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Alteration of Carbohydrate Metabolism in *Fusarium* Infected Wheat Kernels Treated with Fungicides and Its Relation to Baking Technological Parameters and Deoxynivalenol Contamination

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Abstract: Changes of water-soluble carbohydrate (WSC) content such as fructose, glucose, sucrose, maltose, nystose, raffinose, stachyose and fructan were analyzed in wheat kernels in *Fusarium* epidemic and non-epidemic seasons. In both season types, eight commercial fungicides were applied and three wheat varieties with differing *Fusarium* resistance were tested. In the epidemic year, the average total amount of WSC was above 1.6% which was 2 times higher than in the non-epidemic year (0.7%). Sucrose, maltose, raffinose and fructan components determined the increased WSC value, but the most substantial change was observed in maltose content where its average amount was 28 times higher in the epidemic year. Fungicide application also significantly increased all the carbohydrate components except maltose, where significant reduction was observed. WSC components had strong correlation with several farinograph or extensograph parameters, but only the maltose content showed positive strong correlation (r = 0.9) with deoxynivalenol (DON) toxin that was highly affected by the applied fungicide. The changes of WSC indicate altered carbohydrate synthesis along with abnormal degradation processes and thus have impaction on the baking features. It seems that the sugar metabolism interacts with DON synthesis and the results give important additional information to the altered metabolism of the attacked plant.

Keywords: water soluble carbohydrates; *Fusarium* infection; maltose; fungicide; quality; deoxynivalenol

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1. Introduction

Water-soluble carbohydrates are minor components in wheat endosperm with total values of 1–2% [1,2]. The most abundant soluble carbohydrates in wheat are glucose, fructose, galactose as monosaccharides, sucrose, maltose as disaccharides, and raffinose as trisaccharide [3,4]. Fructans, the oligosaccharides of fructose with the degree of polymerization 2–10, are also present in remarkable amounts in wheat grain [5].

Simple carbohydrates play nonstructural roles in plant life involving nutrients, hormone-like signaling molecules or regulators of metabolism, stress responses, and growth and development [6]. The content levels of soluble carbohydrates and their compositions in grain change during wheat kernel development from anthesis until maturity. This carbohydrate content level is negatively correlated with storage compounds such as starch and protein. Studies showed [7,8] that fructan is the major carbohydrate with a maximum value of 35% in the early developing grain, but the monosaccharides (glucose, fructose)

and sucrose also reach their highest concentration in this period (3% and 7%, respectively). Once grain filling started, these compounds were consumed while large amounts of starch, protein and fiber components were deposited simultaneously.

Partially due to climate change, plant survival under environmental stresses and the role of WSC in abiotic stress has been extensively investigated. Many studies, including [9,10], have shown that abiotic stress such as salt, drought, or freezing causes increased water soluble carbohydrate concentration. Stress tolerant varieties in those studies accumulated higher carbohydrate content than that of the intolerant ones. In most of the previous studies, the main goal was to characterize the role of carbohydrates in the stress responses. That goal has resulted in a focus on the vegetative parts of plants (leaves and seedlings), and only a few studies have investigated changes of sugar metabolism in grains. Those few with grain observations have shown interactions between environmental conditions and sugar concentration in cereal grain too, even the tendencies are the same. For example, ref. [11] reported an increase of sucrose and reducing other sugars in heat-stressed wheat grain during grain filling, and another [12] supported this finding.

Fusarium head blight (FHB) FGB also influence the carbohydrate metabolism in wheat, but only a limited number of research papers was found on this topic. *Fusarium* infection can cause direct damage to kernels, resulting in yield loss and poor flour quality varying for resistance level and protective capacity of the fungicides [13]. *Fusarium* infection also leads an increased carbohydrate content in both wheat shoots and kernels similar to the effects described for abiotic stresses [14–16], but in leaf diseases we do not have toxin problems.

Simple carbohydrates occurring in the wheat endosperm do not play a significant part in technological quality of wheat flour, especially not in rheological properties. The only exception could be maltose, as it supposedly forms mostly from the degradation of starch by the activity of α-amylase and β-amylase [1]. In this way the amount of maltose is in negative correlation with Hagberg falling number (HFN) [1,17,18]. We should add that for *Fusarium* the higher rate of soluble carbohydrates may originate from the inhibition of starch synthesis, therefore, the water soluble sugars cannot be used and remain soluble form. In the bread making procedure, simple carbohydrates act as a substrate for yeast during fermentation and can be involved in caramelization and Maillard reactions [19].

In earlier studies, significant differences were found in technological parameters between treated and untreated wheat samples when severe *Fusarium* infection occurred [13,20]. Among the quality traits, HFN values depended significantly on the applied fungicide and thus it was correlated to the extent of infection.

The purpose of this study was to investigate the background of these changes from the point of view of water-soluble carbohydrate content. Aside from carbohydrate changes, the correlation between technological parameters, DON content and carbohydrate content was also followed to identify possible connections between soluble sugar content and DON contamination.

2. Materials and Methods

2.1. Field Experiments

Flour samples were collected from farm-scale fungicide trials as published in [20]. Three wheat varieties with differing *Fusarium* resistance were examined: the *Fusarium* sensitive (GK Kalasz, S), a moderately susceptible (GK Bekes, MS), and a moderately resistant (GK Feny, MR) cultivar. The wheat varieties were tested for 2 years (2010–2011) at Kiszombor, the test site of the Cereal Research Non-Profit Company (46°10 N, 20°25 E, altitude 83 m). In May and June of 2010 there were nearly 300 mm rain measured at high temperatures, and a severe *Fusarium* Head Blight (FHB) epidemic developed. The next year, 2011, was a dry year with 100 mm of precipitation in the same period. The epidemic level was low, only sporadic infections developed.

