly by the lowest bread volume with the highest bread crumb hardness and the highest acrylamide content (296.9 cm^3, 14.3 N and 17.8 $\mu g\ 100^{-1}$ g, respectively), and was rated as the least favorable.

Browning products obtained from the Maillard reaction influence sensory perception. Bread crust obtained from flours with higher protein and amino acid contents was darker. Localization and fertilization, but not cultivar, were factors that significantly influenced the lightness *L* of the tested bread crusts (Table 7). The highest value of brightness *L* of the crust was obtained for the bread samples obtained from Wielichowo (with also a lower value of protein content, asparagine content in grain, and acrylamide content in bread), and with N + 30S treatment (with lower acrylamide content) (Table 7). Li et al. [68] indicated that the environmental factor and cultivar, but not sulfur fertilization, influence the brightness of the bread crust. Bread obtained from grain cultivated with sulfur was also characterized by a smaller share of green and a greater share of red in the color of the crumb, higher brightness of the crust, and a higher share of red tone.

Asparagine content influenced the acrylamide content in bread (r = 0.768) (Table S3). Bread from flours with higher asparagine content was also characterized by darker crumb color and a greater share of red color (a^*crumb) (r = −0.733 and r = 0.897, respectively). In this work, we did not observe the influence of starch damage on the acrylamide content in bread when the results of all cultivars, fertilization, and location were compared. However, bread obtained from flour with higher starch damage was characterized by darker crust color, with a greater share of red, and a smaller share of yellow (correlation with L^* value r = −0.607, a^* r = 0.548, and b^* r = −0.545, respectively).

The results of a principal component analysis (PCA) demonstrated that the first two principal components (PC1 and PC2) accounted for 8.3% of the variation (Figure 5). PC1 explained 51.4% and PC2 explained 30.9% of the variation. Figure 5 shows that cultivars 'Hondia' and 'Hybery F_1' with the lowest asparagine content, starch damage, water absorption of flour, bread crumb hardness, acrylamide content, and the highest loaf volume belong to one group, while the remaining cultivars form three separate groups. Comparing the position of the cases on the chart with the forms of components and factor loadings, it can be concluded that the cv. 'Hondia' is negatively correlated with PC1 and positively with PC2, while the cv. 'Hybery F_1' is negatively correlated with PC1 and

PC2. However, the cultivars 'Bonanza', 'Belissa', and 'Pokusa' were positively correlated with PC1.

