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THE CONTENT AND QUALITY OF STARCH IN DIFFERENT WHEAT VARIETIES GROWING IN EXPERIMENTAL CONDITIONS

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ABSTRACT

Wheat is the most important bread crop and the primary food source of human nutrition in our climate conditions. Its dominant constituent, i.e., starch typically comprises 20-30 % amylose and 80-70 % amylopectin. Due to positive impacts on human health, starches with elevated contents of amylose are of interest to scientific communities and plant breeders. Based on the modern trends of nutrition, the current study was aimed to evaluate the quality of various wheat grains in terms of starch content and its composition. For this purpose, grains of total 10 wheat varieties (Vígľašská tvrdá, Mironovská, Viglanka, Puqa, Scorpion, Vígľašská červená, Bonavita, Zirnita, Durgalova and Badurka) of three species (common, colored, emmer) obtained from the Research Institute of Plant Production in Piešťany (Slovak Republic) were analysed. The content of starch was determined by Ewers polarimetric method and the amylose/amylopectin ratio was assessed using a commercial kit (Megazyme Co., Wicklow, Ireland). We observed statistically significant differences in the starch content between common wheat varieties, implying higher amount of starch in modern varieties, and between color wheat varieties. Significantly different amylose contents were found only among colored varieties which were negatively correlated to the amount of starch. Taken together, according to our results it can be concluded that the amount and nutritional composition of wheat grain starch is influenced by the variety of wheat.

Keywords: *Triticum* sp., polysaccharide, amylose, amylopectin

INTRODUCTION

From economic and agronomic point of view, cereals represent the most important group of crops in the structure of plant production. Due to the most abundant sources of proteins, energy, and other valuable components with biological activities to humans, they are widely consumed around the world **(Čurná and Lacko-Bartošová, 2017)**.
Among them, wheat is the prevailing staple cereal in temperate regions in which it provides from 20 % to 50 % of the total calorie intake (**Shewry and Hey, 2015a**). Also, it is an important source of health-promoting compounds, such as starch, proteins, vitamins (mainly B vitamins), dietary fiber, and various phytochemicals **(Shewry and Hey, 2015b)** significantly influencing the color of grains **(Lachman *et al.*, 2017)**. Although colored wheat has attracted the attention of many breeders and baking industry, the lines have displayed low yield due to linkage drag **(Martinek *et al.*, 2014)**. Hence, colored wheat lines with satisfactory yield potential and regional adaptation have been still developing **(Garg *et al.*, 2016)**. The hexaploid "bread" or "common" wheat (*Triticum aestivum* L.) is the major wheat species accounting for approximately 95 % of world wheat production **(Peng *et al.*, 2011)**. Emmer wheat (*Triticum dicoccon* Schrank), a tetraploid species with hulled grain has been largely cultivated during seven millenia in the Middle-East, Central and West Asia and Europe **(Zaharieva *et al.*, 2010)**. The wheat is rich in resistant starch, fibre, minerals, antioxidant compounds, carotenoids, and poor in fats. It has been recognized as a very healthy cereal and is recommended in the diet for people suffering from allergies, colitis, diabetes and high blood cholesterol **(Čurná and Lacko-Bartošová, 2017)**. Additionally, it serves as a progenitor of cultivated wheat and it is considered as a potential source of genes for important agronomical traits including grain quality **(Taneva *et al.*, 2015)**.
Starch, as the main component of wheat comprising about 60–75 % of grain and 70–80 % of flour, has a number of food and industrial applications **(Shevkani *et al.*, 2017)** It is the major energy storage carbohydrate in higher plants and the second most abundant biopolymer on earth (after cellulose; **Geigenberg, 2011**). Structurally, starch is composed of a mixture of two major glucan polymers, i.e., amylose and amylopectin **(Jiang *et al.*, 2011)** which represent approximately 98–99 % of the dry weight **(Tester *et al.*, 2004)**. Minor components of starch include endogenous lipids and proteins, intermediate constituents, and phosphate monoester **(Ai and Jane, 2018)**. In linear amylose, the glucose units are joined through *α*-(1,4)-glycosidic linkages while amylopectin mainly consists of long chains of *α*-(1,4)-linked D-glucopyranosyl units with occasional branching *α*-(1,6)-linkages forming its branched structure **(Zhang *et al.*, 2017)**. According to **Tester *et al.* (2004)**, the ratio of the two starch polysaccharides depends on the botanical origin of the starch. In general, starches from most food crops contain approximately 70–80 % amylopectin and roughly 20–30 % amylose **(Hofman *et al.*, 2016)**. The 'waxy' starches contain less than 15 % amylose, and its content in 'high' (amylo-) amylose starches is greater than 40 % **(Tester *et al.*, 2004)**.
Due to the ability to affect and modify the texture, quality and stability of starch-based food products, starches with different amylose and amylopectin content have attracted more and more attention of scientific communities **(Schirmer *et al.*, 2013)**. A comprehensive understanding of chemical compositions, structures, and functional properties of starch is an essential step for proper utilization of the valuable ingredient to develop high-quality and nutrient-rich foods **(Ai and Jane, 2018)**.
Therefore, the aim of our study was to determine the content of starch and its amylose/amylopectin ratios in different common, colored and emmer wheat varieties growing in experimental conditions.

