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Effect of Climatic Conditions on Wheat Starch Granule Size Distribution, Gelatinization and Flour Pasting Properties

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Abstract: The aim of this study was to determine the effects of different varieties, year and location of growing, and their interactions, on wheat starch and flour properties, and to analyze the relationship between these attributes. The set of 92 wheat samples chosen to cover wide ranges in the parameters commonly used for the assessment of wheat flour and starch properties was reduced to a representative set of 27 samples. The obtained results showed that wheat variety and year significantly affected maximum viscosity (MV), alpha–amylase activity (AA), amylose content (AM), volume proportion of A-, B- and C-starch granules, as well as gelatinization temperatures, while the year and location by year interaction had a significant effect on the falling number (FN). In this work, a number of significant correlations were observed among analyzed starch and flour properties. AA was mostly influenced by the changes in packing of starch granules and granule size distribution, while gelatinization temperatures were affected by particle size distribution. Additionally, when testing the suitability of the parameters for the estimation of alpha–amylase activity, it was determined that Amylograph was more reliable in predicting alpha–amylase than FN because it provided a better description of the state of flour starch complex.

Keywords: starch structure; alpha–amylase activity; pasting; gelatinization; starch granule size distribution; wheat variety

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1. Introduction

Bread wheat (*Triticum aestivum* L.) is identified as one of the world's most important cereal crops and it is cultivated around the world in diverse environments which can affect productivity and quality. It is predicted that the demand for wheat will increase by 50% until 2050, so the identification of wheat varieties of acceptable yield and constant technological traits is of high importance for breeders [1]. It has long been recognized that wheat quality varies considerably as a result of the genotype, environment, which plays a major role in the expression of the genotype, as well as the interaction between genotype and environmental conditions [2,3]. Weather conditions during wheat development, especially temperature and precipitation, have a significant impact on the metabolic processes of the plant which results in inconsistent wheat yield and technological quality [4,5]. Even though climatic factors such as the temperature and water level during plant development cannot be controlled, physicochemical properties and the composition of wheat grains can be controlled to some extent by applying adequate agricultural practice or developing wheat varieties less susceptible to environmental conditions [6]. Therefore, a better understanding of the influence of growing conditions on grain quality is of great importance for breeders to develop new strategies toward developing new varieties more resistant to adverse climate factors, with a high yield potential and consistent quality to meet the market needs [7].

Starch is an important part of wheat endosperm, not only because starch presents the most abundant component of a wheat grain, comprising approximately 80% of the endosperm dry mass, but also because wheat starch has a notable role in determining the quality of the final products. Physicochemical properties and the structure of starch granules are influenced by many factors. The most important is the genetic factor, but environmental conditions during cultivation are also of high importance [8]. Environmental factors, such as the temperature and rainfall distribution play important roles in starch accumulation. High temperatures during grain–filling reduce starch content by affecting its biosynthesis and accumulation, and thus diminish the final weight of the wheat grain [9]. Exposure to high temperatures after flowering alters starch structure, granule size distribution, pasting and gelatinization properties, while the extent of these changes depends on the severity and developmental stage in which the stress occurs [10–12].

Although the impact of the variety and environmental factors has been studied for a long time, there are still some contradictory views on which factor has the greatest influence on wheat flour and starch structure and properties. These inconsistencies point out the need to investigate the effect of the genotype, the effect of the environment (climatic conditions and growing location) and their interactions, as well the properties of varieties developed under different growing conditions. A study on the effects of the genotype and growing conditions on the physicochemical properties of starch from wheat could provide an insight into the heritability of these attributes and valuable information for breeders to enhance the possibility of predicting and identifying wheat varieties of improved starch structure and properties. Additionally, it may also help to identify better wheat varieties that are less susceptible to varying environmental conditions and have consistent baking quality traits, because the seasonal fluctuations of product quality are not acceptable in breeding programs. Therefore, the main objective in the present work was to evaluate the effect of the variety and the growing conditions (year and location of growing), as well as their interactions on the indicators of alpha–amylase activity, amylose content, granule size distribution, and gelatinization properties of wheat starch, and to analyze the relationship between these quality traits. Our additional goal was to revise the suitability of the practical indicators, such as the Amylograph maximum viscosity and falling number for estimating the level of alpha–amylase activity, and test whether the resulting values of the proposed methods indicate the same technological quality status of wheat flour as has been generally accepted.