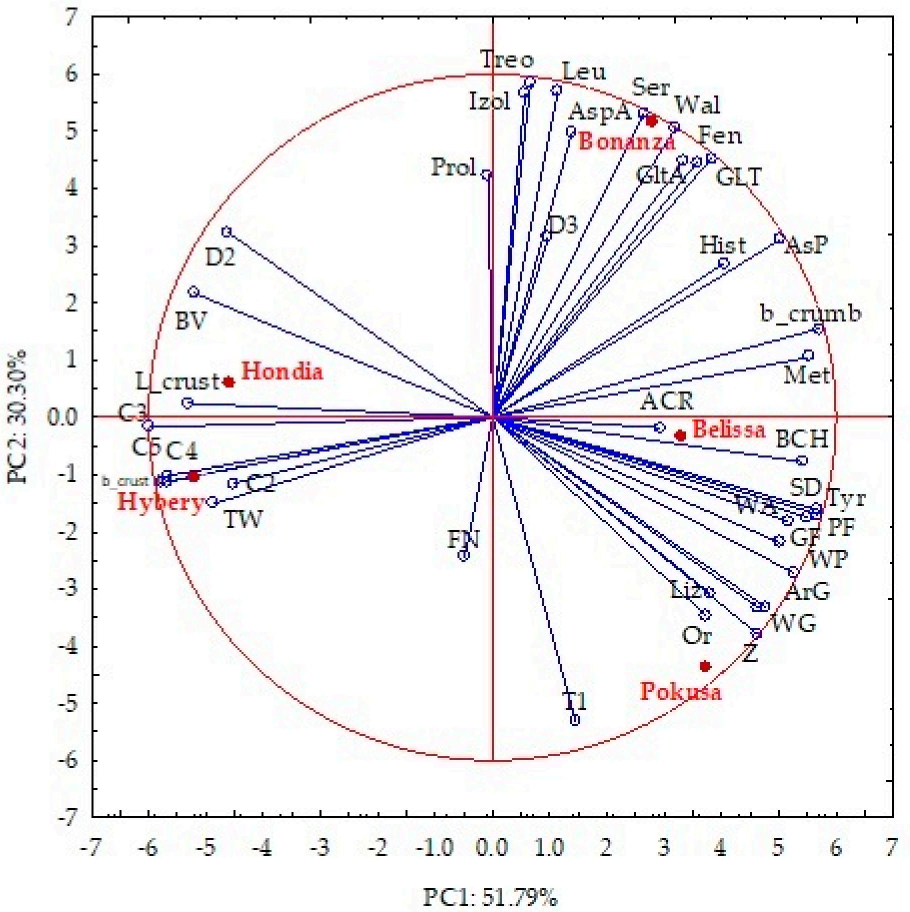


Figure 5. Variable graph of PCA showing the quality parameters and cultivars of wheat. Explanations: TW—test weight, FN—falling number, Z—Zeleny index, WP—wheat protein content, PF—flour protein content, WG—wheat gluten content, SD—starch damage, WA—water absorption, T1—development time, BV—bread volume, BCH—bread crumb hardness, ACR—acrylamide content, Treo—threonine, Leu—leucine, Ser—, Wal—valine, Fen—phenylalanine, Izol—isoleucine, Prol—proline, GltA—glutamic acid, GLT—glutamine, Hist—histidine, AsP—asparagine, AspA—aspartic acid, Met—methionine, Tyr—tyrosine, ArG—arginine, Liz—lysine, Or—ornithine.

Figure 5 shows that cultivars 'Hondia' and 'Hybery F_1' are classified in the left section of the chart, which indicates relatively higher values of bread volume (BV), brightness of crust (*L*crust), starch gelatinization (C3), amylolytic activity (C4), retrogradation (C5), and protein weakening (C2), while featuring low values of amino acids, total protein, and gluten content. However, cultivars 'Bonanza', 'Belissa', and 'Pokusa' scored low values of parameters such as the starch–amylolytic complex and higher amino acid values than the previously mentioned cultivars. The cv. 'Bonanza' is characterized by the highest value of aspartic acid, threonine, leucine, serine, and valine among the three cultivars mentioned. The cv. 'Pokusa' is characterized by the highest protein content both in grain and flour regarding gluten content and arginine, and the most favorable features characterizing the quality of gluten proteins, i.e., the Zeleny sedimentation index and water absorption of flour. The obtained results indicate a positive correlation of the starch damage (SD) with water absorption (WA) and bread crumb hardness (BCH), and a negative correlation with the brightness of a crust (*L*crust) and starch retrogradation (C5). Cultivars 'Belissa', 'Bonanza', and 'Pokusa' were characterized by higher average values of starch damage (SD), water absorption (WA), and bread crumb hardness (BCH). Bread obtained from cultivars 'Hondia' and 'Hybery F_1' had the lowest acrylamide content and bread from cultivars 'Bonanza', 'Belissa' and, 'Pokusa' had the highest acrylamide content due to the high protein content and amino acid content, including asparagine and aspartic acid, and higher access to maltose and dextrin products during fermentation as a result of high starch damage.

4. Conclusions

To our knowledge, this work presents the first comprehensive analysis related to the accumulation of asparagine in grain and acrylamide content in bread, including the assessment of the baking value of five winter wheat varieties cultivated in Poland.