MATERIAL AND METHODS

Plant material

The plant material used in this study included 10 wheat varieties obtained from the Research Institute of Plant Production in Piešťany, Slovak Republic. Among them, four common varieties: Vígľašská tvrdá (old country variety), Mironovská (old country variety), Viglanka (registration year: 2010), Puqa (2015); three colored varieties: blue aleurone wheat Scorpion (2011), red wheat Vígľašská červená (old country variety), Bonavita (2011) with yellow endosperm, and emmer wheats Zirnita (2017), Durgalova (2017) and Badurka (2019) have been evaluated. Field experiments were established by randomized complete block design in two replications during the 2015/2016 vegetative season. Experimental unit consisted of a 1 m2 plot. In Table 1, month precipitation and average temperatures during the growing season of the different wheat varieties are presented.

Table 1 Average precipitation and temperature during the growing season of the wheat varieties from experimental field.

Month	10	11	12	1	2	3	4	5	6	7	Average
Precipitations [mm]	57.3	27.6	14.8	31.0	57.2	12.0	44.8	17.6	54.4	124.3	44.1
Temperature [°C]	9.6	6.3	2.7	-1.2	5.3	6.2	11.0	15.8	20.1	21.5	9.73

Determination of starch content

The starch content was determined by Ewers polarimetric method (STN EN ISO 10520; 1997) which is based on the partial acid hydrolysis of starch followed by measurement of the optical rotation of the resulting solution. According to this method (STN EN ISO 10520; 1997), a portion of 5 g of a homogenised sample was weighed in a 100 ml Kohlrausch volumetric flask and its content was mixed with 25 mL of 1.124 % HCl solution. After addition of another 25 mL of 1.124 % HCl solution, the suspension was heated on a boiling water bath for 15 min (after 3 min the content of a volumetric flask was mixed to avoid coagulation). Once the hydrolysis had been finished, 20 mL of 1.124 % HCl solution was added. After fast cooling (using a stream of flowing water), clarification was performed using 5 mL of Carrez I (30 % $ZnSO_4$ solution) and 5 mL of Carrez II (15 % $K_4[Fe(CN)_6]$ solution) solutions. Finally, a volumetric flask was filled up by distilled water to the final volume of 100 mL, its content was properly mixed, and filtered using a filtration funnel. The obtained filtrate was then transferred to a polarisation tube (2 dm) and measured using a polarimeter (Automatic digital polarimeter P 3001 RS; Krűss; A. Krűss Optronic Germany). The optical rotations of all samples were measured at 20 °C by using a sample cell of 200 mm optical path length.

Determination of amylose/amylopectin ratio

For the determination of the amylose/amylopectin ratio a commercial kit (Megazyme Co., Wicklow, Ireland) was used in accordance with the producer's recommendations and Spekol 11 (Carl Zeiss, Jena, Federal Republic of Germany) was used for measurement. This enzymatic method is based on the specific formation and precipitation of AP–Concanavalin A (Con A) complexes, after a pre-treatment of the sample to solubilize resistant starch and to remove lipids and free D-glucose. The test kit includes relative standard deviations of <5 % for pure starches.

Statistic analysis

Statistical analysis was performed using Statgraphics X64 software by using multifactor analysis of variance (ANOVA). Significant differences were evaluated by Fisher's least significant difference (LSD) test at $P < 0.05$. Data were analyzed statistically by Pearson correlations at the 0.95 probability level.