Natural *Fusarium* contamination was enhanced by maize as previous crop. Fungicide treatments were carried out at full flowering. Eight commercial fungicides were selected for testing (Table 1) that were chosen from the suggested fungicides against FHB by the

fungicide producers and earlier experiences. The control plots (or UTC as untreated control), designated as treatment 9, were not treated by chemicals. The experiments were carried out in triplicate.

Table 1. Active ingredients of the fungicides and their abbreviations.

Commercial Name and Application Rate (L/ha)	Active Ingredient (g/L)	Abbreviation of Active Ingredient
Alert S 1.0	fluzilazole 125 + carbendazim 250	FC
Cherokee 2.0	ciproconazole 50 + propioconazole 62.5 + chloronitrile 375	CPC
Prosaro 1.0	prothioconazole 125, tebuconazole 125	PT
Caramba 1.2	metconazole 90	M90
Eminent 1.0	tetraconazole 125	TET
Falcon 0.8	tebuconazole 167 + spiroxamine 250 + triadimenol 43	TST
Folicur Solo 1.0	tebuconazole 250	T250
Juwel TT 1.0	epoxyconazole 83 + kresoxym-methyl 83 + fenpropimorf 317	EKF

3 kg grain was separated from each subplot after harvest, which served as experimental material for the quality analysis. White flour was ground by Brabender Senior Laboratory Mill (four break and four reduction rolls, sieve size < 195 µm) after conditioning wheat samples. DON analyses were made within 3–4 months after harvest. The same happened with the flour quality data published in 2017 [13] at internet edition. Flour samples for sugar analyses were kept in a dry, cool environment until usage at 4 °C and were analyzed within one year after harvest. We had this storage in refrigerator and dry to keep the original physiological status as far as possible. This was applied in both years.

2.2. Determination of Simple Carbohydrates

The extraction of the carbohydrates was carried out according to [21] with minor modification. Finely ground, 50 mg white flour samples were extracted with 3 mL of methanol/water (80/20, *v*/*v*) for 2 h with a vertical shaker at 20 °C to prevent the effect of the endogenous enzymes. After centrifugation (11,200× *g*, 10 min) the supernatants were filtered through a 0.22 µm PTFE membrane filter (Phenomenex, Bologna, Italy). Ribitol (10 µL, 0.5 mg/mL) as internal standard was added to 1 mL of the filtrates, and 5 µL of the final solution was injected into the LC-MS system. LC-MS/MS analyses were carried out on a Shimadzu Nexera XR HPLC system (Duisburg, Germany) coupled to a TSQ Quantum Access triple quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with an H-ESI probe. Liquid chromatographic separation was performed using a SeQuant (Merck, Darmstadt, Germany) ZIC-HILIC column (3.5 µm, 150 × 2.1 mm) column equipped with a SeQuant (Merck, Darmstadt, Germany) ZIC-HILIC guard column (20 × 2.1 mm) thermostated at 25 °C. Mobile phase A consisted of 5 mM ammonium-acetate containing 0.1% formic acid, while acetonitrile containing 0.1% formic acid served as mobile phase B, adapted from [22]. The gradient elution was performed as follows: 0 min, 80% B; 0.5 min, 80% B; 8.5 min, 40% B; 10.5 min, 40% B; 11 min, 80% B; 20.0 min, 80% B. The mobile phase flow rate was maintained at 0.2 mL/min and the injection volume was 5 µL. The general MS conditions were set as follows: spray voltage, 4500 V; vaporizer temperature, 250 °C; sheath gas (nitrogen) pressure, 50 psi; auxiliary gas (nitrogen) flow, 10 arbitrary units; ion transfer capillary temperature, 200 °C; collision gas (argon) pressure, 1.5 mTorr. Electrospray ionization was operated at negative mode. The carbohydrates were detected as formylated molecules $[M + HCOO]^-$. Mass spectrometric detection of the carbohydrates was carried out in multiple reaction monitoring (MRM) mode. MRM transitions were 197 > 151, 225 > 180, 387 > 180, 549 > 180, and 711 > 383 for ribitol, mono-, di-, tri- and tetra saccharides, respectively. The acquired data were processed using Xcalibur™ version 2.2.1 and Trace Finder version 3.3 (Thermo Fisher Scientific, Budapest, Hungary). All carbohydrate standards, ammonium-acetate and formic acid were purchased from Sigma

(Darmstadt, Germany). Water and acetonitrile (HPLC-grade) were obtained from VWR International, Debrecen, Hungary.

2.3. Determination of Fructan Content

Total fructan content was determined with the enzymatic/spectrophotometric AOAC method 999.03 [23] using commercially available enzymatic kits (Fructan HK Assay kit, Megazyme, Bray Business Park, Bray, Co., Wicklow, Ireland) in accordance with the manufacturer's instructions. 1.0 g of flour with 40 mL hot distilled water (~80 °C) was cooked on a hot-plate, magnetic stirrer for 15 min. After cooling to room temperature, the solution was quantitatively transferred to 50 mL volumetric flask and was adjusted to volume with distilled water. Aliquot was filtered through Whatman No. I filter and analyze immediately. 0.2 mL aliquot was dispensed into glass test-tubes and 0.2 mL sucrose/maltase mixture (prepared according to Megazyme (Wicklow, Ireland) instruction) was added. The mixture was incubated at 40 °C for 30 min. 0.5 mL 100 mM sodium acetate buffer (pH 4.5) was added to each solution. After mixing, 0.2 mL aliquot was dispensed to two plastic spectrophotometer cuvettes (2.5 mL). 0.1 mL fructanase solution was added to one of the cuvettes, 0.1 mL 100 mM sodium acetate was added to the second cuvette. After mixing, the covered cuvettes were incubated at 40 °C for 30 min in a dry hot-block heater. 2.0 mL distilled water, 0.2 mL buffer pH 7.6 and 0.1 mL $NADP^+$/ATP solution were added to each cuvette, mixed, and after 3 min. absorbances were read at 340 nm. Finally, 0.02 mL of HK/PGI/G-6-PDH suspension was added to the cuvettes and after the reaction stopped, absorbances were read again at 340 nm. Fructan was calculated according to the instruction handbook. All enzyme solutions, buffer pH 7.6, $NADP^+$/ATP solution and HK/PGI/G-6-PDH suspension were provided by the Fructan HK Kit (Megazyme, Wicklow, Ireland).