2. Materials and Methods

2.1. Wheat Samples

Ninety-two wheat grain samples (*Triticum aestivum*) of eight different varieties were grown at seven locations in the province Vojvodina in Serbia, of which thirty-nine of the wheat samples were grown in year 1, forty were grown in year 2 and thirteen were cropped in year 3. The average minimum and maximum temperatures during the grain filling period (from 1 May to 16 July) in year 1 did not differ significantly between the observed locations, while the average temperatures for the observed period were slightly above or similar to long-term average temperatures (Supplement Table S1). The number of days with daily temperatures above 30 °C ranged between 8 and 19, depending on the location. The average amount of precipitation for the observed period in year 1 was less than the long-term average, which indicates that the observed period in year 1 was characterized by a precipitation deficit. The average minimum and maximum temperatures during grain filling in year 2 did not differ significantly between the observed locations. However, the average temperatures in year 2 were markedly higher compared to year 1 and the average long-term temperatures. Moreover, year 2 had the greatest number of days with daily temperatures above 30 °C across all the observed locations during grain filling, ranging between 28 and 38 days. Therefore, year 2 could be regarded as a year with pronounced heat-stress and deficient precipitation. Year 3 was characterized by a lower average, minimum and maximum temperatures compared to year 1, while the amount of precipitation was higher compared to years 1 and 2, but corresponded to the average amount of precipitation during the long-term period. The number of days with daily temperatures above 30 °C ranged between 11 and 26, depending on the location. The wheat samples were harvested, cleaned and milled using a laboratory mill MLU 202 (Bühler, Uzwil, Switzerland), according to AACC Methods 26–31, to obtain wheat flour [13].

2.2. Alpha–Amylase Activity

Alpha–amylase activity of the wheat flour was determined using the Megazyme Ceralpha assay kit (Megazyme International Inc., Wicklow, Ireland). The results of alpha–amylase activities per g sample were expressed in Ceralpha Units (CU) on a dry basis.

2.3. Amylograph Peak Viscosity

The maximum Amylograph viscosity of wheat flour was determined using an Amylograph (Brabender Co., Duisburg, Germany), according to ICC Method 126/1 [14].

2.4. Falling Number

The determination of the Hagberg falling number values was performed according to ICC Methods 107/1 [14].

2.5. Amylose Content

Total amylose content was determined using the Megazyme amylose/amylopectin assay kit (Megazyme International Inc., Ireland).

2.6. Starch Isolation

The wheat flour samples (10 g) were transferred to Glutomatic (PerkinElmer, Upplands Väsby, Sweden) apparatus in order to separate starch from gluten, according to ICC Method 155 [15]. The obtained starch suspension was collected and filtered through a sieve with a pore diameter of 63 µm, and the material retained on the cloth was discarded. The filtrate was left to precipitate and then centrifuged for 10 min at 3000× *g* to sediment the crude starch. The supernatant was discarded, and the formed upper yellow-pigmented layer was removed carefully using a spatula. The remaining starch fraction was purified by re-suspending with 10 mL of distilled water and centrifugation. Four such purification cycles were conducted to obtain pure starch. The starch was finally collected and air-dried at room temperature for 48 h.

2.7. Granule Size Analysis

The size distribution of the isolated starch granules was measured using laser light scattering (Mastersizer 2000, Malvern Instruments, Malvern, UK) in the wet cell mode. Prior to analysis, each starch sample was re-suspended with distilled water and vortexed. The sample was transferred into the particle size analyzer dispersion tank until an optimal obscuration was attained, and the measurement was then conducted. The volumes of the starch granules were calculated on the assumption that all granules were spherical in shape.

2.8. Gelatinization Properties

Gelatinization characteristics of the isolated starches were determined using a differential scanning calorimeter (DSC) device (204 F1 Phoenix, Netzsch, Selb, Germany). Starch (3.5 mg) was loaded into a 25 µL capacity aluminum pan and distilled water was added to achieve a starch–water suspension containing 70% water. The sample pans were then hermetically sealed and allowed to stand for 2 h at room temperature before heating in the DSC device. An empty pan was used as a reference. The temperature regime involved heating from 20 to 110 °C at a scanning rate of 10 °C/min. The gelatinization onset temperature (To), peak temperature (Tp), end temperature (Te), and enthalpy of gelatinization (ΔH) were determined. The enthalpies were calculated on the starch dry weight basis.