Our research showed a significant influence of localization (environment) and cultivar and their interaction on the asparagine and acrylamide content and technological parameters of grain. The influence of sulfur application on the asparagine content in grain and on the baking value has not been confirmed. However, we found a negative effect of sulfur fertilization on the acrylamide content in bread. The obtained results indicate that, on the one hand, there is no effect of sulfur on the asparagine content in grain and, on the other hand, a decrease in acrylamide in bread may be surprising because the highly significant positive correlation between these features was confirmed in our research. Probably, the effectiveness of sulfur in reducing the asparagine in the endosperm depends on the wheat cultivar and the location of the crop, which requires further research and confirmation.

The food industry requires cultivars with reduced free asparagine content and favorable baking properties, and which show stable characteristics regardless of weather conditions. The obtained localization × cultivar interactions indicate different responses of wheat cultivars depending on habitat conditions. The interaction was confirmed in the asparagine and acrylamide content and bread-making quality. In our research, the asparagine content in tested cultivars ranged from 235 to 588 mg kg^{-1}, the acrylamide content from 8.0 to 23.4 μg 100^{-1} g, and the protein content in wheat grain from 12.3 to 17.6% d.m. (Table S2). Considering the quality requirements of the baking industry (high protein content) and food safety requirements (low asparagine and acrylamide content), the most valuable cultivar is 'Pokusa'. Cv. 'Pokusa' is characterized by high protein content in grain (15.8% d.m.) and one of the lowest asparagine contents in grain (397 mg kg^{-1}) and acrylamide content in bread (14.2 μg 100^{-1} g). From the point of view of the health of the raw material, we can also recommend the 'Hybery F_1' cultivar. Bread obtained from this cultivar has the lowest acrylamide content (13.9 μg 100^{-1} g), but the grain is characterized by the lowest protein content (14.6% d.m.) and gluten content (29.7%) among tested cultivars.

A novel aspect of our research is the analysis of the influence of the amylolytic complex and starch damage on the acrylamide content in bread. Our results indicate a significant negative correlation of starch quality parameters with asparagine and acrylamide content. The results also indicate differences in cultivars. Among others, cv. 'Belissa', with the highest falling number and starch damage values, is characterized by a higher asparagine content in grain and acrylamide content in bread. Further research should therefore consider the aspect of starch properties on the formation of asparagine in grain and acrylamide in bread.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14020207/s1. Table S1: The ratio of the factorial SS to the total sum of squares illustrating the percentage share of the total variance for the qualitative parameters of grain of the examined wheat samples. Table S2: Protein and asparagine content in wheat, starch damage of flour, and acrylamide content of bread depend on cultivar, localization, and fertilization. Table S3: Correlation between asparagine concentration, acrylamide content, and quality parameters. Table S4: The ratio of the factorial SS to the total sum of squares illustrating the percentage share of the total variance for the qualitative parameters of the flour obtained from the grain of the examined wheat samples. Table S5: The ratio of the factorial SS to the total sum of squares illustrating the percentage share of the total variance for the qualitative parameters for the bread obtained in the baking trial.

Author Contributions: Conceptualization, A.S. and G.P.; methodology, A.S., G.P. and M.R.; validation, A.S. and O.Ś.; formal analysis A.S.; investigation, A.S., E.A. and O.Ś.; resources, A.S. and M.R.; data curation, A.S. and D.K.; writing—original draft preparation, A.S.; writing—review and editing, A.P.-C. and M.R.; visualization, A.S.; supervision, A.S.; project administration, A.S.; funding acquisition, A.S. and G.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research is supported by Institute of Agricultural and Food Biotechnology—State Research Institute (statutory research activity in 2022–2023—grant number ZZ-107-01) supported by the Polish Ministry of Education and Science.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data are contained within the article or supplementary material. The data presented in this study are available in the supplementary material. Table S1: The ratio of the factorial SS to the total sum of squares illustrating the percentage share of the total variance for the qualitative parameters of grain of the examined wheat samples. Table S2: Protein and asparagine content in wheat, starch damage of flour, and acrylamide content of bread depend on cultivar, localization, and fertilization. Table S3: Correlation between asparagine concentration, acrylamide content, and quality parameters. Table S4: The ratio of the factorial SS to the total sum of squares illustrating the percentage share of the total variance for the qualitative parameters of the flour obtained from the grain of the examined wheat samples. Table S5: The ratio of the factorial SS to the total sum of squares illustrating the percentage share of the total variance for the qualitative parameters for the bread obtained in the baking trial.