RESULTS AND DISCUSSION

Determination of starch and amylose contents in bread (common) wheat varieties

Between common varieties of wheat, significant differences ($F= 23.32$; $P < 0.0007$) in the total content of starch were detected. Statistical evaluation is given in Table 2. The total starch content in analysed wheat varieties ranged from 53.61 % to 66.11 %, with an average around 59.86 % (Figure 1). The highest content of starch had Viglanka and, conversely, its lowest content was determined in the variety Vígľašská tvrdá. From our results it is evident that modern common varieties of wheat which were bred after the year 2010 had a higher average starch content (64.12 %) than the old ones bred in the 20th century (57.3 %) suggesting a relationship between starch content and the year of wheat registration. Indeed, prolonged and intensive breeding of wheat has produced modern high-yielding wheat cultivars with different compositions (particularly in their content and composition of starch and other components) as compared to traditional types of wheat **(Morris and Sands, 2006; Shewry *et al.*, 2015a)**.

Table 2 ANOVA for multiple regression of starch content in common wheat varieties

Sources of variance	Sum of squares	Df	Mean squares	F-value	P-value
Within groups	133,582	5	26,7164	23,32	0,0007
Between groups	6,87415	6	1,14569		
Total	140,456	11			

According to **Rakszegi *et al.* (2008)**, most of the older varieties have high protein contents and low starch contents which is consistent with lower yields. Conversely, most of the modern varieties had hard kernel texture and lower protein content in the sense of the modern emphasis on breeding high-yielding breadmaking varieties which are associated with increased accumulation of starch and hard endosperm texture. However, higher starch content can be expected to result in reduced proportions of other grain components, mainly health-promoting mineral micronutrients **(Shewry *et al.*, 2016, Shewry *et al.*, 2017)**. As compared to old common wheats, significant lower micronutrient content, especially zinc and iron concentrations in modern cultivars have been demonstrated in the research by **Fan et al. (2008)**.

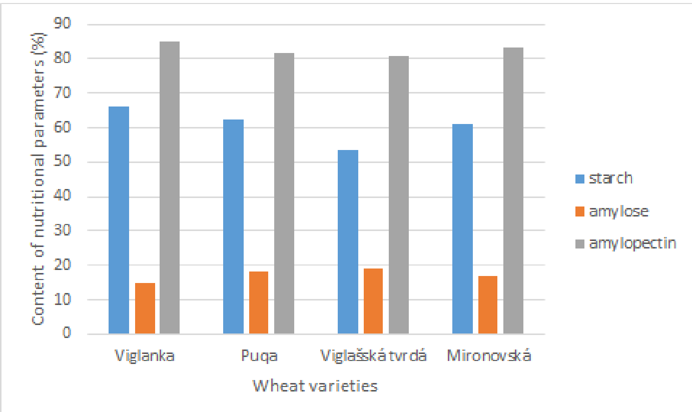

Figure 1 Content of total starch and ratio amylose/amylopectin in common wheat varieties

With regard to the chemical composition of total starch, the content of amylose did not demonstrably differ ($F= 1.60$; $P= 0.29$) between common varieties. Data from statistical evaluation are summarised in Table 3. In the varieties, the amylose content varied from 14.77 % to 19.05 % and in average 16.91 %. The variety Vígľašská tvrdá (19.05 %) excelled by the highest amylose content in grain in the evaluated set followed by the varieties Puqa (18.37 %), Mironovská (16.77 %) and Viglanka (14.77 %; Figure 1). By contrast to our results, other authors (**Singh *et al.*, 2008; Singh *et al.*, 2010**; **Shevkani *et al.*, 2011**; **Wang *et al.*, 2014**) have reported higher concentrations of amylose (26.8 % - 34.7 %; 18.2 % - 28.8 %; 27.4 % - 37.2 %; 27.1 %, respectively) in wheat grains. However, this discrepancy can be attributed to different wheat varieties analysed in our and those studies.