2.9. Statistical Analysis

The collected data are presented using the mean values in Supplement Table S2. The experimental data used for the analysis are derived using the full factorial (3 level, 3 parameter) design involving the 27 samples. Principal component analysis (PCA) was applied effectively to classify and separate the different samples. The following second-order polynomial (SOP) model was fitted to the exploratory data. Eleven models of the accompanying structure are produced to relate 11 responses (Y) and three factors (X), for each of the growing conditions:

$$Y_k = \beta_{k0} + \sum_{i=1}^{3} \beta_{ki} \cdot X_i + \sum_{i=1}^{3} \beta_{kii} \cdot X_i^2 + \sum_{i=1, j=i+1}^{3} \beta_{kij} \cdot X_1 \cdot X_2, k = 1 - 11 \quad (1)$$

where: β_{k0}, β_{ki}, β_{kii}, β_{k12} are constant regression coefficients; Y_k, either the MV, FN, AA, AM, C, B, A, To, Tp, Te or ΔH; while X_1 is wheat variety (Var); X_2 is the location of growth (Loc); and X_3 is the year of growth. The assessment of PCA and ANOVA analysis of the acquired outcomes was performed using the Statistica software, version 12 (Statistica, StatSoft Inc., 2012, Tulsa, OK, USA)®.

2.10. The Accuracy of the Models

The numerical verification of the developed models was tested using the coefficient of determination (r^2), reduced chi-square (χ^2), mean bias error (MBE), root mean square error (RMSE), mean percentage error (MPE) and Akaike's Information Criterion (AIC). These commonly used parameters can be calculated as follows:

$$\chi^2 = \frac{\sum_{i=1}^{N} \left(x_{\exp,i} - x_{pre,i}\right)^2}{N - n} \quad (2)$$

$$\text{RMSE} = \left[\frac{1}{N} \cdot \sum_{i=1}^{N} \left(x_{pre,i} - x_{\exp,i}\right)^2\right]^{1/2} \quad (3)$$

$$\text{MBE} = \frac{1}{N} \cdot \sum_{i=1}^{N} \left(x_{pre,i} - x_{\exp,i}\right) \quad (4)$$

$$\text{MPE} = \frac{100}{N} \cdot \sum_{i=1}^{N} \left(\frac{\left|x_{pre,i} - x_{\exp,i}\right|}{x_{\exp,i}}\right) \quad (5)$$

$$\text{AIC} = N \cdot \log \sum_{i=1}^{N} \frac{\left(x_{pre,i} - x_{\exp,i}\right)^2}{N} + 2 \cdot n \quad (6)$$

where $x_{\exp,i}$ stands for the experimental values and $x_{pre,i}$ is the predicted value calculated using the model for these measurements. N and n are the number of observations and constants, respectively.

3. Results

The set of 92 wheat samples was chosen to cover wide ranges in the parameters commonly used for the assessment of wheat flour and starch properties. Correlation analysis was conducted to point out the relationship between the observed parameters. The maximum viscosity (MV) and falling number (FN) demonstrated a similar negative correlation with alpha–amylase activity (AA), r = −0.587 and −0.620, respectively. MV and FN, which are commonly used as the predictors of wheat flour quality, were positively correlated to each other (r = 0.650). Moreover, an inverse relationship between amylose content (AM) and MV (r = −0.484) was observed. In order to determine the contribution of the year of harvest, growing location and variety to the starch and flour quality indexes, the number of wheat samples was reduced and the relations between the same parameters as in the initial set of wheat samples were tested. For that purpose, three wheat varieties (Apač, Pobeda and Zvezdana) grown across three locations (Bačka Topola, BT, (45°49′ N, 19°39′ E), Sremska Mitrovica, SM, (44°58′ N, 19°38′ E) and Sombor, SO, (44°47′ N, 19°05′ E)) during three consecutive years (year 1, year 2 and year 3) were selected for further analysis. Since similar correlation coefficients between PV and FN with AA (−0.610 and −0.332, respectively) were obtained as in the case of the whole set sample (AA/PV = −0.585; AA/FN = −0.620), it was considered that the reduced set sample could represent the initial one. By reducing the number of wheat samples, it was possible to investigate a greater number of parameters to confirm this hypothesis.