Acknowledgments: This research was based upon the work carried out in the frame of COST Action ACRYRED—"Reducing Acrylamide Exposure of Consumers by a Cereals Supply-chain Approach Targeting Asparagine", grant number CA 21149, where the author Anna Szafrańska is a WG1 and WG2 Member, and Marek Roszko is a WG5 Member.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. FAOSTAT. *Crops and Livestock Products*; FAOSTAT: Rome, Italy, 2023. Available online: https://www.fao.org/faostat/en/#data/QCL (accessed on 10 December 2023).
2. Huang, T.; Xu, M.; Lee, A.; Cho, S.; Qi, L. Consumption of whole grains and cereal fiber and total and cause-specific mortality: Prospective analysis of 367,442 individuals. *BMC Med.* **2015**, *13*, 59, Erratum in *BMC Med.* **2015**, *13*, 85. [CrossRef]
3. Zyzak, D.V.; Sanders, R.A.; Stojanovic, M.; Tallmadge, D.H.; Eberhart, B.L.; Ewald, D.K.; Gruber, D.C.; Morsch, T.R.; Strothers, M.A.; Rizzi, G.P.; et al. Acrylamide formation mechanism in heated foods. *J. Agric. Food Chem.* **2003**, *51*, 4782–4787. [CrossRef]
4. Becalski, A.; Lau, B.P.; Lewis, D.; Seaman, S.W. Acrylamide in foods: Occurrence, sources, and modeling. *J. Agric. Food Chem.* **2003**, *51*, 802–808. [CrossRef]
5. Claus, A.; Carle, R.; Schieber, A. Acrylamide in cereal products: A review. *J. Cereal Sci.* **2008**, *47*, 118–133. [CrossRef]
6. Amrein, T.M.; Schőnbächler, B.; Rohner, F.; Lukac, H.; Schneider, H.; Keiser, A.; Escher, F.; Amadò, R. Potential for acrylamide formation in potatoes: Data from the 2003 harvest. *Eur. Food Res. Technol.* **2004**, *219*, 572–578. [CrossRef]
7. Claus, A.; Schreiter, P.; Weber, A.; Graeff, S.; Herrmann, W.; Claupein, W.; Schieber, A.; Carle, R. Influence of Agronomic Factors and Extraction Rate on the Acrylamide Contents in Yeast-Leavened Breads. *J. Agric. Food Chem.* **2006**, *54*, 8968–8976. [CrossRef] [PubMed]
8. Taeymans, D.; Wood, J.; Ashby, P.; Blank, I.; Studer, A.; Stadler, R.H.; Gondé, P.; Van Eijck, P.; Lalljie, S.; Lingnert, H.; et al. A review of acrylamide: An industry perspective on research, analysis, formation, and control. *Crit. Rev. Food Sci. Nutr.* **2004**, *44*, 323–347. [CrossRef] [PubMed]
9. Curtis, T.Y.; Powers, S.J.; Wanga, R.; Halford, N.G. Effects of variety, year of cultivation and sulphur supply on the accumulation of free asparagine in the grain of commercial wheat varieties. *Food Chem.* **2018**, *239*, 304–313. [CrossRef] [PubMed]
10. Rapp, M.; Schwadorf, K.; Leiser, W.L.; Würschum, T.; Longin, C.F.H. Assessing the variation and genetic architecture of asparagine content in wheat: What can plant breeding contribute to a reduction in the acrylamide precursor? *Theor. Appl. Genet.* **2018**, *131*, 2427–2437. [CrossRef] [PubMed]
11. Malunga, L.N.; Ames, N.P.; Masatcioglu, M.T.; Khorshidi, A.S.; Thandapilly, S.J.; Cuthbert, R.D.; Sopiwnyk, E.; Scanlon, M.G. Free asparagine concentrations in Canadian hard red spring wheat cultivars. *Can. J. Plant Sci.* **2019**, *99*, 338–347. [CrossRef]
12. Lea, P.J.; Sodek, L.; Parry, M.A.J.; Shewry, P.R.; Halford, N.G. Asparagine in plants. *Ann. Appl. Biol.* **2007**, *150*, 1–26. [CrossRef]
13. Navrotskyi, S.; Baenziger, P.S.; Regassa, T.; Guttieri, M.J.; Rose, D.J. Variation in asparagine concentration in Nebraska wheat. *Cereal Chem.* **2018**, *95*, 264–273. [CrossRef]
14. Mansfield, J.; Gebauer, S.; Dathe, K.; Ulbrich-Hofmann, R. Secretory phospholipase A2 from *Arabidopsis thaliana*: Insights into the three-dimensional structure and the amino acids involved in catalysis. *Biochemistry* **2006**, *45*, 5687–5694. [CrossRef]