2.4. Wheat Quality Analysis Methods

The physical properties of wheat kernels such as wheat hardness, moisture content and 1000 kernel weight were determined by Perten Single Kernel Characterization System 4100 (Perten Instruments AB—Segeltorp, Sweden). Wet gluten was determined according to the ICC method 106/2. The dough rheological properties were examined by Brabender Farinograph according to ICC method 115/1. Farinograph quality number, dough development time, water absorption were assessed. Extensograph parameters (extensograph energy at 135 min, extensograph extensibility, maximum resistance to extensibility) were measured by Brabender Extensograph (Brabender GmbH & Co., Duisburg, Germany) according to ICC 114/1 method using 300 g of flour. Sedimentation values were obtained by using the Zeleny ICC method 116/1. Falling number "Hagberg" was measured according to the ICC method 107/1 using falling number apparatus (Perten Instruments, Segeltorp, Sweden).

2.5. DON Analyses

6 g of grain samples were milled with Laboratory Mill 3310 (Perten Instruments, Segeltorp, Sweden). 1 g flour was used for DON extraction with 4 mL of acetonitrile/water (84/16, v/v) mixture for 2.5 h with a vertical shaker. Following centrifugation (10,000 rpm, 10 min), 2.5 mL of the extract was passed through an activated charcoal/neutral alumina SPE column at a flow rate of 1 mL/min. Thereafter, 1.5 mL of the clear extract was transferred to a vial and evaporated to dryness at 40 °C under vacuum. The residue was dissolved in 500 µL of acetonitrile/water solution (20/80, v/v). HPLC analyses was performed on Agilent Infinity 1260 (Agilent Technologies, Santa Clara, CA, USA). DON was separated on a Zorbax SB-Aq (4.6 × 50 × 3.5 µm) column (Agilent, Santa Clara, CA, USA) equipped with a Zorbax (Agilent, Santa Clara, CA, USA) SB-Aq guard column (4.6 × 12.5 × 5 µm) thermostated at 40 °C. The mobile phase A was water, while mobile phase B was acetonitrile. The gradient elution was performed as follows: 0 min, 5% B; 5 min, 15% B; 8 min, 15% B; 10 min, 5% B; 12 min, 5% B. The flow rate was set to 1 mL/min.

The injection volume was 5 µL. DON was monitored at 219 nm. The method of DON analysis is given in details in [24].

2.6. Statistical Analysis

The analysis of variance (ANOVA) and PCA (Principal Component Analysis) was calculated by STATISTICA 12 (developed by StatSoft Inc., 2013, Tulsa, OK, USA) software. The treatment means were compared using Fischer's protected LSD test at $p < 0.05$. The Pearson's correlations were made according to the built-in functions of Analysis ToolPak of Microsoft Excel version 1997–2003.

3. Results

The two experimental seasons covered varied *Fusarium* infection severities due to differing precipitation and environmental conditions. In 2010, *Fusarium* infection was severe in the experimental field, the average rate of *Fusarium* damaged kernel (FDK) was 8.5%, while in the next year it was only 0.4%, while the naturally infected control in the susceptible cultivar showed 22.50%, this was reduced by the best fungicide 7.38%. The most resistant variety gave 7.75% in the untreated, and only 0.32% for the best fungicide [13]. The results of WSC contents were significantly different between the two seasons.

3.1. Concentrations of Water-Soluble Carbohydrate Components

The WSC content was significantly higher in 2010 than in 2011, and there were also significant changes between the fungicide treatments (Tables 2 and 3). Comparing the average content of the various WSC components of the epidemic year to the non-epidemic, significant increase was experienced in sucrose, maltose, raffinose and fructan content. The average total WSC content in 2010 was 1.6%, while it was below 0.8% in 2011, which means double difference between the epidemic and non-epidemic season.

In the epidemic year (2010), there were significant differences between the UTC and the fungicide treated samples, except in two cases (sucrose, stachyose). The fungicide treated samples contained increased fructose, glucose, nystose, raffinose and fructan content compared to the UTC. In the cases of fructose and fructan, their content was significantly higher in all treated samples (117–152 mg/kg and 7583–9433 mg/kg, respectively) than in UTC (92 mg/kg and 7283 mg/kg), and samples treated with PT and TST had the highest fructose (152 mg/kg, 143 mg/kg) and fructan (9433 mg/kg, 8933 mg/kg) content.

Table 2. Water soluble carbohydrate content in wheat flour samples treated with various fungicides in a *Fusarium* epidemic year (2010); results are the average of three wheat varieties.