3.1. Principal Component Analysis

Principal component analysis is a mathematical procedure used as a central tool in exploratory data analysis. PCA applied to the given dataset (Supplement Table S2) has shown a differentiation between the samples according to the observed parameters (Figure 1). Wheat samples are separated by wheat variety (red color for Apač variety, blue color for Pobeda variety and green color for Zvezdana), location (BT, SM and SO) and production year (year 1, year 2, and year 3). The first two principal components, accounting for 62.76% of the total variability for the wheat samples grown under different conditions, can be considered sufficient for data representation.

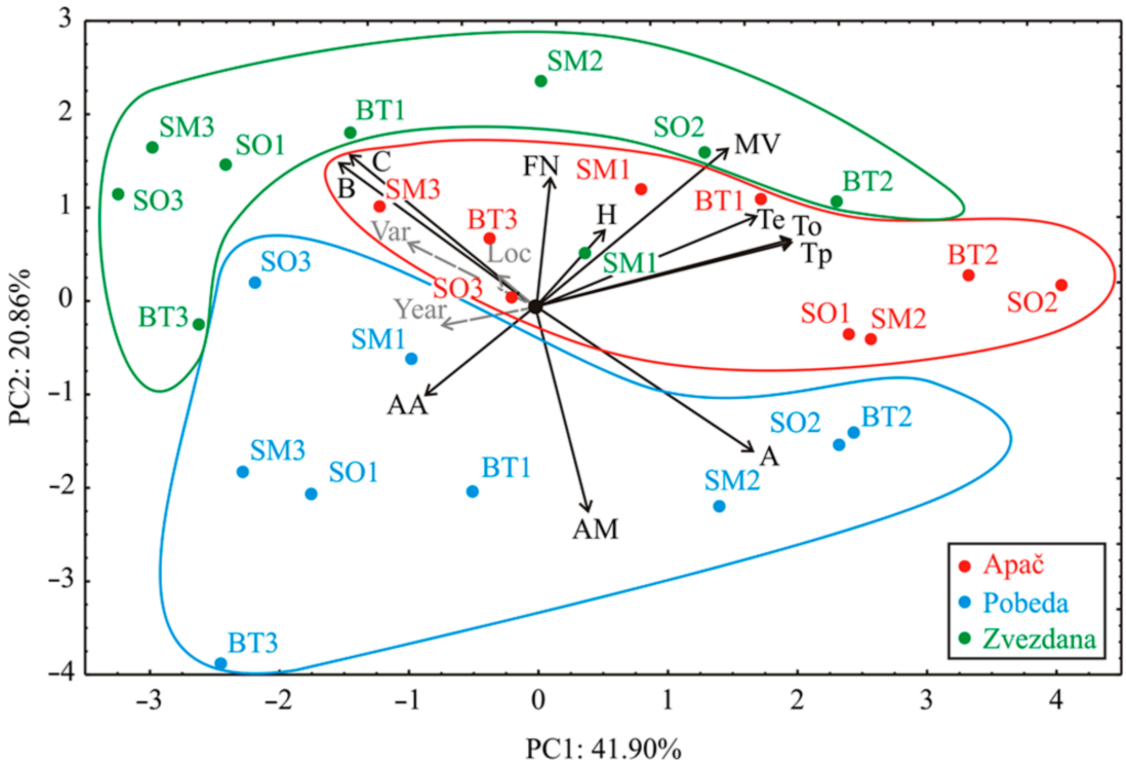

Figure 1. Biplot graphic of three wheat varieties, grown at three different locations in three years.

Considering the map of the PCA performed on the data, the variables contributing negatively according to the first principal component are B-granule (which explained 11.0% of the total variance, based on the correlations) and C-granule (10.0%). The variables which showed a positive influence toward the first principal component are MV (contributed 10.2% of total variance, based on correlations), A-granule (13.4%), To (17.8%), Tp (18.2%) and Te (13.7%). The variables AM (24.2% of the total variance) and A-granule (12.4%) showed a negative impact on the second principle component, while the variables B-granule (11.2%), C-granule (12.5%) and MV (13.8%) showed a positive influence on the second principal component calculation. The PCA graphic showed quite good discrimination between the samples of the three different wheat varieties grown at three different locations in three years (Figure 1). The Pobeda, Zvezdana and Apač samples grown in Bačka Topola, Sombor and Sremska Mitrovica in year 3, and are located in the left part of the PCA biplot graphic. Most of the wheat samples grown in year 1 are located in the central region of the PCA diagram, while the samples grown in year 2 are located in the right part of the PCA graph. The Zvezdana wheat samples are positioned on the upper part of the graph, the Apač samples are positioned at the central part and the Pobeda samples are located at the lower part of the graph. According to PCA analysis, the first principal coordinate describes the difference between the samples, based on variances in the growing conditions observed at different years, while the second principal component shows the variations between the samples caused by the variety of the wheat. According to correlation analysis (Supplement Table S3), AA was negatively correlated to MV ($p < 0.01$) (Figure 1). Significant positive correlations were observed between MV and gelatinization temperatures, To, Tp and Te ($p < 0.01$). AM was negatively correlated with C-granule and B-granule, while being positively related to A-granule ($p < 0.05$). The B-granule and C-granule were negatively correlated to To and Tp ($p < 0.05$), while the A-granule positively correlated to To and Tp ($p < 0.01$).