15. Stockmann, F.; Weber, E.A.; Schreiter, P.; Merkt, N.; Claupein, W.; Graeff-Hönninger, S. Impact of Nitrogen and Sulphur Supply on the Potential of Acrylamide Formation in Organically and Conventionally Grown Winter Wheat. *Agronomy* **2018**, *8*, 284. [CrossRef]
16. Curtis, T.Y.; Halford, N.G. Reducing the acrylamide-forming potential of wheat. *Food Energy Secur.* **2016**, *5*, 153–164. [CrossRef]
17. Halford, N.G.; Muttucumaru, N.; Curtis, T.Y.; Parry, M.A.J. Genetic and agronomic approaches to decreasing acrylamide precursors in crop plants. *Food Addit. Contam.* **2007**, *24* (Suppl. S1), 26–36. [CrossRef] [PubMed]
18. Lerner, S.E.; Seghezzo, M.L.; Molfese, E.R.; Ponzio, N.R.; Cogliatti, M.; Rogers, W.J. N- and S-fertiliser effects on grain composition, industrial quality and end-use in durum wheat. *J. Cereal Sci.* **2006**, *44*, 2–11. [CrossRef]
19. Sinclair, T.R.; Horie, T. Leaf Nitrogen, Photosynthesis, and Crop Use Efficiency: A Review. *Crop Sci.* **1989**, *29*, 90–97. [CrossRef]
20. Jamal, A.; Moon, Y.-S.; Abdin, M.Z. Sulphur–A general overview and interaction with nitrogen. *Aust. J. Crop Sci.* **2010**, *4*, 523–529.
21. Cambell, W.H. Nitrate reductase structure, function and regulation: Bridging the Gap between Biochemistry and Physiology. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1999**, *50*, 277–303. [CrossRef]
22. Mandal, U.K.; Singh, G.; Victor, U.S.; Sharma, K.L. Green manuring: Its effect on soil properties and crop growth under rice–wheat cropping system. *Eur. J. Agron.* **2003**, *19*, 225–237. [CrossRef]
23. Swamy, U.; Wang, M.; Tripathy, J.N.; Kim, S.-K.; Hirasawa, M.; Knaff, D.B.; Allen, J.P. Structure of Spinach Nitrite Reductase: Implications for Multi-electron Reactions by the Iron−Sulphur:Siroheme Cofactor. *Biochemistry* **2005**, *44*, 16054–16063. [CrossRef] [PubMed]
24. Salvagiotti, F.; Castellarin, J.M.; Miralles, D.J.; Pedrol, H.M. Sulphur fertilization improves nitrogen use efficiency in wheat by increasing nitrogen uptake. *Field Crops Res.* **2009**, *113*, 170–177. [CrossRef]
25. Yu, Z.; Juhasz, A.; Islam, S.; Diepeveen, D.; Shang, J.; Wang, P.; Ma, W. Impact of mid-season sulphur defciency on wheat nitrogen metabolism and biosynthesis of grain protein. *Sci. Rep.* **2018**, *8*, 2499. [CrossRef] [PubMed]
26. De Ruiterr, J.M.; Martinn, R.J. Management of nitrogen and sulphur fertilizer for improved bread wheat (*Triticum aestivum*) quality. *N. Z. J. Crop Hortic. Sci.* **2001**, *29*, 287–299. [CrossRef]
27. Aulakh, M.S.; Malhi, S.S. Interaction of Nitrogen with Other Nutrients and Water: Effects on Crop Yield and Quality, Nutrient Use Efficiency, Carbon Sequestration and Environmental Pollution. *Adv. Agron.* **2004**, *86*, 341–409. [CrossRef]
28. Brosnan, J.T.; Brosnan, M.E. The sulphur-containing amino acids: An overview. *J Nutr.* **2006**, *136* (Suppl. 6), 1636S–1640S. [CrossRef]
29. Győri, Z. Sulphur Content of Winter Wheat Grain in Long Term Field Experiments. *Commun. Soil Sci. Plant Anal.* **2005**, *36*, 373–382. [CrossRef]