Table 3 ANOVA for multiple regression of amylose content in common wheat varieties

Sources of variance	Sum of squares	Df	Mean squares	F-value	P-value
Within groups	20,0317	5	4,00634	1,60	0,2909
Between groups	15,0464	6	2,50773		
Total	35,078	11			

In general, amylose to amylopectin ratio is one of the main factors affecting starch digestibility which has influence not only on gastric health and postprandial blood glucose levels but also the lipid metabolism **(Shevanki et al., 2017)**. It is known that amylose tends to form secondary structures that are hard to disperse, both in the starch granule and after food processing **(Englyst and Englyst, 2005)** thus resulting in the formation of resistant (slowly digested) starch **(Shewry *et al*., 2016)**. On the other hand, starches with high content of amylopectin are digested and absorbed more quickly than those enriched in amylose and produce larger postprandial glucose and insulin responses. This fact was confirmed in the study by **Byrnes et al. (1995)** who have reported the development of insulin resistance in rats fed with high amylopectin diet (i.e., quickly digested starch). In contrast, it was shown that chronic consumption of high-amylose foods normalizes the insulin response of hyperinsulinemic human subjects indicating the potential benefit of such diet for diabetics **(Behall and Howe, 1995)**. On the basis of all the aspects it is obvious that the determination of the amylose/amylopectin ratio of wheat starch as indicator of glycemic index is crucial for nutrition and human health.

Moreover, the ratio of the two α-glucans in starch granules as well as their molecular structure influence e.g. the solubility, gelatinization temperature, viscosity, gelation and retrogradation properties of starch and therefore represent major parameters for the quality, texture and stability of starch- or flour-based products **(Blazek and Copeland, 2008)**. For example, the physicochemical properties of waxy (1 % of amylose) and high-amylose wheat (37.5 % of amylose) starches were investigated and compared to those of normal bread-wheat starch (26.5 % of amylose) by **Hung *et al.* (2007)**. The authors have found higher gelatinization temperature, transition enthalpy and crystallinity of granules in the waxy wheat starch as compared to the normal and high-amylose wheat starches.

Determination of starch and amylose contents in colored wheat varieties

In colored wheat, the content of starch was significantly different (F= 252.12; P < 0.0001) between investigated varieties. In the Table 4, data from statistical analysis are presented. As demonstrated in the Figure 2, Scorpion variety displayed the highest starch content (68.50 %) whilst the lowest content was reported in the old variety Vígľašská červená (52.23 %). In accordance with our findings, the same starch content (67.0 %) in Scorpion variety was determined by **Hřivna *et al.* (2018)**. The average total content of starch in the analysed colored varieties was 60.37 %, dissimilarly to average starch content of 72.6 % reported recently in two red wheat cultivars **(Ma *et al.*, 2017)**. Moreover, another research collective **(Zanoletti *et al.*, 2017)** also analyzed colored wheat, specifically purple wheat which was provided by Molino Quaglia S.p.A. The starch content in this sample also showed a high value of 76.17 %.

Table 4 ANOVA for multiple regression of starch content in colored wheat varieties

Sources of variance	Sum of squares	Df	Mean squares	F-value	P-value
Within groups	452,717	6	75,4529	252,12	0,0000
Between groups	2,0949	7	0,299271		
Total	454,812	13			

Further, the statistically significant differences (F= 6.57; P < 0.01) in the content of amylose among the colored varieties are demonstrated in Table 5. This study showed negative correlation relationships (r= -0.6928*) between amylose content and starch content in wheat color varieties. The amylose content in colored wheat ranged from 17.83 % to 22.93 % (for Scorpion and Viglašská červená, respectively), with an average around 20.38 %.

Table 5 ANOVA for multiple regression of amylose content in colored wheat varieties

Sources of variance	Sum of squares	Df	Mean squares	F-value	P-value
Within groups	47,6905	6	7,94842	6,57	0,0129
Between groups	8,47355	7	1,21051		
Total	56,1641	13			

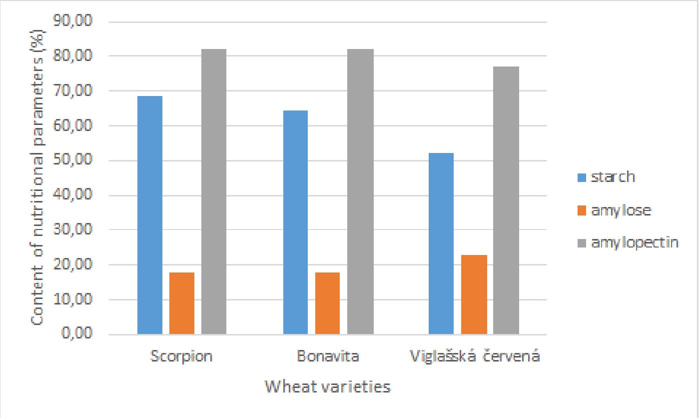

Figure 2 Content of total starch and ratio amylose/amylopectin in colored wheat varieties