3.2. Second-Order Polynomial (SOP) Model

The ANOVA analysis of the SOP model showed significant effects of independent variables on the responses, and which of the responses (MV, FN, AA, AM, C-granule, B-granule, A-granule, To, Tp, Te, and H) were significantly affected by the independent variable combinations (Table 1).

Table 1. The ANOVA calculation of the SOP model for predicting the flour properties according to variety and growing conditions.

	df	MV	FN	AA	AM	C-Granule	B-Granule	A-Granule	To	Tp	Te	ΔH
Var	1	85,598	6035 ***	0.004 *	2219 **	9720 **	31,454 *	75,962 *	7113 *	5416 *	5187 *	3426 *
Var^2	1	2,120,185 *	4997***	0.001	20,106 *	4063	12,055 **	20,531 ***	3708 **	3325 *	3708 *	1575 **
Loc	1	5032	23	0.000	0.461	2792	0.500	2722	0.285	0.067	0.045	1469 **
Loc^2	1	17,478	5448 ***	0.000	0.011	4078	0.003	4848	0.072	0.225	0.762	0.363
Y	1	279,371 **	24,674 *	0.004 *	0.000	3655	2217	10,082	3361 **	3861 *	0.918	1060 ***
Y^2	1	1,826,621 *	15,726 *	0.010 *	1962 **	26,280 *	28,749 *	121,117 *	16,118 *	12,336 *	5139 *	0.127
Var × Loc	1	24,928	624	0.001	0.148	0.769	7610 ***	13,425	0.062	0.012	0.075	0.043
Var × Y	1	14,811	1071.4	0.000	0.218	2148	0.523	0.474	0.627	0.001	0.079	0.091
Loc × Y	1	158,598 **	8168.8 **	0.000	0.817	2759	0.061	4605	0.179	0.006	0.036	0.281
Error	17	593,039.3	23,721.8	0.007	6030	24,227	42,633	82,131	9452	5797	6586	5378

* Significant at $p < 0.01$ level. ** Significant at $p < 0.05$. *** Significant at $p < 0.10$. Error terms have been found to be statistically insignificant. df—degrees of freedom; Y—year; Var—wheat variety; Loc—location of wheat growing.

MV was mostly affected by the quadratic terms of Var and Y in the SOP model ($p < 0.01$), and also by the linear term of Y and Loc × Y interaction ($p < 0.05$). Among the tested varieties, Apač exhibited the highest values of MV (740–2000 AU), while Pobeda showed the lowest values of MV at all locations in three years (160–1350 AU) (Supplement Table S2). Concerning the harvest year, the wheat samples from year 2 had the highest average values of MV (1499 AU), while the wheat samples from year 3 exhibited the lowest average values of MV (734 AU) (Supplement Table S2). The linear and the quadratic term of Y were most influential in the SOP model for the FN calculation ($p < 0.01$), while the Loc × Y interaction was also influential ($p < 0.05$). The highest values of FN had wheat samples grown in year 2 ranging between 425 and 528 s, while the lowest values had samples from year 1 ranging between 313 and 421 s (Supplement Table S2). AA was mostly affected by the quadratic term of Y and the linear terms of Var and Y ($p < 0.01$). The lowest average level of AA was found in samples from year 2, while a slight increase in AA was observed in all wheat samples grown in year 3. Among the tested varieties, Zvezdana had the lowest level of AA, while Apač exhibited the highest level of AA (Supplement Table S2).