	Fructose		Glucose		Sucrose		Maltose		Nystose		Raffinose		Stachyose		Fructan		Total	
Fungicide	**(mg/kg)**		**(mg/kg)**		**(mg/kg)**		**(mg/kg)**		**(mg/kg)**		**(mg/kg)**		**(mg/kg)**		**(mg/kg)**		**(mg/kg)**	
TST *	143	d	262	a	2101	a,b	2670	a	42	d	577	d	4	a	8933	f	14,732	a
PT	152	e	271	a,b	2340	b	2235	a	50	e	655	d	4	a	9433	g	15,139	a
M90	125	b,c	362	b,c	2225	a,b	4718	b	35	a–c	348	b,c	5	a	7817	c	15,634	a,b
TET	123	b,c	384	c	2007	a,b	4858	b,c	33	a,b	281	a–c	3	a	8200	e	15,888	a,b
T250	131	c,d	368	b,c	2242	a,b	4837	b,c	37	a–d	366	c	3	a	8167	e	16,152	a,b
CPC	128	b,c	396	c	1708	a	5887	b,c	39	c,d	297	a–c	3	a	7967	d	16,425	a,b
FC	117	b	332	a–c	2275	a,b	5497	b,c	38	b–d	305	a–c	4	a	7933	d	16,500	a,b
EKF	118	b,c	362	b,c	1830	a,b	6746	c	33	a,b	270	a,b	3	a	7583	b	16,944	a,b
UTC	92	a	279	a,b	2039	a,b	10,377	d	32	a	257	a	4	a	7283	a	20,363	b
Mean	125		335		2085		5314		37		373		4		8146		16,420	
LSD 5% **	13		98		577		1996		5		88		2		102		4784	

* TST = tebuconazole + spiroxamine + triadimenol; PT = prothioconazole + tebuconazole; M90 = metconazole; TET = tetraconazole; T250 = tebuconazole; CPC = ciproconazole + propioconazole + chloronitrile; FC = fluzilazole + carbendazim; EKF = epoxyconazole + kresoxym-methyl + fenpropimorf; UTC = untreated control); ** between treatments (fungicides and UTC, 9 treatments); Values followed by different letters are significantly different where significance is performed by Fisher LSD test.

Table 3. Simple carbohydrate content in wheat flour samples treated with various fungicides in a non-epidemic year (2011); results are the average of three wheat varieties.

	Fructose		Glucose		Sucrose		Maltose		Nystose		Raffinose		Stachyose		Fructan		Total	
Fungicide	**(mg/kg)**		**(mg/kg)**		**(mg/kg)**		**(mg/kg)**		**(mg/kg)**		**(mg/kg)**		**(mg/kg)**		**(mg/kg)**		**(mg/kg)**	
TST *	101	a	280	a	681	a	135	a	74	a	37	a	1	a	5217	b	6526	a
PT	108	a,b	291	a	1035	a–c	168	a,b	76	a	65	c	2	a	5233	b	6979	a
M90	117	a–c	274	a	1304	c	153	a	110	a	59	b,c	3	a	5033	a	7053	a
TET	110	a,b	373	b	760	a,b	326	c	72	a	41	a,b	1	a	5483	d	7166	a
T250	128	c,d	319	a	955	a–c	315	b,c	90	a	47	a–c	2	a	5367	c,d	7223	a
CPC	142	d,e	296	a	1224	c	166	a,b	97	a	41	a,b	4	a	5383	c,d	7352	a
FC	150	e	300	a	1202	b,c	178	a–c	108	a	57	b,c	4	a	5717	e	7715	a
EKF	119	b,c	275	a	1281	c	165	a,b	115	a	46	a,b	3	a	5733	e	7737	a
UTC	119	b,c	289	a	913	a–c	114	a	76	a	45	a,b	2	a	5317	b,c	6875	a
Mean	122		300		1039		191		91		49		3		5387		7177	
LSD 5% **	17		48		456		151		45		18		3		130		1413	

* TST = tebuconazole + spiroxamine + triadimenol; PT = prothioconazole + tebuconazole; M90 = metconazole; TET = tetraconazole; T250 = tebuconazole; CPC = ciproconazole + propioconazole + chloronitrile; FC = fluzilazole + carbendazim; EKF = epoxyconazole + kresoxym-methyl + fenpropimorf; UTC = untreated control); ** between treatments (fungicides and UTC, 9 treatments); Values followed by different letters are significantly different where significance is performed by Fisher LSD test.

There were significantly higher nystose contents in 5 treated samples (TST, PT, T250, CPC and FC) in 2010 with 37–50 mg/kg values compared to UTC (32 mg/kg). The highest values were measured in PT and TST treated samples (50 and 42 mg/kg, respectively). Similar results could be observed in the raffinose content: TST, M90, PT and T250 treated samples had significantly higher raffinose content (348–655 mg/kg) than the naturally infected control (UTC: 257 mg/kg), and the highest values were again measured in the PT and TST treated samples with 655 mg/kg and 577 mg/kg. In glucose measurements there were not any significant differences between the treated and untreated samples, except in two fungicides (TET, CPC) where the amount of glucose was significantly higher (384 and 396 mg/kg) than in the UTC sample (279 mg/kg). Other fungicides also resulted in increased glucose values (332–368 mg/kg). Reduced glucose content was however measured in PT (271 mg/kg) and TST (262 mg/kg) treated samples, though the reduction was not significant. The highest change was observed in maltose content. In 2010, the average value of maltose was above 0.5% (5314 mg/kg), which was more than 28 times higher than in the non-epidemic year. The maltose content of the UTC samples was the highest (above 1%) (Table 4), and all the treated samples showed significantly lower maltose content (2235–6746 mg/kg). The biggest change comparing to UTC was observed with PT and TST treatments across all three varieties. The sensitive (S) variety contained the highest maltose content (avg. 9290 mg/kg), the moderately sensitive (MS) variety and the moderately resistant (MS) had significantly lower (avg. 3906 mg/kg and 2745 mg/kg, respectively). It is important that even though the reduction of the very high UTC values were highly significant, the maltose content still remained many folds higher compared to the practically healthy experiment in 2011. It is remarkable that at heavy epidemic all fungicides significantly reduced the maltose content, while at sporadic epidemic no changes or in two cases (CPC, FC fungicide) increase was observed.