The trend of the utilization of non-traditionally colored wheat in human nutrition has an increasing tendency since the consumption of colored grains supplies an organism with not only starch but also beneficial pigment substances such as anthocyanins and beta-carotene **(Garg *et al*., 2016)**. Anthocyanins belong to bioactive compounds, and their consumption is linked to numerous beneficial health effects **(Ranilla *et al*., 2010)**. Due to the reduction of risk of oxidative damage, anthocyanins were recently proposed as potential candidates for the treatment of cardiovascular disease or cancer **(Chen *et al*., 2012)**. Positive role of anthocyanins in the treatment of type 2 diabetes has also been suggested **(Ranilla *et al.*, 2010).** Anthocyanins were found to reduce starch digestion and inhibit activities of enzymes linked to postprandial hyperglycemia (α-glucosidase and α-amylase) **(Ranilla *et al.*, *2010;* Matsui *et al*., 2001)**. In the work by **McDougall *et al*. (2005)** the inhibitory activity of several extracts from soft fruit were tested against α-glucosidase. The authors reported that the fractions rich in anthocyanins were effective in inhibiting the enzyme. Additionally, this inhibition effect was also observed in anthocyanins isolated from black rice **(Yao *et al*., 2010)**. The described abilities of anthocyanins start to shed more light to colored wheat varieties as a promising source of health-promoting substances.

Determination of starch and amylose contents in emmer wheat varieties

In this part of the study, statistically significant differences were not found between the content of starch in emmer wheat varieties (F= 1.52; P= 0.40; Table 6). The starch content showed values between 60.56 % and 62.21 % with an average of 61.39 %. The Badurka variety, had the lowest quantity, and the Durgalova variety showed the highest value (Figure 3). Our values were lower than those reported by **Lacko-Bartošová and Lacko-Bartošová (2016**; 75.50 %) but similar to those reported by **Galterio *et al.* (1994; 2003)**, ranging from 52.7 % to 56.8 %, and from 45.7 % to 65.1 %, respectively. These differences can be caused by investigation carried out on new lines with enhanced starch content, which is consistent with the results ranging from 55.4 % to 73.3 % reported by **Galterio *et al.* (2001)**.

Table 6 ANOVA for multiple regression of total starch content in emmer wheat varieties

Sources of variance	Sum of squares	Df	Mean squares	F-value	P-value
Main effects					
A:variety	3,05583	2	1,52792	1,52	0,3967
B:repeated	0,000816667	1	0,000816667	0,00	0,9798
Residual	2,00923	2	1,00462		
Total	5,06588	5			

For amylose content, no statistically significant differences between varieties of emmer wheat (F= 14.21; P= 0.07; Table 7) were found. The amylose content ranged from 21.77 % to 33.68 %, with the average of 27.73 %. The highest value of amylose was obtained for the Badurka variety, on the contrary, the lowest reported value was for the Zirnita variety; Figure 3. The results agree well with the previous study by **Galterio *et al.* (2003**) showing that the content of amylose varied between 21.9 % and 23.1 % with an average of 22.28 %, and study by **Galterio *et al.* (2001)** with the content of amylose from 22.5 % to 29.9 %.

Table 7 ANOVA for multiple regression of amylose content in emmer wheat varieties

Sources of variance	Sum of squares	Df	Mean squares	F-value	P-value
Main effects					
A:variety	113,515	2	56,7576	14,21	0,0658
B:repeated	0,442817	1	0,442817	0,11	0,7709
Residual	7,99023	2	3,99512		
Total	121,948	5			