The quadratic term of Var was most influential for AM evaluation ($p < 0.01$), while the linear terms of Var and the quadratic term of Y were also significant ($p < 0.05$). Pobeda demonstrated the highest average amylose content compared to the other tested varieties. Regarding the testing years, the highest AM content demonstrated wheat flours grown in year 2 (Supplement Table S2). The quadratic term of Y was most influential for C-, B- and A-granule evaluation ($p < 0.01$). The linear term of Var was influential for the C-granule calculation ($p < 0.05$) and also for A- and B-granule evaluations ($p < 0.01$). The quadratic term of Var was influential for the B-granule calculation ($p < 0.01$). The volume proportion of the A-granule was the highest in samples from year 2, while the volume proportion of the B- and C-granule was the lowest in the same year. The volume proportion of the B- and C-granule was the highest in year 3, while the volume proportion of the A-granule was the lowest in the same year (Supplement Table S2). The quadratic term of Y was the most influential for To, Tp and Te calculation ($p < 0.01$). The quadratic term and linear term of Var were also influential for To, Tp and Te calculation ($p < 0.01$). The linear term of Var was most influential for the H calculation ($p < 0.01$), while the quadratic term of Var and the linear term of Loc were also influential for the ΔH calculation ($p < 0.05$). Starch from wheat grown in year 2 exhibited the highest To, Tp and Te, while starches from wheat grown in year 3 exhibited the lowest To, Tp and Te. Concerning the genotype, the Apač variety showed the highest gelatinization temperatures compared starches of Pobeda and Zvezdana wheat varieties (Supplement Table S2). Var × Loc, Var × Y, Loc × Y interactions were not significant for starch thermal properties. Generally, for most parameters, the Var × Loc and Var × Y interactions were not significant.

The quality of the model fit was tested in Table 2, with the higher r^2 values and the lower χ^2, MBE, RMSE and MPE values showing the better fit to the experimental results [16]. Residual analysis of the developed model was also performed. Skewness measures the deviation of the distribution from normal symmetry. If the skewness is clearly different from zero, then the distribution is asymmetrical, while normal distributions are perfectly symmetrical. Kurtosis measures the "peakedness" of a distribution. If the Kurtosis is clearly different than zero, then the distribution is either flatter or more peaked than normal; the Kurtosis of the normal distribution is zero. The average and the standard deviation (SD) were also analyzed and are shown in Table 2. The results showed a good approximation to a normal distribution, around zero, with a probability of 95% (2 × SD), which means that the developed SOP model had a good generalization ability to predict the characteristics of flour and starch structure of wheat samples grown at different growing conditions for the range of observed experimental data.

Table 2. The "goodness of fit" tests for the developed mathematical SOP models.

	χ^2	RMSE	MBE	MPE	AIC	r^2	Skew	Kurt	Mean	StDev	Var
MV	37,065	148.20	0.0	12.69	183.721	0.897	0.15	−0.03	0.0	151	22,809
FN	1483	29.64	0.0	6.12	134.263	0.737	−0.56	−0.09	0.0	30.21	912.4
AA	0.00	0.02	0.0	10.38	41.872	0.803	1.47	3.46	0.0	0.02	0.00
AM	0.38	0.47	0.0	1.43	42.178	0.825	0.27	−0.42	0.0	0.48	0.23
C−granule	1.51	0.95	0.0	8.93	51.256	0.646	−0.04	−0.59	0.0	0.97	0.93
B-granule	2.66	1.26	0.0	5.80	55.839	0.658	0.10	0.10	0.0	1.28	1.64
A-granule	5.13	1.74	0.0	1.78	68.920	0.734	−0.05	0.93	0.0	1.78	3.16
To	0.59	0.59	0.0	0.88	44.464	0.800	0.13	−0.63	0.0	0.60	0.36
Tp	0.36	0.46	0.0	0.65	41.861	0.833	−0.01	−0.82	0.0	0.47	0.22
Te	0.41	0.49	0.0	0.63	36.474	0.716	0.31	−0.82	0.0	0.50	0.25
ΔH	0.34	0.45	0.0	4.14	29.004	0.577	−0.36	−0.69	0.0	0.45	0.21

χ^2, the reduced chi-square; RMSE, the root mean square error; MBE, the mean bias error; MPE, the mean percentage error; AIC, Akaike information criterion; r^2, coefficient of determination; Skew, skewness; Kurt, kurtosis; StDev, standard deviation of residuals; Var, variance of residuals.