In the non-epidemic year (2011), the differences in WSC content were minor between the fungicide treatments (Table 3), and no trends can be observed in these changes (Table A1, Appendix A). Also, in this year the maltose content varied between 101 and 578 mg/kg and it was significantly lower (avg. 191 mg/kg) then in the epidemic year (5314 mg/kg). In case of CPC and FC treatment, higher maltose content was measured but in general, there were no significant differences between fungicide treatments or varieties.

Table 4. Effect of fungicide treatments on maltose content in three wheat varieties, with differing levels of *Fusarium* susceptibility in a severe *Fusarium* epidemic (2010), Kiszombor-Hungary.

Fungicide	Maltose Content (mg/kg) 2010							Maltose Content (mg/kg) 2011						
	S		MS		MR		Mean	S		MS		MR		Mean
TST *	4538	a	2457	a	1016	a	2670	184	a	172	a,b	178	a,b	178
M90	9230	b,c	2436	a	2489	a	4718	177	a	166	a,b	156	a,b	166
TET	7107	a,b	3316	a	4153	a,b	4858	158	a	142	a	158	a,b	153
EKF	13,702	d,e	3398	a	3139	a,b	6746	125	a	211	a,b	168	a,b	168
PT	3893	a	1953	a	860	a	2235	156	a	172	a,b	168	a,b	165
T250	9967	b,c	2893	a	1653	a	4837	139	a	131	a	136	a,b	135
CPC	10,817	c,d	4188	a	2657	a	5887	365	b	302	b	278	a,b	315
FC	9465	b,c	4695	a	2332	a	5497	237	a	163	a,b	578	c	326
UTC	14,898	e	9822	b	6411	b	10,377	113	a	129	a	101	a	114
Mean	9290		3906		2745		5314	181		176		213		191
LSD 5% variety							1153							50
LSD 5% fungicide							1996							87

Means followed by different letters are significantly different where significance is performed by Fisher LSD test. Wheat varieties: sensitive to *Fusarium* (S), moderately sensitive (MS), moderately resistant (MR); * TST = tebuconazole + spiroxamine + triadimenol; PT = prothioconazole + tebuconazole; M90 = metconazole; TET = tetraconazole; T250 = tebuconazole; CPC = ciproconazole + propioconazole + chloronitrile; FC = fluzilazole + carbendazim; EKF = epoxyconazole + kresoxym-methyl + fenpropimorf; UTC = untreated control).

3.2. Results of Correlation Analyses between Carbohydrate Content, Technological Properties and DON Contamination

The relationship between quality traits such as wet gluten (WG), farinograph stability (FS), Hagberg Falling number (HFN), hardness index (HI), thousand kernel weight (TKW), protein (PRO), Zeleny index (ZI), extensograph energy (E135), extensograph extensibility (EXT), maximum resistance to extensibility (RMAX) [13], the DON and the total mass of simple carbohydrates was evaluated with correlation analyses referring to the epidemic year 2010 (Table A2). There were several strong correlations between the content of WSC components and other parameters. The changes in fructose, nystose, raffinose and fructan content showed strong (r = 0.7–0.9) or very strong (r > 0.9) positive relationships to technological parameters such as FS, HFN, E135, RMAX, FS, E135. The change of maltose content has also demonstrated this strong relationship with quality parameters, but in negative correlation. The strongest correlation regarding DON toxin content was measured in the case of maltose content too (Figure 1).

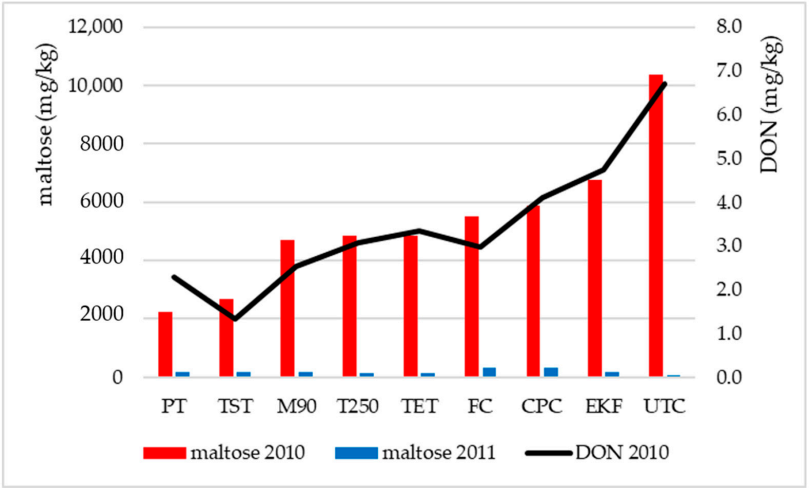

Figure 1. The average total amounts of maltose and DON (deoxynivalenol) toxin content across cultivars in fungicide-treated flour samples; 2010–2011. DON content in 2011 was below detection limit in all treatments.

PCA analysis was also performed to get better understanding about the interrelations between the examined 19 quality traits. The first four principal components (PC) exhibited more than 1.00 eigenvalue (Table 5) and showed maximum variability of 95.66%. PC1 had the highest variability (62.8%) followed by PC2 (20.24%), PC3 (6.55%) and PC4 (6.08%). On the basis of the value of factor loads, the first components represent 15 traits out of 19. Only three traits (protein, glucose, stachyose) belong to the second component, and the sucrose alone is related to PC3. In the system of PC1 and PC2 vectors of studied traits are presented in Figure 2. Four groups of strongly correlated variables can be determined from the graph as small distances (angels) between the vectors proves strong correlation between variables. MAL and DON belong to the first group, the second group is the PRO and STA, the third one is WG and ZI, the fourth is RAF, FS, HFN, E135, NYS, RMAX, FRN, EXT and FRU. Four sugar compounds were largely independent from any group they seem to have a neutral role. Surprisingly, the DON contamination did not show significant correlation with any other sugar compounds.