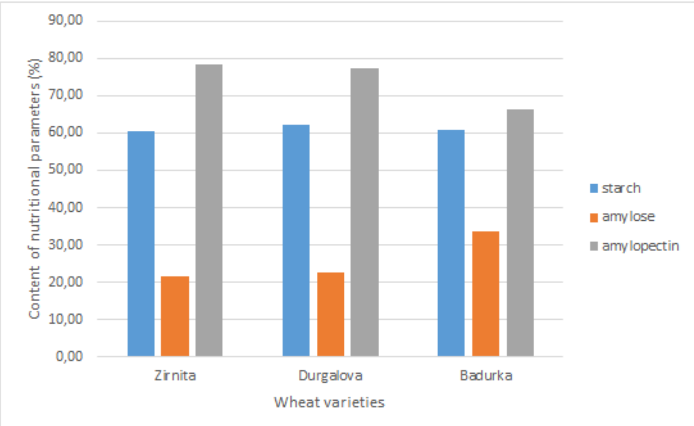

Figure 3 The content of total starch and ratio amylose/amylopectin in emmer wheat varieties

When comparing the three species (common, colored and emmer) of wheat varieties analyzed in the present study, differences in the starch amount and composition can be observed, although not verified by statistical analysis. Emmer varieties displayed the highest average amount of starch and amylose (61.39 % and 27.73 %, respectively), followed by color varieties (60.37 % and 20.38 %) and common varieties (59.86 % and 16.91 %). Certain discrepancies were noticed in the starch content values and the amylose/amylopectin ratio between our data and results from other studies. Yet, it is generally known, that not only the yield but also the quality of seed are determined genetically and environmentally **(Zhang *et al.*, 2008)**. Among the environmental factors, soil water status is considered as the crucial factor affecting the yield and the quality of cereal grains **(He *et al.*, 2012; Lu *et al.*, 2015)**. Soil drought can regulate the activity of enzymes involved in starch biosynthesis (e.g., granule-bound starch synthase, soluble starch synthase and ADP-glucose pyrophosphorylase; **Zhang *et al.*, 2011**) and thus modify starch composition. For example, in a recent work it was revealed that amylose synthesis in wheat grains is more sensitive to soil drought than the amylopectin synthesis **(Zhang *et al.*, 2017)**. Therefore, the source of the dissimilarity in the starch content and composition between our results and other studies can be attributed to both – genetic factors (due to different varieties used in studies) and environmental aspects (such as different growing conditions). In the present study, all the analyzed varieties were grown under the same experimental conditions. Hence, it can be hypothesized that our results might reflect the tendency toward higher starch and amylose content in emmer varieties.

Starch comprises the main source of calories in bread and other wheat-based food products. The rate of starch digestion is important relating to the glycaemic load and the development of type 2 diabetes. As mentioned above, starches containing high amounts of amylose have slower absorption rate, thus the consumption of such foods could possibly have positive effects in terms of glucose and insulin responses. Determination of varieties with the highest amylose content is of considerable importance, as those varieties might be considered as a suitable ingredient for production of cereal-based products in personalized nutrition, noteworthy mainly with regard to diabetic patients. Besides starch and amylose, wheat is a source of other nutritionally important compounds (such as various phytochemicals present mainly in colored varieties) and minerals. Based on previous studies **(Shewry *et al.*, 2016; Shewry *et al*., 2017; Fan *et al.*, 2008)**, inverse relationship between the content of starch and mineral micronutrients like zinc and iron can be anticipated. Therefore, the assessment of the content of beneficial components in similar studies should not be omitted and will be involved in the methodology of our future investigations on wheat.

CONCLUSION

In this scientific study, the nutritional parameters of wheat grain were determined, namely the starch content and the amylose/amylopectin ratio in 10 wheat varieties. The starch content ranged from 53.61 % (Vígľašská tvrdá) to 68.50 % (Scorpion) in the wheat varieties analyzed. In the oldest common varieties (Vígľašská červená and Vígľašská tvrdá) we observed the lowest starch contents (52 - 53 %). On the other hand, modern wheat varieties grown after 2007 had the highest starch content. The amount of amylose in wheat grains of the studied varieties ranger from 14.77 % (Viglanka) to 33.68 % (Badurka) and in colored varieties inversely correlated with the amount of starch. Our data confirm specific content and composition of starch among different wheat varieties. Prolonged and intensive breeding of wheat commodity gave rise to varieties that would be unrecognizable to our ancestors. Raw materials rich in starch are widely used in food industry, although their applications are considerably affected by starch characteristics. Unsurprisingly, breeding efforts have been focused on the enhancement of this parameter. Wheat grains are also a valuable source of other nutritionally interesting substances which should be acknowledged in future research investigations and breeding programmes.

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