4. Discussion

Amylose content was highly affected by the genotype and year of growing. High temperature has been regarded as the primary stressor which affects an increase in amylose content in cereal grains [9,10,12,17–19]. High temperature stress at early grain filling stages in year 2 might affect the starch formation and consequently cause a slight increase in amylose concentration in wheat grains. It has been reported that heat shock during the first days after anthesis has influences on the increase in amylose content [10], while others found that the rise in amylose content was the result of elevated temperatures during terminal growth [17]. The content of amylose increased with increasing accumulation of temperatures above 30 °C [20]. The impact of the environment and cultivar on amylose content in starch was reported by others [21–24]. The distribution of starch granule size was controlled both by genotype and environmental conditions. Climatic conditions such as changes in temperatures have a strong impact on starch granule dimensions and endosperm content [21]. Smaller starch granules tended to be more susceptible to environmental stresses and varied easily than large counterparts. It is known that the formation of large and small starch granules in endosperm occurs during different grain development stages. The synthesis of large granules starts after anthesis and may continue to enlarge throughout the grain filling until they achieve a maximum diameter at physiological maturity. The synthesis of small granules begins late in grain filling and continues growing until 21 days post-anthesis [25]. In that respect, it might be assumed that exposure of wheat plant to elevated temperatures in year 2 caused a decrease in the activity of enzymes that take part in the biosynthesis of starch, suppressed formation of smaller granules and the available substrate could be diverted toward pre-existing large granules [26]. Consequently, the volume proportion of small granules decreased while those of large starch granules increased. The volume proportion of large granules is highly affected when heat stress occurs around the anthesis period [10]. The influence of high temperatures during cereal kernel development on starch granule distribution was reported elsewhere [9,10,12,17,27]. In contrast to our results, Hurkman et al. [9] found that the volume proportion of large granules decreased, while that of smaller granules increased significantly in response to high temperatures. Due to the increase in percent volume of the A−granule in year 2, the content of amylose might have also increased. It could also be assumed that varietal properties of wheat affected the starch granule size distribution, which in turn resulted in changes in the amylose content. A decrease in the volume proportion of large granules and increase in the volume proportion of small granules was observed in wheat starches grown in year 3, which was characterized by lower mean temperatures and higher levels of precipitation than growing years 1 and 2. Dai et al. [28] also reported an increase in the volume proportion of small starch granules and reduction in large starch granules in rainfed treatment. It was explained that the deficit of soil water during the period when small starch granules synthesized rapidly impacted the starch accumulation rate and the activities of the enzymes related to starch biosynthesis. As a result, the percentage volume of smaller starch granules is increased under the rainfed conditions [28]. The effect of the year of growing on gelatinization temperatures was the result of differences in climatic conditions during crucial grain development. Elevated temperatures in year 2 increased the gelatinization temperatures in wheat starches. Higher gelatinization temperatures of starches are related to a higher degree of crystallinity, which contributes to structural stability, thus making the starch granule more resistant to gelatinization [23]. Higher gelatinization temperatures of starches indicate that more energy is required to initiate starch gelatinization [6]. This indicates that the crystalline structure of wheat starches grown at a higher temperature in year 2 was more ordered and hence more resistant to gelatinization than those grown in years 1 and 3. The starch gelatinization temperatures are also affected by particle size distribution, whereby large granules usually have higher gelatinization temperatures than smaller granules. The direct relationship between MV and To, Tp and Te might be the consequence of amylopectin chain length distribution rather than amylose content or starch granule size distribution. It is reported that the higher proportion of longer chains in amylopectin contributes to the higher gelatinization temperature, since these chains form long double helices which require a higher temperature to dissociate [29]. Franco et al. found that wheat starches that had amylopectins of longer branch chains displayed a larger peak viscosity [30].