Table 5. Results of PCA analysis: Principal components, eigenvalues, percentage of total variations and factor loads of variables in case of twenty traits of wheat samples.

Traits	PC1	PC2	PC3	PC4
WG	0.7147	0.6805	0.0454	−0.0329
FS	0.8694	0.0325	−0.3228	−0.2420
HFN	0.9173	0.0074	−0.2980	0.1058
HI	0.8521	0.2814	−0.3088	−0.2196
TKW	0.7709	−0.5076	0.1219	0.1631
PRO	−0.0448	0.9386	−0.1724	−0.2755
ZI	0.7829	0.5708	0.0924	−0.1615
E135	0.9770	−0.0478	0.1815	0.0453
EXT	0.8082	−0.3008	0.2666	−0.3866
RMAX	0.9738	−0.1027	0.1586	0.0979
DON	−0.8021	0.2760	−0.1298	0.4605
FRU	0.8950	−0.3778	−0.0746	−0.0697
GLU	−0.5093	−0.6887	0.2037	−0.4174
SUC	0.5498	0.2623	0.7179	0.3147
MAL	−0.8929	0.3271	−0.0931	0.1715
RAF	0.9502	0.1143	−0.1333	0.1996
NYS	0.8775	−0.0944	−0.1756	0.3503
STA	0.1153	0.9026	0.3473	−0.1227
FRN	0.9180	−0.1720	−0.1169	0.2197
Eigenvalue	11.9316	3.8463	1.2436	1.1543
% of total variance	62.80	20.24	6.55	6.08

Abbreviations: WG—wet gluten, FS—Farinograph stability, HFN—Hagberg Falling number, HI—hardness index, TKW—thousand kernel weight, PRO—protein, ZI—Zeleny index, E135—extensograph energy, EXT—extensograph extensibility, RMAX—maximum resistance to extensibility (RMAX), DON—deoxynivalenol, FRU-fructose, GLU—glucose, SUC—sucrose, MAL—maltose, RAF—raffinose, NYS—nystose, STA—stachyose, FRN—fructan.

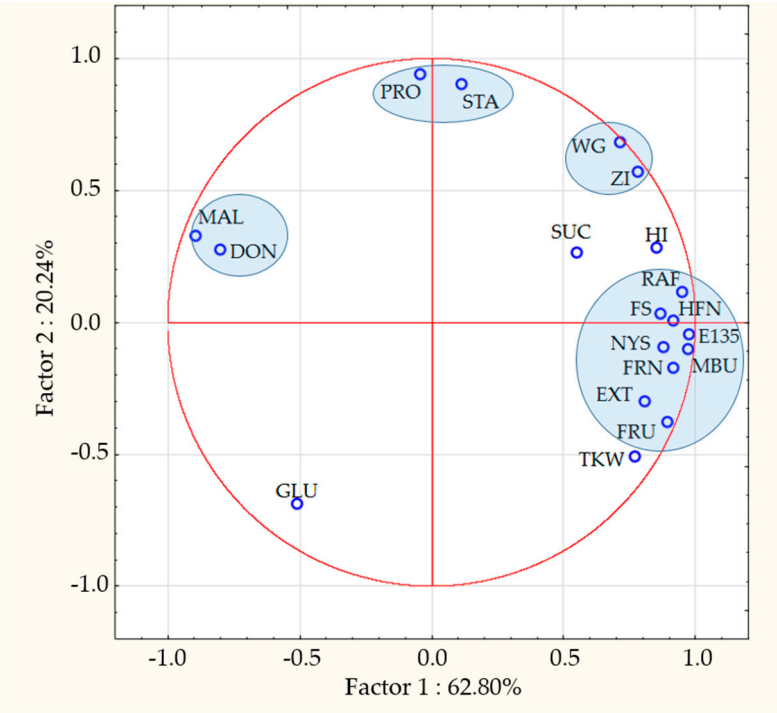

Figure 2. PCA graph of factor coordinates, grouping of the variables in two principal components. WG—wet gluten, FS—Farinograph stability, HFN—Hagberg Falling number, HI—hardness index, TKW—thousand kernel weight, PRO—protein, ZI—Zeleny index, E135—extensograph energy, EXT—extensograph extensibility, RMAX—maximum resistance to extensibility (RMAX), DON—deoxynivalenol, FRU—fructose, GLU—glucose, SUC—sucrose, MAL—maltose, RAF—raffinose, NYS—nystose, STA—stachyose, FRN—fructan.

4. Discussion

It is well proven that infections cause changes in carbohydrate, protein, and starch metabolism of the endosperm, but only a few papers investigated the changes of simple carbohydrate content in wheat kernel after *Fusarium* infection. In most cases, no or slight changes occurred in carbohydrate content [25,26], and one study reported increase in the total sugar content [15]. The present study shows that the severe *Fusarium* infection significantly affects the composition and rate of soluble carbohydrates of grains, but in different ways. Their concentrations were significantly higher in the epidemic year than the non-epidemic (except nystose), but decisively the maltose content was a determining factor in the increased amount. The question is what cause these changes.