30. *National Atmospheric Deposition Program*; NADP Program Office: Leading Ridge, PA, USA; Illinois State Water Survey, University of Illinois: Champaign, IL, USA, 2015.
31. David, M.B.; Gentry, L.E.; Mitchell, C.A. Riverine Response of Sulfate to Declining Atmospheric Sulphur Deposition in Agricultural Watersheds. *J. Environ. Qual.* **2016**, *45*, 1313–1319. [CrossRef]
32. Jukić, M.; Koceva Komlenić, D.; Mastanjević, K.; Mastanjević, K.; Lučan, M.; Popovici, C.; Nakov, G.; Lukinac, J. Influence of damage starch on the quality parameters of wheat dough and bread. *Ukr. Food J.* **2019**, *8*, 512–521. [CrossRef]
33. Putri, N.A.; Shalihah, I.M.; Widyasna, A.F.; Damayanti, R.P. A review of starch damage on physicochemical properties of flour. *Food Scien Tech J.* **2020**, *2*, 1. [CrossRef]
34. Wang, S.; Yu, J.; Xin, Q.; Wang, S.; Copeland, L. Effects of starch damage and yeast fermentation on acrylamide formation in bread. *Food Control* **2017**, *73 Pt B*, 230–236. [CrossRef]
35. Mulla, M.Z.; Bharadwaj, V.R.; Annapure, U.S.; Singhal, R.S. Effect of damaged starch on acrylamide formation in whole wheat flour based Indian traditional staples, *chapattis* and *pooris*. *Food Chem.* **2010**, *120*, 805–809. [CrossRef]
36. *ISO 7971-3:2019*; Cereals Determination of Bulk Density, Called Mass per Hectolitre Part 3: Routine Method. International Organization for Standardization: Geneva, Switzerland, 2019.
37. *ISO 20483:2013*; Cereals and Pulses. Determination of the Nitrogen Content and Calculation of the Crude Protein Content. Kjeldahl Method. International Organization for Standardization:: Geneva, Switzerland, 2013.
38. *ISO 21415-2:2015*; Wheat and Wheat Flour Gluten Content Part 2: Determination of Wet Gluten and Gluten Index by Mechanical Means. International Organization for Standardization: Geneva, Switzerland, 2015.
39. *ISO 5529:2007*; Wheat Determination of the Sedimentation Index Zeleny Test. International Organization for Standardization: Geneva, Switzerland, 2007.
40. *ISO 2171:2007*; Cereals, Pulses and By-Products Determination of Ash Yield by Incineration. International Organization for Standardization: Geneva, Switzerland, 2007.
41. Świder, O.; Roszko, M.Ł.; Wójcicki, M.; Szymczyk, K. Biogenic Amines and Free Amino Acids in Traditional Fermented Vegetables-Dietary Risk Evaluation. *J. Agric. Food Chem.* **2020**, *68*, 856–868. [CrossRef]
42. *ISO 17718:2013*; Wholemeal and Flour from Wheat (*Triticum aestivum* L.) Determination of Rheological Behaviour as a Function of Mixing and Temperature Increase. International Organization for Standardization: Geneva, Switzerland, 2013.
43. *ISO 17715:2013*; Flour from Wheat (*Triticum aestivum* L.) Amperometric Method for Starch Damage Measurement. International Organization for Standardization: Geneva, Switzerland, 2013.
44. Suchowilska, E.; Szafrańska, A.; Słowik, E.; Wiwart, M. Flour from *Triticum polonicum* L. as a potential ingredient in bread production. *Cereal Chem.* **2019**, *96*, 554–563. [CrossRef]