AA was negatively related to MV. The maximum viscosity determined by an Amylograph device is an indicator of the alpha–amylase activity in a flour–water suspension. It is generally accepted that low MV values correspond to a less viscous suspension, with a significant degree of starch depolymerisation caused by the presence of alpha–amylase [31]. Regardless of the significant correlation between these two parameters being established, changes in the alpha–amylase activity were not notable to the extent as those at the maximum viscosity. AA values were in the range of 0.06–0.22 CU/g, while the MV ranged from 160 to 2000 AU. It is well known that alpha–amylase has notable influence on wheat flour processing and bread production, and hence a certain level of this enzyme is desirable to achieve the optimal quality properties of baked products, while excess alpha–amylase has a detrimental effect on baked bread. Although AA was higher in year 3 than in years 1 and 2, the level of alpha–amylase activity was not sufficiently elevated to cause changes in the wheat flour and deteriorate the wheat processing quality, which was confirmed in a preliminary study [32]. Moreover, the level of alpha–amylase activity remained within the optimal level for bread-making, which was confirmed by the baking test showing no impairment in the end-use quality characteristics [33]. During the last decades it was considered that wheat flours with MV values of approximately 450–650 AU were optimal for bread-making [34]. However, a great number of tested wheat flour samples demonstrated very high MV values (>1000 AU), which is believed to be beyond optimal for wheat processing. Since intense climate changes in the recent decades have resulted in the development of wheat varieties which are more adapted to adverse climatic variations during the growing season, the redefining the optimal values of the processing quality indexes and the relationship between wheat components and end-use quality has become necessary [35]. In the present study, variations in the MV were not solely the result of changes in the level of alpha–amylase activity, but were also governed by the influence of variety, year and interaction between the location and year. Heritability observed for the MV indicated that this wheat flour trait is suitable for further improvement by breeding. This stands for the Pobeda variety, which expressed the lowest values of this parameter regardless of the growing location and year of harvest. Rhymer et al. [36] reported that variation in starch-pasting properties between environments was related to the growing year, indicating a predominant climatic factor. As suggested by Tomić et al. [33], variations in the MV might be the result of changes in the packing of starch granules and granule size distribution. A low inverse relationship between AA and FN was noticed, in agreement with the others [37]. FN is a general index in the cereal grain trade, used worldwide for an indirect assessment of the alpha–amylase activity in a grain. It reflects the biochemical and physiological processes taking places in wheat grain. The optimal FN range specified for bread-making varies from country to country, but it is believed that flours with an FN which lies within the range of 250–350 s should produce bread loaves with a higher specific volume [38]. Despite the fact that both FN and MV were used for the indirect assessment of the alpha–amylase activity by measuring starch-pasting properties, differences in correlation coefficients with AA were obtained. The correlation between the alpha–amylase activity and FN was poorer than expected. It should be emphasized that FN is also affected by other grain features, i.e., starch and protein content, which contribute to the flour-pasting property and viscosity, and are the result of interactions between the variety, environment and management practices [38]. As Derkx and Mares [39] reported, in the case when different wheat varieties are grown under diverse growing conditions, these interactions are more pronounced, and thus a strong relationship between the FN and alpha–amylase activity would most likely be lacking. FN was predominantly affected by the year, while the interaction between the growing location and year also had a significant influence on the FN, which is contradictory to the most of literature data in which the influence of the variety and environment are the dominant factors influencing the FN. Barnard and Smith (2012) reported that FN can notably vary from year to year and across various environments [40]. In a study of Rozbicki et al. [35], the most influential factor affecting FN was the interaction between the year and location. Although the level of alpha–amylase activity was not significantly increased, it seems that the Amylograph method provided a better description of the state of a flour starch complex and therefore was more reliable in prediction of the level of alpha–amylase activity and bread-making potential of wheat flour. On the other hand, FN cannot be considered a reliable and unequivocal predictor of the alpha–amylase activity of wheat cultivars. The absence of significant interactions between variety and location, and between variety and year for most of the measured wheat starch and flour traits indicated consistent genotypic trends regardless of variations in the environmental conditions.

5. Conclusions

The presented data showed that wheat variety and year of growing had a significant influence on most of the measured starch and wheat flour traits: MV, AA, AM, volume proportion of A-, B- and C-starch granules and gelatinization temperatures (To, Tp and Te). FN was significantly affected by year, as well by the interaction between the location and year. The location-by-year interaction was also influential for MV. Although a negative correlation was found between AA and MV, variations in MV were not solely the consequence of changes in the level of AA, but likely the result of changes in the packing of the starch granules and granule size distribution. The direct relationship between AM and A-granules and the inverse relation between AM and B- and C-granules confirmed that large starch granules are richer in the content of amylose, while small granules possess a relatively low amylose content. Gelatinization temperatures were influenced by particle size distribution, whereby large granules had higher gelatinization temperatures than their smaller counterparts. Additionally, we determined that the Amylograph method was a more reliable indicator of alpha amylolitic activity in wheat flour, and describes the state of the starch compound better than the FN.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13061551/s1, Table S1: Meteorological data of the examined locations in three years; Table S2: Experimental results of the flour properties according to the variety and growing conditions; Table S3: Correlation table of the flour properties.

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