There are several processes that can lead to the altered carbohydrate concentration. On the one part, *Fusarium* infection can cause impaired synthesis of carbohydrate components. It has been shown that fungal mycelium can cause mechanical blocking of vascular bundles, which can lead to incomplete supply of grain constituents, but the presence of mycotoxin itself such as DON toxin can also cause impaired protein synthesis of grain components [27–29]. There is also an assumption that *Fusarium* spp. secretes enzymes, such as proteases or carbohydrates, which can cause degradation of various grain components [30,31]. Also, as one study reported that powdery mildew suppressed the transformation of sugar into starch, and it can be hypothesized that *Fusarium* infection has a similar negative influence on grain carbohydrate metabolism [32]. In the case of *Fusarium,* the change of the sink-source relation may give an idea as the *Fusarium* attacked grains can build in led sugars in starch production, therefore a part of the sugars remains in the leaves and the young developing grains. We observed that severely infected heads caused a significantly later ripening of leaves showing that the assimilates in leaves could not be translocated and utilized by the developing grains. However, this does not explain the explicit role of the maltose.

On the other hand, environmental circumstances such as rainy weather before harvest could also contribute to the increased maltose level through high α-amylase activity (together with reduced HFN values). Therefore, biotic and abiotic stress along with the genetic background of the plants determine the simple carbohydrate content of the grain in a complex way [32,33].

According to present study, the variation of maltose content gives some explanations about the background. Maltose is mainly derived from hydrolysis of starch, which occurs due to high α-amylase activity and/or the presence of exoenzymes [34]. As 2010 was a wet year, the degradation of the starch could contribute to the increased maltose content, the increased disease severity yielded more DON, but parallel with this the increased a-amylase increased the maltose content on the other side. Therefore, the presence of high maltose content indicates irregular degradation process. Since the correlation analysis showed very strong relationship (r = 0.905) between the maltose and DON content, it shows surely that *Fusarium* infection is the factor behind these changes. It was a surprising result how strongly the maltose content confirms the severity of *Fusarium* infection, but the consideration above may give an explanation. In the more susceptible the variety, a much higher amount of maltose was produced, this seems to be a logical consequence of the event. It is clear for us that this finding needs further support and research to draw a more general conclusion.

PCA analysis proves also that there is mutual correlation between WSC, DON and flour quality parameters and Figure 2 shows well the strong correlation between the DON and maltose content. As maltose mostly originates from the starch degradation due to α-amylase activity, and in diseased kernels under humid conditions this process has a higher probability, this can explain the close correlation between the DON contamination and maltose content. In this respect we should test, how far the high falling number can influence the resistance to toxin accumulation. Therefore, we will check the data sets of the past two decades and new tests will be started. As all the technological quality traits belong to one principal component (PC1) along with most of the WSC and the DON content, it can be assumed that PCA1 factor is mainly responsible for all the correlation between the traits. As the DON and maltose content are closest among the tested sugars to DON, it seems that maltose content can be a biomarker signalizing the presence of DON.

For the fungicides we can conclude that all significantly decreased the amount of free sugars, but even the best fungicide could not restore the original free sugar level identified in the non-epidemic year 2011. The finding that the most effective fungicide could reduce the DON level close to zero in the most resistant variety GK Feny that is close to full success, but the higher maltose content signalizes that the quality could not be restored at this efficacy. In this respect the maltose metabolism needs further research. As fungicide reduced the rate of infection, the WSC content as well as rheological parameters changed significantly compared to untreated samples closer to the normality [13]. On the other side, the fungicide treatments without epidemic caused only slight but significant increase in maltose content at six fungicides, in two cases no change was recorded. We suppose that maltose can be a biomarker also for marking the quality problems. As this is variety and environment depending, further research is needed to have a better understanding this complex process. This Janus face of the maltose problem (DON and quality) seems to be an important research task for the future.

5. Conclusions

Biotic stressors such as *Fusarium* infection cause significant changes to the soluble carbohydrate content in wheat kernel. The total WSC content was significantly (twofold) higher in the epidemic year than in non-epidemic year, with maltose content as the main determinant. Maltose content was more than 28 times higher in the naturally infected control kernels and was highly affected by the applied fungicide and also with the *Fusarium* susceptibility of wheat varieties. DON toxin, WSC content and technological quality traits correlate strongly in case of severe *Fusarium* infection. The changes indicate altered

carbohydrate metabolism where one side the produced WS carbohydrates couldn't be translocated to starch and so accumulated in the grain (double concentration), and on the other side with abnormal (starch) degradation processes. The two processes may go at the same time with different intensity in time. It seems therefore, that maltose can be a biomarker for DON a quality characterization of the given wheat lot. So, the results can have also practical significance beyond scientific findings of the paper and serve as outgoing position for further research.

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Appendix A

Table A1. Pearson's correlations between simple carbohydrate content, wheat quality parameters and DON toxin after responses of fungicide treatments, 2011.