45. Roszko, M.Ł.; Szczepańska, M.; Szymczyk, K.; Rzepkowska, M. Dietary risk evaluation of acrylamide intake with bread in Poland, determined by two comparable cleanup procedures. *Food Addit. Contam. Part B Surveill.* **2020**, *13*, 1–9. [CrossRef]
46. ISO/IEC 17025. General Requirements for the Competence of Testing and Calibrarion Laboratories. International Organization for Standardization: Geneva, Switzerland, 2017.
47. Xie, Y.; Malunga, L.N.; Ames, N.P.; Waterer, J.; Khorshidi, A.S.; Scanlon, M.G. Effects of growing environment, genotype, and commercial fertilization levels on free asparagine concentration in Western Canadian wheat. *Cereal Chem.* **2021**, *98*, 89–99. [CrossRef]
48. Oddy, J.; Elmore, J.S.; Halford, N.G. Accounting for environmental variation in the free asparagine content of wheat grain. *J. Food Compos. Anal.* **2023**, *120*, 105333. [CrossRef]
49. Malunga, L.N.; Ames, N.; Khorshidi, A.S.; Thandapilly, S.J.; Yan, W.; Dyck, A.; Waterer, J.; Malcolmson, L.; Cuthbert, R.; Sopiwnyk, E.; et al. Association of asparagine concentration in wheat with cultivar, location, fertilizer, and their interaction. *Food Chem.* **2021**, *344*, 128630. [CrossRef]
50. Tafuri, A.; Zuccaro, M.; Ravaglia, S.; Pirona, R.; Masci, S.; Sestili, F.; Lafiandra, D.; Ceriotti, A.; Baldoni, E. Exploring variability of free asparagine content in the grain of bread wheat (*Triticum aestivum* L.) varieties cultivated in Italy to reduce acrylamide-forming potential. *Plants* **2023**, *2*, 1349. [CrossRef]
51. Klikocka, H.; Cybulska, M.; Barczak, B.; Narolski, B.; Szostak, B.; Kobiałka, A.; Nowak, A.; Wójcik, E. The effect of sulphur and nitrogen fertilization on grain yield and technological quality of spring wheat. *Plant Soil Environ.* **2016**, *62*, 230–236. [CrossRef]
52. Wilson, T.L.; Guttieri, M.J.; Nelson, N.O.; Fritz, A.; Tilley, M. Nitrogen and sulphur effects on hard winter wheat quality and asparagine concentration. *J. Cereal Sci.* **2020**, *93*, 102969. [CrossRef]
53. Muttucumaru, N.; Halford, N.G.; Elmore, J.S.; Dodson, A.T.; Parry, M.; Shewry, P.R.; Mottram, D.S. The formation of high levels of acrylamide during the processing of flour derived from sulfate-deprived wheat. *J. Agric. Food Chem.* **2006**, *54*, 8951–8955. [CrossRef]
54. Weber, E.A.; Koller, W.D.; Graeff, S.; Hermann, W.; Merkt, N.; Claupein, W. Impact of different nitrogen fertilizers and an additional sulfur supply on grain yield, quality, and the potential of acrylamide formation on winter wheat. *J. Plant Nutr. Soil Sci.* **2008**, *171*, 643–655. [CrossRef]