	WG	FS	HFN	HI	TKW	PRO	ZI	E135	EXT	MBU	DON	FRU	GLU	SUC	MAL	RAF	NYS	STA
FS	0.010																	
HFN	−0.296	−0.071																
HI	0.195	−0.123	−0.822 ***															
TKW	0.214	−0.410	0.628 *	−0.575														
PRO	0.869 ***	−0.235	−0.285	0.260	0.383													
ZI	0.582	−0.277	−0.006	0.168	0.448	0.826 ***												
E135	0.181	−0.002	0.179	−0.206	0.634 *	0.469	0.628 *											
EXT	0.630 *	0.103	−0.676 **	0.402	−0.212	0.576	0.289	0.218										
MBU	−0.315	0.074	0.668 **	−0.529	0.666 *	−0.169	0.100	0.599 *	−0.576									
DON	−0.328	−0.085	0.329	−0.333	0.218	−0.523	−0.654 *	−0.173	−0.286	0.338								
FRU	−0.244	0.709 **	−0.106	−0.193	−0.298	−0.460	−0.495	−0.088	−0.160	0.247	0.190							
GLU	0.523	0.321	0.192	−0.028	−0.033	0.198	0.086	−0.359	−0.097	−0.124	−0.050	−0.026						
SUC	−0.342	0.435	0.182	−0.602 *	0.071	−0.287	−0.226	0.339	−0.014	0.296	−0.033	0.568	−0.480					
MAL	0.443	0.388	0.159	−0.113	−0.214	0.055	0.052	−0.384	0.106	−0.317	−0.123	0.023	0.830 ***	−0.256				
RAF	−0.311	0.244	0.347	−0.563	0.169	−0.377	−0.481	0.188	0.086	0.228	0.580	0.103	−0.272	0.512	−0.133			
NYS	−0.433	0.454	−0.029	−0.361	−0.317	−0.413	−0.323	0.008	−0.010	−0.033	−0.229	0.570	−0.444	0.895 ***	−0.137	0.294	1.000	
STA	−0.381	0.606 *	0.018	−0.383	−0.087	−0.383	−0.329	0.271	−0.106	0.392	0.045	0.846 ***	−0.397	0.889 ***	−0.284	0.330	0.807 ***	
FRN	−0.843 ***	0.295	0.304	−0.327	−0.362	−0.832 ***	−0.587 *	−0.359	−0.650 *	0.169	0.058	0.460	−0.224	0.501	−0.115	0.169	0.680 **	0.529

Correlations *** $p \leq 0.01$, ** $p \leq 0.05$, * $p \leq 0.10$; Abbreviations are the following: WG: wet gluten, FS: Farinograph stability, HFN: Hagberg Falling number, HI: Hardness index, TKW: thousand kernel weight, PRO: protein, ZI: Zeleny index, E135: Extensograph energy at 135min, EXT: Extensograph stability, MBU: Extensograph maximum resistance to extensibility, DON: deoxynivalenol, FRU: fructan, GLU: glucose, SUC: sucrose, MAL: maltose, RAF: raffinose, NYS: nystose, STA: stachyose, FRN: fructan.

Table A2. Pearson's correlations between simple carbohydrate content, wheat quality parameters and DON toxin after responses of fungicide treatments, 2010.

	WG	FS	HFN	HI	TKW	PRO	ZI	E135	EXT	MBU	DON	FRU	GLU	SUC	MAL	RAF	NYS	STA
FS	0.650 *																	
HFN	0.638 *	0.814 ***																
HI	0.796 **	0.862 ***	0.899 ***															
TKW	0.229	0.604 *	0.652 *	0.450														
PRO	0.599 *	0.119	−0.027	0.320	−0.588 *													
ZI	0.962 ***	0.746 **	0.640 *	0.811 ***	0.333	0.536												
E135	0.684 **	0.796 **	0.838 ***	0.752 **	0.826 ***	−0.137	0.755 **											
EXT	0.416	0.705 **	0.630 *	0.609 *	0.723 **	−0.266	0.536	0.841 ***										
MBU	0.639 *	0.781 **	0.850 ***	0.734 **	0.858 ***	−0.200	0.709 **	0.996 ***	0.825 ***									
DON	−0.406	−0.699 **	−0.696 **	−0.711 **	−0.687 **	0.203	−0.526	−0.792 **	−0.949 ***	−0.781 **								
FRU	0.366	0.814 ***	0.804 ***	0.655 *	0.841 ***	−0.340	0.502	0.867 ***	0.829 ***	0.881 ***	−0.821 ***							
GLU	−0.820 ***	−0.380	−0.623 *	−0.640 *	−0.079	−0.528	−0.676 **	−0.444	−0.006	−0.433	0.048	−0.153						
SUC	0.589 *	0.162	0.349	0.263	0.398	0.007	0.578	0.664 *	0.447	0.649 *	−0.332	0.310	−0.460					
MAL	−0.391	−0.747 **	−0.784 **	−0.672 **	−0.787 **	0.300	−0.535	−0.876 ***	−0.890 ***	−0.884 ***	0.905 ***	−0.957 ***	0.130	−0.428				
RAF	0.736 **	0.819 ***	0.916 ***	0.815 ***	0.680 **	0.048	0.771 **	0.900 ***	0.611 *	0.904 ***	−0.613 *	0.836 ***	−0.661	0.513	−0.783 **			
NYS	0.549	0.795 **	0.859 ***	0.659 *	0.742 **	−0.187	0.579	0.855 ***	0.574	0.875 ***	−0.498	0.821 ***	−0.524	0.441	−0.726 **	0.913 ***		
STA	0.693 **	0.057	−0.024	0.255	−0.345	0.829 ***	0.671 **	0.119	−0.069	0.058	0.075	−0.231	−0.527	0.502	0.110	0.155	−0.085	
FRN	0.489	0.747 **	0.903 ***	0.714 **	0.785 **	−0.225	0.569	0.873 ***	0.643 *	0.898 ***	−0.672 **	0.914 ***	−0.451	0.447	−0.886 ***	0.935 ***	0.898 ***	−0.079

Correlations *** $p \leq 0.01$, ** $p \leq 0.05$, * $p \leq 0.10$; Abbreviations are the following: WG: wet gluten, FS: Farinograph stability, HFN: Hagberg Falling number, HI: Hardness index, TKW: thousand kernel weight, PRO: protein, ZI: Zeleny index, E135: Extensograph energy at 135min, EXT: Extensograph stability, MBU: Extensograph maximum resistance to extensibility, DON: deoxynivalenol, FRU: fructan, GLU: glucose, SUC: sucrose, MAL: maltose, RAF: raffinose, NYS: nystose, STA: stachyose, FRN: fructan.

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