55. Ohm, J.-B.; Mergoum, M.; Simsek, S. Variation of free asparagine concentration and association with quality parameters for hard red spring wheat grown in North Dakota. *Cereal Chem.* **2017**, *94*, 712–716. [CrossRef]
56. Corol, D.I.; Ravel, C.; Rakszegi, M.; Charmet, G.; Bedo, Z.; Beale, M.H.; Shewry, P.R.; Ward, J.L. 1H-NMR screening for the high-throughput determination of genotype and environmental effects on the content of asparagine in wheat grain. *Plant Biotechnol. J.* **2016**, *14*, 128–139. [CrossRef]
57. Emebiri, L.C. Genetic variation and possible SNP markers for breeding wheat with low-grain asparagine, the major precursor for acrylamide formation in heat-processed products. *J. Sci. Food Agric.* **2014**, *94*, 1422–1429. [CrossRef]
58. Bonfil, D.J.; Posner, E.S. Can bread wheat quality be determined by gluten index? *J. Cereal Sci.* **2012**, *56*, 115–118. [CrossRef]
59. Dabija, A.; Codină, G.G.; Fradinho, P. Effect of yellow pea flour addition on wheat flour dough and bread quality. *Rom Biotechnol. Lett.* **2017**, *22*, 12888–12897.
60. Wiwart, M.; Szafrańska, A.; Suchowilska, E. Grain of Hybrids Between Spelt (*Triticum spelta* L.) and Bread Wheat (*Triticum aestivum* L.) as a New Raw Material for Breadmaking. *Pol. J. Food Nutr. Sci.* **2023**, *73*, 265–277. [CrossRef]
61. Litvyak, V. Size and morphological features of native starch granules of different botanical origin. *Ukr. Food J.* **2018**, *7*, 563–576. [CrossRef]
62. Liu, X.; Li, E.; Jiang, Y.; Tian, Y. Milling process of starch. In *Physical Modification of Starch*; Sui, Z., Kong, X., Eds.; Springer Nature: Singapore, 2018; pp. 155–174. [CrossRef]
63. Ma, S.; Wang, X.-X.; Zheng, X.-L.; Bian, K.; Bao, Q.-D. Effect of mechanically damaged starch from wheat flour on the quality of frozen dough and steamed bread. *Food Chem.* **2016**, *202*, 120–124. [CrossRef]
64. Codină, G.G.; Sarion, C.; Dabija, A. Effects of Dry Sourdough on Bread-Making Quality and Acrylamide Content. *Agronomy* **2021**, *11*, 1977. [CrossRef]
65. Sadd, P.A.; Hamlet, C.G.; Liang, L. Effectiveness of methods for reducing acrylamide in bakery products. *J. Agric. Food Chem.* **2008**, *56*, 6154–6161. [CrossRef]
66. Claeys, W.L.; De Vleeschouwer, K.; Hendrickx, M.E. Quantifying the formation of carcinogens during food processing: Acrylamide. *Trends Food Sci. Technol.* **2005**, *16*, 181–193. [CrossRef]
67. Shahsavani, S.; Gholami, A. Effect of Sulphur Fertilization on Breadmaking Quality of Three Winter Wheat Varieties. *Pak. J. Biol. Sci.* **2008**, *11*, 2134–2138. [CrossRef]
68. Li, X.; Tyl, C.E.; Kaiser, D.E.; Annor, G.A. Effect of sulphur fertilization rates on wheat (*Triticum aestivum* L.) functionality. *J. Cereal Sci.* **2019**, *87*, 292–300. [CrossRef]